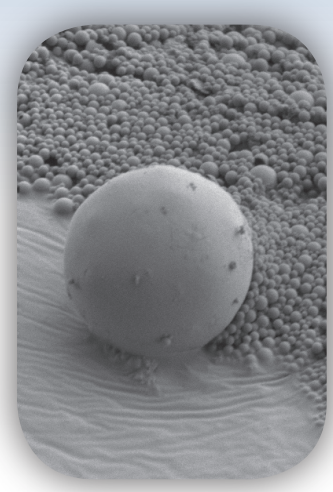
7th International Conference on the

Scientific and Clinical Applications of Magnetic Carriers

Conference Program and Abstract Booklet



University of British Columbia
Vancouver, Canada
May 20 - 24, 2008

http://www.magneticmicrosphere.com/

Welcome Message

It is my great pleasure to welcome you all to another, and already 7th International Conference on the Scientific and Clinical Applications of Magnetic Carriers. Since 1996, we have met in Rostock, Cleveland, Tallahassee, Lyon, Krems, and now Vancouver. We are excited to continue presenting new and interesting research in the small but wonderful world of magnetic particles. One of our primary aims is to promote collaborations so that our research area will continue to flourish.

As in the past, we wish to cultivate a familiar atmosphere not only during the talks, but also during breaks, lunches, the boat trip, and the yellow school bus tour. The beautiful campus of the University of British Columbia in the wonderful city of Vancouver provides us with the perfect surroundings in which to interact, learn and grow!

If you have any questions about anything, please don't hesitate to ask any of our volunteers at the registration desk. They are here to help.

I wish you all a wonderful conference and a great time.

Urs Hafeli, Chairman
Faculty of Pharmaceutical Sciences
University of British Columbia

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Free Wireless Internet Service

We have arranged for free wireless internet access during the 7th International Conference on the Scientific and Clinical Applications of Magnetic Carriers. By using this access, you are agreeing to the rules outlined in the privacy statement below.

Access to Free Internet Service

To get access to free internet access, either use the available computers in the SUB main floor or your own wireless-able laptop. On your own laptop, choose from the available networks or SSID "ubc". Open your internet browser and enter the following username and password.

Username: magmeet
Password: magnetic1

Privacy Statement

The meeting organizer has sponsored you for a Campus-wide Login (CWL) guest account during the conference week. It is important that you read, understand and agree to the terms of this service before using your CWL guest account.

The University of British Columbia's Campus-wide Login (CWL) supports the protection of privacy and the freedom of information throughout the campus in accordance with:

-UBC Policy #118, Records Management
(http://www.universitycounsel.ubc.ca/policies/policies.html)

-UBC Policy #106, Access to and Security of Administrative Information Systems
(http://www.universitycounsel.ubc.ca/policies/policies.html)

All applicable Canadian federal, provincial and local laws and statutes, including the Freedom of Information and Protection of Privacy Act R.S.B.C. 1996 c. 165, sections 26(a) and 26(c).

For further information on Freedom of Information at UBC, please contact the Freedom of Information (FOI) Coordinator. (http://www.universitycounsel.ubc.ca/contact/)

In using your CWL login, the user bears the primary responsibility for the material that he or she chooses to access, send or display. Always completely close all web browser windows when you finish using a web-based service.

The computer facilities may not be used in any manner that contravenes the above policies, laws or statutes. Those who do not adhere to these guidelines may be subject to the suspension of computing privileges in addition to any other applicable penalties or discipline.

For more detailed information, please refer to the Campus-Wide Login Caveats and Limitations document (http://www.it.ubc.ca/cwl/support/policies/caveats.shtml). It has further details on information collected, personal information you choose to share, legal restrictions, exceptions and the CWL security measures.

Sponsors

We thank our sponsors for their support of this conference. Without their generous contributions this meeting would not be possible.

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The City of Vancouver

Vancouver is situated along the beautiful coastline of the Pacific Ocean in the southwest corner of Canada. With a metropolitan population of over two million, Vancouver is the largest city in British Columbia and the third largest in Canada. People have immigrated to the city from many countries making Vancouver a wonderful mixing pot of different ethnic backgrounds.

Not only does Vancouver have a bustling city district but also vast wilderness and mountains outside city limits. Warm and wet weather coming from the Pacific Ocean create a lush habitat capable of supporting the area's temperate rainforests. This landscape leads to many outdoor activities that a great number of residents and visitors embark upon. Hiking, mountain biking and skiing are a few of the many options available just across the Burrard Inlet that separates Downtown Vancouver from the mountains that lie beyond.

Vancouver was chosen to host the 2010 Olympic games for its beauty, accessibility and range of activities available both in the city and the surroundings. Events will take place at venues in Vancouver as well as the popular Whistler Ski Resort to the north. This event is sure to make Vancouver a city known worldwide for its spectacular outdoor activities and progressive urban lifestyle.

The Vancouver based, University of British Columbia is situated on the west tip of the Point Grey Peninsula, approximately 30 minutes from downtown. Before entering campus you will pass through Pacific Spirit Park filled with walking and biking trails that offer a great look at the local habitat. Once on campus you will be presented with an array of state of the art buildings conducted teaching and research in areas of biotechnology, agriculture, commerce and more. The campus is used by over forty thousand students and more than seven thousand faculty members making it quite a busy place on the average afternoon. The campus has made a pledge to become a world model for sustainable practices and has a number of buildings that have been awarded for their green building accomplishments.

For more information http://www.city.vancouver.bc.ca/

Vancouver Tourism

Wild Whales Vancouver has graciously offered a 20% discount to all conference attendees that wish to go on a whale and wildlife watching boat ride during or shortly after the conference. Just mention our conference and you will receive the discount. Wild Whales Vancouver has adviced participants reserve a tour prior to the conference to be sure a spot is available.
http://www.whalesvancouver.ca/
Phone: 604-699-2011

Big Bus Tours has kindly offered a discount rate of 20% off. All you have to do is enter the word "hafeli" (without quotes) into the coupon code box in the checkout area of
their website, and the discount will be applied. This is a great way to see the city if you want to cover a lot of ground.
http://www.bigbus.ca/

Stanley Park is a popular tourist attraction with its 400 hectares (1,000 acres) of forested and grassy areas directly west of downtown Vancouver. The park is filled with trails for biking and hiking, including one that follows the perimeter of the park with stunning views of the North Shore and the Burrard Inlet. Rental bikes are available for affordable rates on the west end of downtown Vancouver. In addition, the Vancouver Aquarium is located in Stanley Park where you can get a look at Vancouver's native sea life.
http://www.city.vancouver.bc.ca/Parks/parks/stanley/

The Museum of Anthropology offers a fantastic look into the history of past and present human societies. The building is filled with large totem poles and carved building posts from many of the First Nations artists of British Columbia. In addition, the Museum features an extensive system of visible storage, allowing the public to view over 13,000 objects from around the world. The objects are arranged according to culture area, and information on each item is available in books nearby.
http://www.moa.ubc.ca/

World famous Whistler is a great area to spend some time after the conference. From the beautiful scenery to great skiing you will find Whistler has a little bit of it all. Blackcomb ski resort is still open during the conference dates if you are an avid skier or snowboarder. Bike rentals and trails also allow visitors to explore the forest on wheels rather than on foot. It is a 2-3 hour drive to Whistler from Vancouver so plan accordingly!
http://www.whistler.com/

Granville Island is a small shopping districted located below the Granville St. Bridge. Once an industrial area, this island has been turned into a public market populated with craft shops and local farmers selling their current crops. Granville Island Brewing Co. (though mostly brewed in Kelowna, BC) also has a small facility on the island where tours and tastings are held. The island provides a great afternoon of food and shopping for visitors and locals alike!
http://www.granvilleisland.com/

The UBC Botanical Gardens encompasses approximately 44 hectares of UBC's campus. Over 8000 different plant species can be viewed in a number of locations around campus that reflect different areas of the world including alpine, asian and native gardens. Whether you are looking for a short stroll through a beautiful garden or a full afternoon exploring the different plants that populate the world, the UBC Botanical Gardens are a great experience for people of all ages.
http://www.ubcbotanicalgarden.org/

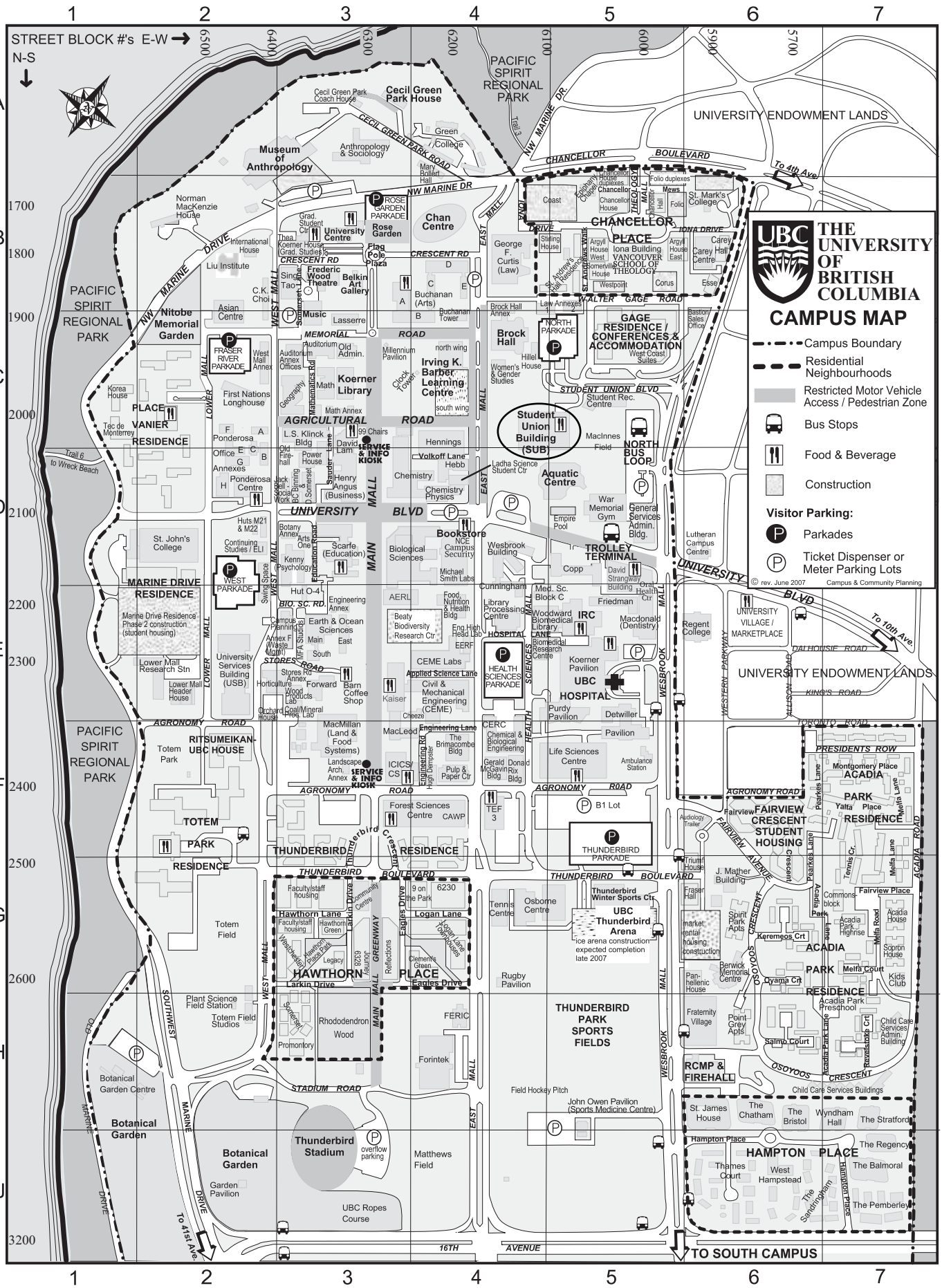
Grouse Mountain provides an excellent viewpoint of Vancouver and the surrounding ocean. Take the skyride lift to the top of the mountain to be treated with the spectacular view. There are also plenty of hiking trails and the famous Grouse Grind, a 2.9 km hike straight up the face of the mountain.
http://www.grousemountain.com/

The Capilano Suspension Bridge is Vancouver's oldest tourist attraction with its 137 m bridge suspended 70 m above the Capilano River. Visitors can also explore the treetops through a series of structure built 30 m above the forest floor. First Nations carving demonstrations and musical entertainment occur daily at the First Nations Cultural Centre.
http://www.capbridge.com/

Kayaking in Indian Arm provides a day of great scenery and a good time. Rental kayaks are available in the city of Deep Cove and can be rented hourly or daily. There are camping locations at the waters edge only accessible by boat as well as many islands to explore.
http://www.deepcovekayak.com/

UBC Map

The buildings relevant to our conference are highlighted on the next page.



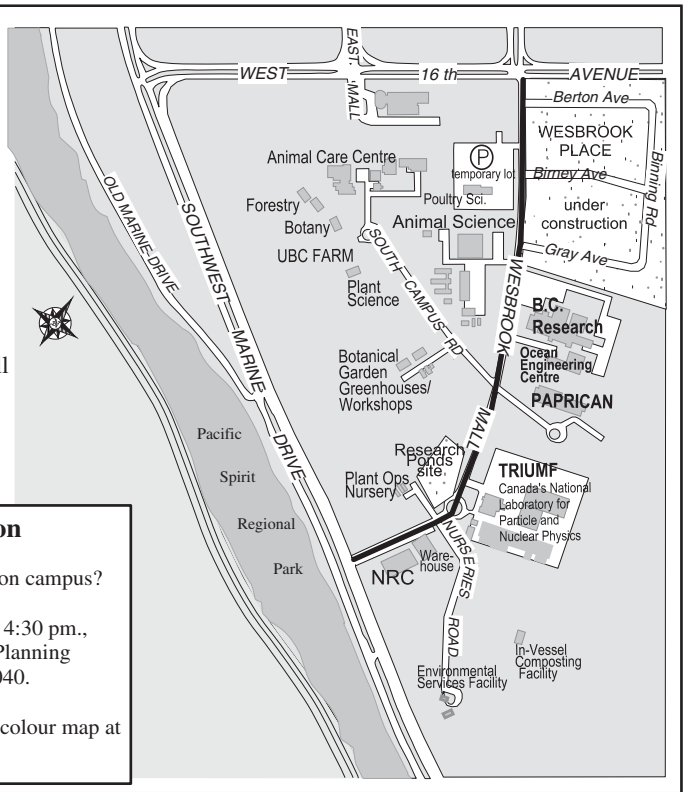
UBC Map Directory

Site or Building Name & Address	Grid
Acadia/Fairview Common Block, 2707 Tennis Cres	G7
Acadia House, 2700-2720 Acadia Rd	G7
Acadia Park Residence	F/H-6/7
Acadia Park Highrise, 2725 Melfa Rd	G7
Acadia Park Preschool, 2750 Acadia Park Lane	H7
Animal Care Centre, 6199 S. Campus Rd	South Campus
Animal Science S. Campus Bldgs, 3473 Wesbrook Mall	South Campus
Anthropology & Sociology Bldg, 6303 NW Marine Dr	A3
Aquatic Centre, 6121 University Blvd	D5
Aquatic Ecosystems Research Lab (AERL), 2202 Main Mall	E3
Arts One Bldg, 6358 University Blvd	D3
Asian Centre, 1871 West Mall	.B2
Audiology & Speech Sciences Classroom Trailer, 5830 Fairview Ave	F6
Auditorium, 6344 Memorial Rd	C3
Auditorium Annex Offices, 1924 West Mall	C3
Barn Coffee Shop, 2323 Main Mall	E3
B.C. Research Inc., 3650 Wesbrook Mall	South Campus
B.C. Binning Studios (formerly Hut M-17), 6373 University Blvd	D3
Beaty Biodiversity Research Ctr, 2212 Main Mall (under construction)	E3/4
Belkin (Morris & Helen) Art Gallery, 1825 Main Mall	B3
Berwick Memorial Centre, 2765 Osoyoos Cres	G6
Biological Sciences Bldg [Science Faculty office], 6270 University Blvd	D3
Biomedical Research Ctr, 2222 Health Sciences Mall	E4
Biotechnology Laboratory, 2125 East Mall	D4
Bollert (Mary) Hall, 6253 NW Marine Dr	A4
Bookstore, 6200 University Blvd	D4
Botanical Garden Centre/Gatehouse, 6804 SW Marine Dr	H1
Botanical Garden Pavilion (enter at Gatehouse, 6804 SW Marine Dr)	J2
Botan. Gard. Greenhses/ Workshops, 6088 S. Campus Rd	South Campus
Botany Annex, 6386 University Blvd	D3
Botany Greenhouses & Trailer, 6182 S. Campus Rd	South Campus
Brimacombe Building, 2355 East Mall	F4
Brock Hall and Brock Hall Annex, 1874 East Mall	C4
Buchanan Building (Blocks A, B, C, D, & E) [Arts], 1866 Main Mall	B3/4
Buchanan Tower, 1873 East Mall	C4
C.K. Choi Building for the Institute of Asian Research, 1855 West Mall	B2
Campus & Community Planning, 2210 West Mall	E3
Campus Security, 2133 East Mall	D4
Carey Hall, 1815 Wesbrook Mall	B6
Carey Centre, 5920 Iona Drive	B6
Cecil Green Park Coach House, 6323 Cecil Green Park Rd	.A3
Cecil Green Park House, 6251 Cecil Green Park Rd	A3
CEME — *see Civil & Mechanical Engineering Building*	
Centre for Women's & Gender Studies, 1896 East Mall	C4
Chan Centre for the Performing Arts, 6265 Crescent Rd	B4
Chancellor Place	B5
Chemical & Biological Engineering Bldg, 2360 East Mall	F4
Chemistry Building, 2036 Main Mall	D3
Chemistry Physics Building, 6221 University Blvd	D4
Child Care Services Admin. Bldg, 2881 Acadia Rd	H7
Child Care Services Bldgs, 5580-5690 Osoyoos Cresc	H7
Civil & Mechanical Engineering Bldg (CEME), 6250 Applied Science Lane	E4
Civil & Mechanical Eng. Labs, 2275 East Mall	E4
Coal & Mineral Processing Lab, 2332 West Mall	E3
Continuing Studies Bldg [English Language Institute], 2121 West Mall	D2
Copp (D.H.) Building, 2146 Health Sciences Mall	.D5
Cunningham (George) Building [Pharmaceutical Sc.], 2146 East Mall	E4
Curtis (George F.) Building [Law], 1822 East Mall	B4
David Lam Learning Centre, 6326 Agricultural Rd	C3
David Lam Management Research Ctr, 2033 Main Mall	C3
Donald Rix Building, 2389 Health Sciences Mall	F4
Dorothy Somerset Studios (formerly Hut M-18), 6361 University Blvd	D3
Earth & Ocean Sciences (EOS) - East, 2219 Main Mall	E3
Earth & Ocean Sciences (EOS) - Main and South, 6339 Stores Rd	E3
Earthquake Engineering Research Facility (EERF), 2235 East Mall	E4
Engineering Annex, 6298 Biological Sciences Rd	E3
Engineering High Head Room Lab, 2225 East Mall	E4
Environmental Services Facility, 6025 Nurseries Rd	South Campus
Faculty of Law Annexes 1 and 2, 6050 and 6020 Walter Gage Rd	B4/5
Fairview Crescent Student Housing, 2600-2804 Fairview Cres	F6
FERIC (Forest Eng. Res. Institute), 2601 East Mall	H4
Fire Department, 2992 Wesbrook Mall	H6
First Nations Longhouse, 1985 West Mall	C2
Flag Pole Plaza (Main Mall & Crescent Rd)	B3
Food, Nutrition and Health Bldg, 2205 East Mall	E4
Forest Sciences Centre [Faculty of Forestry], 2424 Main Mall	F4
Forest Sciences Greenhouse, 6186 S. Campus Rd	South Campus
Forestry Field House, 6186 S. Campus Rd	South Campus
Forintek Western Research Facility, 2665 East Mall	H4
Forward (Frank) Building, 6350 Stores Rd	E3
Fraser Hall, 2550 Wesbrook Mall	G6
Fraternity Village, 2880 Wesbrook Mall	H6
Frederic Wood Theatre, 6354 Crescent Rd	B3
Friedman Bldg, 2177 Wesbrook Mall	E5
Gage Residence, 5959 Student Union Blvd	C5
General Services Administration Bldg (GSAB), 2075 Wesbrook Mall	D5
Geography Building, 1984 West Mall	C3
Gerald McGavin Building, 2386 East Mall	F4
Graduate Student Centre (Thea Koerner House), 6371 Crescent Rd	B3
Green College, 6201 Cecil Green Park Rd	A4
Hampton Place	H/J-6/7
Hawthorn Place	G/H3
Hebb Building, 2045 East Mall	D4
Hennings Building, 6224 Agricultural Rd	C4
Henry Angus Building [Sauder School of Business], 2053 Main Mall	D3
Hillel House, 6145 Student Union Blvd	C4
Horticulture Building/Greenhouse, 6394 Stores Rd	E2/3
Hugh Dempster Pavilion, 6245 Agronomy Rd	F4
Hut M-21 & Hut M-22, 2109 West Mall	D2
Hut O-4, 6365 Biological Sciences Rd	E3
ICICS/CS (Institute for Computing, Information & Cognitive Systems/ Computer Science - formerly CICSR/CS), 2366 Main Mall	F4
Instructional Resources Centre (IRC), 2194 Health Sciences Mall	E5
International House, 1783 West Mall	B2
Irving K. Barber Learning Ctr (former Main Library)	C4
Jack Bell Building for the School of Social Work, 2080 West Mall	D3
John Owen Pavilion & Allan McGavin Sports Medicine Centre, 3055 Wesbrook Mall	H5
Kaiser (Fred) Building [Faculty of Applied Science], 2332 Main Mall	E3
Kenny (Douglas T) Building, 2136 West Mall	D3
Kids Club, 2855 Acadia Rd	G7
Klinck (Leonard S.) Bldg, 6356 Agricultural Rd	C3
Koerner (Walter C.) Library, 1958 Main Mall	C3
Korea House (in Place Vanier), 1935 Lower Mall	C1
Ladha Science Student Ctr, 2055 East Mall	D4
Landscape Architecture Annex, 2371 Main Mall	F3
Lasserre (Frederic) Building, 6333 Memorial Rd	C3
Leon and Thea Koerner University Centre, 6331 Crescent Rd	B3
Library Processing Centre, 2206 East Mall	E4
Life Sciences Centre, 2350 Health Sciences Mall	F5
Liu Institute for Global Issues, 6476 NW Marine Dr	B2
Lower Mall Header House, 2269 Lower Mall	E2
Lower Mall Research Station, 2259 Lower Mall	E2
Macdonald (J.B.) Building [Dentistry], 2199 Wesbrook Mall	E5
MacLeod (Hector) Building, 2356 Main Mall	F3
MacMillan (H.R.) Bldg [Faculty of Land & Food Systems], 2357 Main Mall	F3
Main Library (new Irving K. Barber Learning Ctr), 1956 Main Mall	C4
Marine Drive Residence (student housing), 2205 Lower Mall	D2
Mathematics Annex, 1986 Mathematics Rd	C3
Mathematics Building, 1984 Mathematics Rd	C3
Mather (James) Building, 5804 Fairview Ave	G6
Medical Sciences Block C, 2176 Health Sc. Mall	E4
M.F.A. Studios (formerly B.C. Binning MFA Studios), 6363 Stores Rd	E3
Michael Smith Laboratories, 2185 East Mall	D4
Museum of Anthropology, 6393 NW Marine Dr	A2/3
Music Building, 6361 Memorial Rd	B/C3
Networks of Ctrs of Excellence (NCE), 2125 East Mall	D4
99 Chairs/Trek Express, 2015 Main Mall	C3
Nitobe Memorial Garden, 1903 West Mall	B/C2
Norman MacKenzie House, 6565 NW Marine Dr	B2
NRC Institute for Fuel Cell Innovation, 4250 Wesbrook Mall	South Campus
Ocean Engineering Centre, 3760 Wesbrook Mall	South Campus
Old Administration Building, 6328 Memorial Rd	C3
Old Barn Community Centre, 6308 Thunderbird Blvd	G3
Old Firehall, 2038 West Mall	D3
Orchard House (formerly Header House), 2336 West Mall	E2
Osborne (Robert F.) Centre/Gym, 6108 Thunderbird Blvd	G4
Panhellenic House, 2770 Wesbrook Mall	G6
PAPRICAN Building, 3800 Wesbrook Mall	South Campus
Place Vanier Residence, 1935 Lower Mall	C/D2
Plant Ops Nursery/Greenhouses, 6116/6136 Nurseries Rd	South Campus
Plant Science Field Building, 6182 S. Campus Rd	South Campus
Plant Science Field Station & Garage, 2613 West Mall	H2
Plant Operations Annex F (Waste Mgmt), 6381 Stores Rd	E2/3
Point Grey Apartments, 2875 Osoyoos Cresc	H6
Police (RCMP) & Fire Department, 2990/2992 Wesbrook Mall	H6
Ponderosa Centre, 2071 West Mall	D2
Ponderosa Office Annexes: A, B, & C, 2011-2029 West Mall	C/D2
Ponderosa Office Annexes: E to H, 2008-2074 Lower Mall	C/D2
Power House, 2040 West Mall	D3
Pulp and Paper Centre, 2385 East Mall	F4
Ritsumeikan-UBC House, 6460 Agronomy Rd	F2
Rodney Graham Millennium Pavilion	C3
Rose Garden	B3
Rugby Pavilion, 2584 East Mall	G4
Scarfe (Neville) Building [Education], 2125 Main Mall	D3
Sing Tao Building, 6388 Crescent Rd	B3
Sopron House, 2730 Acadia Rd	G7
South Campus Warehouse, 6116 Nurseries Rd	South Campus
Spirit Park Apartments, 2705-2725 Osoyoos Cresc	G8
St. Andrew's Hall/Residence, 6040 Iona Dr	B5
St. John's College, 2111 Lower Mall	D2
St. Mark's College, 5935 Iona Dr.	B6
Stores Road Annex, 6368 Stores Rd	E3
Student Recreation Ctr, 6000 Student Union Blvd	C5
Student Union Bldg (SUB), 6138 Student Union Blvd	C4
Tec de Monterrey (in Place Vanier), 1935 Lower Mall	C1
Technology Enterprise Facility 3 (TEF3), 6190 Agronomy Rd	F4
Thea Koerner House [Graduate Studies], 6371 Crescent Rd	B3
Thunderbird Residence, 6335 Thunderbird Cresc	F3/4
Thunderbird Stadium, 6288 Stadium Rd	J3
Thunderbird Winter Sports Ctr, 6066 Thunderbird Blvd	G5
Totem Field Studios, 2613 West Mall	H2
Totem Park Residence, 2525 West Mall	F/G2
TRIUMF, 4004 Wesbrook Mall	South Campus
Triumf House (TRIUMF Visitor's Residence), 5835 Thunderbird Blvd	G6
UBC Hospital, 2211 Wesbrook Mall	E5
UBC Tennis Centre, 6160 Thunderbird Blvd	G4
UBC Thunderbird Arena, 2555 Wesbrook Mall	G5
University Centre (Leon & Thea Koerner), 6331 Crescent Rd	B3
University Services Building (USB), 2329 West Mall	E2
Vancouver School of Theology, 6000 Iona Drive	B5
Walter H. Gage Residence, 5959 Student Union Blvd	C5
War Memorial Gymnasium, 6081 University Blvd	D5
Wesbrook Bldg, 6174 University Blvd	D4
Wesbrook Place neighbourhood (under construction)	South Campus
West Mall Annex, 1933 West Mall	C2
West Mall Swing Space Bldg, 2175 West Mall	D2
Wood Products Laboratory, 2324 West Mall	E3
Woodward Biomedical Library, 2198 Health Sciences Mall	E4/5

SOUTH CAMPUS MAP

© rev. June 2007
Campus & Community Planning
www.planning.ubc.ca

Note:
— Local traffic only along Wesbrook Mall on South Campus



Map Information

Need help finding your way on campus?

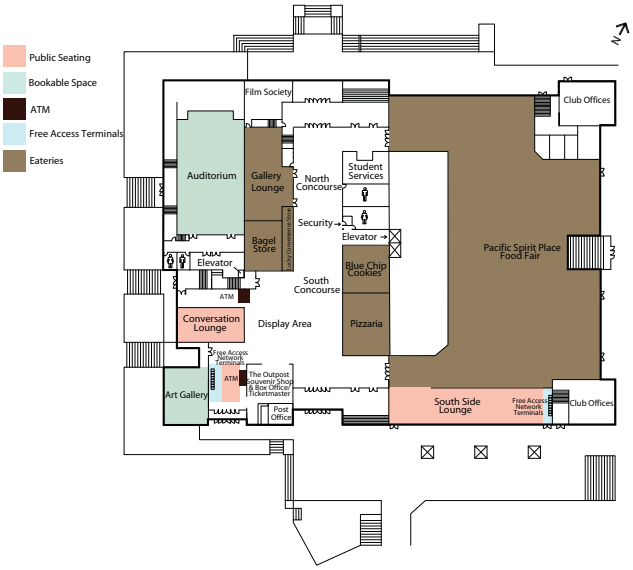
Monday to Friday, 8:30 am - 4:30 pm., call Campus & Community Planning Map Info Line at 604-827-5040.

Or use the online searchable colour map at www.maps.ubc.ca

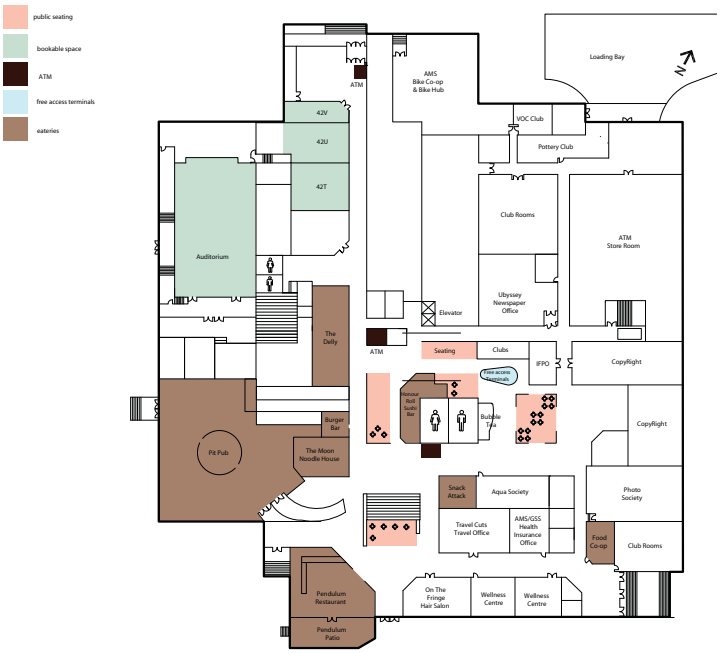
Conference Location - Student Union Building (SUB) Map

All talks are in the Auditorium on the Main floor of the SUB, and all the poster sessions, exhibits, coffee breaks and lunches are upstairs in the inner court and ball room.

Main Floor



Lower Floor



Upper Floor

Vancouver Travel Information

Public Transportation

Vancouver features a great bus system provided by Translink. You can plan your route and find out the bus schedule at http://www.translink.bc.ca/ or http://www.google.ca/transit/.

Cab Services

Black Top & Checker Cabs (604) 681-2181
Maclure's Cabs (604) 683-6666
Yellow Cab (604) 681-1111

Social Program

And as always, we will not let science prevent us from having fun together and learning about and enjoying Vancouver to the fullest.

May 20

A welcome reception sponsored by **Stem Cell Technologies** will be held upstairs in the Student Union Building (SUB). It is open to all participants of the conference and will start on Tuesday at 19:00 (7 PM) and go until about 22:00 (10 PM). We will have a jazz duo play (Daniel and Conrad), with a flutist as a guest (Shona). Conference registration will be available beginning at 18:30 (6:30 PM).

In addition, participants will have the opportunity to walk to the Museum of Anthropology (200 m away) and enjoy the exhibit. The museum is world renowned for its collection of totem poles from British Columbia and more recent art of societies from around the globe. The exhibit is open on Tuesday evenings from 5 - 9 PM for a reduced rate of $5. It is well worth the visit.

May 21

After the talks, the poster session will begin around 18:10 (6:10 PM) upstairs in the SUB. Beer and pretzels have been graciously sponsored by **Diagnostic Biosensors**. The rest of the evening is free. Go out and explore Vancouver - you might especially like to try out the closest restaurants on West 10th Ave or Broadway, just a couple of minutes away by bus.

May 22

Starting at 9:00 (9 AM), we will have a full day complimentary tour for spouses. It will include the visit of some parts of the city as well as of the scenic surroundings of Vancouver. Please tell us on the previous day if your spouse would like to take part in this old tradition at our meetings!

After the talks, the poster session will begin around 17:00 (5:00 PM) upstairs in the SUB. Beer and pretzels have been graciously sponsored by **Magnisense**.

After the poster session, we will be picked up by bus punctually at 18:30 (6:30 PM) in front of the Gage Towers. We will board the M.V. Brittania for our traditional dinner cruise at the North foot of Denman Street downtown. We will be back at UBC around 23:00 (11 PM).

May 23

On this evening, we will take a tour of the city and Stanley Park in yellow school buses. We will end up on the beautiful English Bay beach, on the South bottom of Denman Street, a great area for food and night life. And not too far for a stroll on Davie, Denman and Robson to other parts of downtown.

May 24

The meeting will end at 4 PM. Please take the opportunity to explore beautiful British Columbia on your own! You might also be interested in a whale watching tour, exploring the wilderness surrounding the city or visiting one of the many museums in Vancouver.

Conference Proceedings

The proceedings of the Symposium will be published as full papers in the well indexed "Journal of Magnetism and Magnetic Materials". The cost of the proceedings (incl. mailing) is included in the registration fee. As in previous years, reviewing and publishing of the JMMM issue will take some time. We expect that each conference participant will receive the special JMMM issue in the first quarter of 2009.

Talk and Poster Abstracts

Tuesday, May 20, 2008

15:00	Registration desk opens at **Gage Towers** (until 18:00 - 6 PM)
17:00	Possibility to visit the **Anthropology Museum** nearby from 17:00-21:00 (5-9 PM) for a reduced fee of $5 - **highly recommended !**
18:30	Registration desk opens in the **student union building** (**SUB**) upstairs (until 22:00 - 10 PM) / **Posters can be put up** (pins will be provided)
19:00	**Informal reception and welcome cocktail (apero) in the SUB upstairs with Jazz music** - *sponsored by StemCell Technologies*

Today's Social Program

A welcome reception sponsored by **Stem Cell Technologies** will be held upstairs in the Student Union Building (SUB). It is open to all participants of the conference and will start on Tuesday at 19:00 (7 PM) and go until about 22:00 (10 PM). We will have a jazz duo play (Daniel and Conrad), with a flutist as a guest (Shona). Conference registration will be available beginning at 18:30 (6:30 PM).

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Talk Abstracts - Wednesday, May 21, 2008

7:30	**Registration desk opens / Posters can be put up** (pins will be provided)			
	Opening Session			
9:00		Opening of the conference		
9:05	Burt, Helen	Welcome by the Associate Dean of Research, Faculty of Pharmaceutical Sciences, UBC	Canada	
9:10	Hafeli, Urs	Short review of the last 2 years of magnetic carriers	Canada	
9:30	**Zimmerman, Peter**	**Diagnosis of Malaria using Magnetic Separation**	**U.S.A.**	**Invited talk 1**
10:10	**Coffee break / poster session / exhibitors**			
10:50	**Session 1: Highlights from Different Areas** - *Chair: Axel Rosengart (U.S.A.)*			
10:55	Wang, Jian-Ping	Physical design and synthesis of new multifunctional nanoparticles and nanoparticle-crystals for biomedical applications	U.S.A.	Talk 1
11:10	Rahn, Helene	Tomographic Examination of Magnetic Nanoparticles used as Drug Carriers	Germany	Talk 2
11:25	Nikitin, Maxim	Highly Sensitive Room-Temperature Method of Non-Invasive in vivo Detection of Magnetic Nanoparticles	Russia	Talk 3
11:40	Palfreyman, Justin	Digital Biomagnetism: Electrodeposited Multilayer Magnetic Tags	U.K.	Talk 4
11:55	Ritter, James	Isolated Swine Heart Ventricle Perfusion Model for Implant Assisted-Magnetic Drug Targeting	U.S.A.	Talk 5
12:10	Zabow, Gary	Engineered microparticles for MRI	U.S.A.	Talk 6
12:25	**Lunch upstairs**			
13:55	**Pankhurst, Quentin**	**In vivo sensing, moving and heating of magnetic nanoparticles in the human body**	**U.K.**	**Invited talk 2**
14:35	**Session 2: Magnetic Drug Delivery / Gene Delivery** - *Chairs: Quentin Pankhurst (U.K.) and Peter Zimmerman (U.S.A.)*			
14:40	Mykhaylyk, Olga	Nucleic acid delivery to magnetically labelled cells	Germany	Talk 7
14:55	Rovers, Stefan	Externally triggered on-demand drug delivery from polymer matrices induced by a magnetic field	The Netherlands	Talk 8
15:10	Chang, Jin	Application of Tat and Folate Mediated Magnetic Nanoparticles in the Therapy of Epilepsy	China	Talk 9
15:25	Kuznetsov, Oleg	Magnetically guided transport of anti-cancer drugs using nanostructured ferrocarbon particles – animal and clinical trials	Russia	Talk 10
15:40	Rosengart, Axel	Magnetic Nanospheres for Targeted Thrombolysis: Flow studies and in vivo results	U.S.A.	Talk 11
15:55	Shapiro, Benjamin	Dynamic Control of Magnetic Fields to Focus Drug-Coated Nano-Particles to Deep Tissue Tumors	U.S.A.	Talk 12
16:10	**Coffee break / posters / exhibitors** - *coffee break sponsored by CDRD*			
16:40	Kroll, Torsten	Nanoparticle driven imatinib delivery into target cells	Germany	Talk 13
16:55	Leslie-Pelecky, Diandra	Biodistribution, Clearance, and Biocompatibility of a Novel Iron-Based Magnetic Nanoparticle Drug Delivery Formulation	U.S.A.	Talk 14
17:10	Mertz, Carol	Exploiting the Electromagnetic Response of Polymer Composite Nanocarriers for Controlled Drug Release	U.S.A.	Talk 15
17:25	Darton, Nicholas	The in-flow capture of superparamagnetic nanoparticles in microcapillary film as a potential model for targeting of gene therapeutics	U.K.	Talk 16
17:40	Pradhan, Pallab	Targeted temperature sensitive magnetic liposomes for thermo-chemotherapy of cancer	India	Talk 17
17:55	Farrow, Neil	Improved magnetic nanoparticle-based gene transfection using oscillating magnet arrays	U.K.	Talk 18
18:10	**Poster session with beer and pretzels - PLEASE RATE POSTERS** - *sponsored by Diagnostic Biosensors*			
19:40	**Free evening - use it to meet old friends, discuss new collaborations, and enjoy UBC and Vancouver on your own!**			

Today's Social Program

After the talks, the poster session will begin around 18:10 (6:10 PM) upstairs in the SUB. Beer and pretzels have been graciously sponsored by **Diagnostic Biosensors**. The rest of the evening is free. Go out and explore Vancouver - you might especially like to try out the closest restaurants on West 10th Ave or Broadway, just a couple of minutes away by bus.

Diagnosis of Malaria using Magnetic Separation

Peter Zimmerman

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An important impediment to effective malaria treatment and control is the absence of low-cost diagnostic tools and strategies capable of evaluating infection status rapidly in rural settings where the majority of malaria cases are encountered. As a result, generalized treatment of malarial and bacterial infections follows symptoms-based diagnosis. This approach is certain to contribute to selection favoring drug resistant parasites and bacteria. Although the conventional blood smear serves as the "gold standard" tool for malaria diagnosis, it is widely acknowledged that molecular tools are faster, such as antigen-based rapid diagnostic tools or provide significantly greater sensitivity and specificity (PCR). However, molecular techniques are expensive, do not assess *Plasmodium vivax*, *P. malariae* or *P. ovale* with specificity of *P. falciparum*, and have been observed to be less sensitive than the blood smear. In an attempt to overcome some problems inherent to blood smear microscopy, we have developed a magnet-based approach to concentrate malaria parasites and augment detection of malaria-infected erythrocytes by microscopy. This system, malaria magnetic deposition microscopy (MDM), exploits the fact that *Plasmodium* species parasites produce a crystalline by-product, hemozoin, from heme liberated during hemoglobin digestion. Unlike previous systems requiring elution of cells from steel mesh, MDM captures parasitized erythrocytes in a narrow magnetic field and deposits them directly onto a small region of a polyester slide, which is then immediately ready for fixation and staining. By concentrating parasites, MDM increases the sensitivity of diagnosis and decreases the time it takes to read the slide. The results of MDM application to concentrate parasites of all four human malaria parasite species in the laboratory, an efficient capture of *P. falciparum* gametocytes in the field study in Papua-New Guinea, and their potential importance to malaria prevention will be discussed.

PHYSICAL DESIGN AND SYNTHESIS OF NEW MULTIFUNCTIONAL NANOPARTICLES AND NANOPARTICLE-CRYSTALS FOR BIOMEDICAL APPLICATIONS

Jian-Ping Wang, University of Minnesota

We will first review and discuss the desirable properties of magnetic nanoparticles for kinds of biomedical applications. Nanoparticles with high-magnetic moment, proper anisotropy constant and smart functions (e.g. vs. temperature) will be emphasized. Then, we will report a newly-developed general gas-phase method to fabricate nanoparticles with selected crystal structures and a variety of heterostructures, which can't be made by well-developed wet methods. Our newly-developed gas-phase method produces the magnetic particles with the following advantages: 1) phase-selective and forming desirable magnetic phases without post-annealing, especially for alloy and compound magnetic nanoparticles with more than two elements for intelligent functions; 2) free-standing and contamination-free nanoparticles; 3) forming desirable heterostructures with multifunctions; 4) transferable to solution and become water-soluble by surface modification.

First we report a preparation method that can tune the crystal structure and magnetic properties of nanoparticles and at the same time provide materials without surface adsorbates [1]. In this method gas phase aggregation is used to generate nanoparticles. Crystal structure control is achieved by directly manipulating the energy carried by nucleating atoms and growing species using magnetron plasma as the adjustable energy source. At three different densities of plasma, FePt nanocrystals with three different kinds of crystal structures, A1 icosahedron, A1 octahedron and $L1_0$ octahedron were prepared, respectively. High-resolution TEM images clearly show the different arrangements of atoms and magnetic measurements reveal that they have very different magnetic performance. These materials can be used as different types of nanoscale magnets having the same chemical composition. So far, wet methods can't produce these mondodispersed tiny hard magnetic nanoparticles. The technique reported here can be easily extended to other magnetic as well as magnetic-related nanocrystals fabrication thus to provide a general platform for technological and fundamental study at the nanometer scale [2].

Heterostructured nanoparticles are highly desirable in applications such as medical imaging, drug delivery, thermal cancer treatment and etc., which combine different properties of the individual component structure into one single nanoparticle and have possible enhancement of each properties. Different from the previous two-step method used in both the chemical approach and physical vapor deposition approach, a "one-step" method is developed to synthesize heterostructured nanoparticles direct from gas phase. It has been demonstrated that a variety of heterostructures can be produced by using the method, including core-shell, multi-core-multi-shell, dumbbell, and sphere with nodules. Results have been obtained from different materials systems including Co and Au, Fe and Ag, FeCo and Si, and FeCo and Au/Ag/Cu [3-6].

Superparamagnetic nanoparticles are limited by size, typically ranging from several nm to 20 nm. For most of biomedical applications, it is desirable to have large superparamagnetic nanoparticles. We proposed and demonstrated a new gas-phase synthesis method to make 100 nm giant superparamagnetic nanoparticle-crystals, which will tremendously reduce the dose of magnetic nanoparticles in a variety of applications [7].

These gas-phase synthesized nanparticles are transferred into water by PEG and PVA coating. Finally, applications and advantages of these water-soluable and bio-compatible high-magnetic-moment nanoparticles will be reported in magnetic hyperthermia, MRI imaging, and biomagnetic sensing.

[1] J. M. Qiu and J. P. Wang, APPLIED PHYSICS LETTERS 88 (19): Art. No. 192505 (2006)
[2] J. M. Qiu and J. P. Wang, ADVANCED MATERIALS, 19 (13), 1703 (2007),
Highlighted in **NATURE**, http://www.nature.com/nature/journal/v447/n7147/full/447888a.html
[3] J. M. Bai, Y. H. Xu, Thomas John, J. P. Wang, NANOTECHNOLOGY, 18, 065701 (2007)
[4] J. M. Bai, Y. Xu and J. P. Wang, IEEE TRANSACTIONS ON MAGNETICS 43: 3340 (2007)
[5] Y. Xu and J. P. Wang, APPLIED PHYSICS LETTERS 91: 233107 (2007)
[6] Y. Xu and J. P. Wang, ADVANCED MATERIALS, 20: 994 (2008)
[7] J. M. Bai, Y. Xu and J. P. Wang, NATURE NANOTECHNOLOGY, submitted, (2008)
Acknowledgement: National Science Foundation, Biomedical Engineering; Nanobio Initiative, U of Minnesota; Mayo Clinic and Minnesota Medical Foundation; Medical Device Center, Center for Active Nanostructures, U of Minnesota;

Tomographic Examination of Magnetic Nanoparticles used as Drug Carriers

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Magnetic nanoparticles suspended in an appropriate carrier liquid, so called ferrofluids, may be used for biomedical applications. Ferrofluids are being investigated for minimally invasive cancer treatment procedures with the aim of treating the cancer locally, reducing the side effects. To convert chemotherapy into a localized treatment, the drugs are ionically bound to the nanoparticles, which are led to the tumour by an external magnetic field. This therapeutic approach is called magnetic drug targeting (MDT). As the nanoparticles are acting as pure drug carriers, the correct amount and distribution within the tissue are important factors for the success of MDT. To study this, several techniques have been applied. While microscopy of histological cuts gives 2-dimensional information about the particle distribution, 3-dimensional information can be achieved by means of micro-computed tomography (µCT), without the need to section the tumour.

Figure 1 shows a reconstructed tomographic slice of a tumour sample, which has been treated with MDT and measured by µCT. The examination has been performed on a synchrotron beamline at HASYLAB (DESY/Hamburg) at photon energy of 20 kV and spatial resolution of approximately 10 µm. Using image processing techniques, the distribution of particles can be examined and related to experimental conditions used during the MDT process. The different constituents of the tumour sample can be separated. The tomographic examination reveals that the particles are contained within a well-defined tubular shape, which is part of the vascular system of the tissue. The results have shown an accumulation of the nanoparticles, and therefore drugs within the vessels in the tumour. This localisation is essential for the success of this form of cancer treatment.

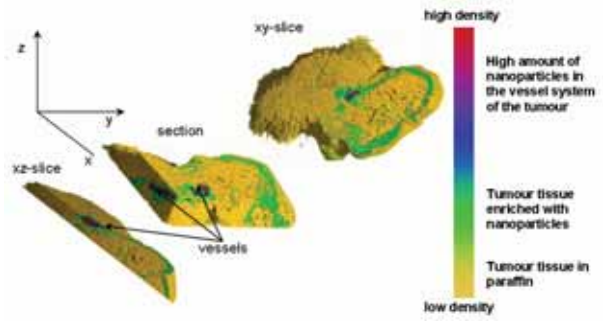

Figure 1: Rendered image of a tumour slice measured with DiTo.

Highly Sensitive Room-Temperature Method of Non-Invasive *in vivo* Detection of Magnetic Nanoparticles

M.P. Nikitin[1*], P.M. Vetoshko[2], P.I. Nikitin[3], N.A. Brusentsov[4]

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The detection of magnetic nanoparticles (MP) *in vivo* is very important for many biomedical applications. At present, expensive cryogenic MRI scanner, SQUIDs or popular fluorescent labels were used to study MP in tissues of a living animal. Other methods usually are not compatible with the life of the animal. However, the fluorescence and MRI scanner provide only qualitative data.

In the present work a novel room-temperature non-invasive method of MP detection in vivo has been realized and tested for different biomedical applications. The method provides real-time quantitative measurements of MP concentration in blood or tissues of a living organism. This method is based on non-linear magnetization of MP [1,2]. Its sensitivity of few ng of MP in 0.1-0.5 ml volume is on the level of radioactive technique for MP with ^{59}Fe isotope [3].

The developed new class of instruments has a remote probe with a special coil system that allows external MP excitation at several frequencies and measuring response at combinatorial frequencies [1]. The output signal linearly depends on MP quantity in wide dynamic range of up to five orders of magnitude. An example of the remote probe for model detection of MP on the other side of a hand is shown in Fig. 1. The influence of parameters of the coil and electronic systems on spatial resolution and the depth where MP could be detected has been established. The developed instruments were tested to study magnetic drug delivery to various parts of mice bodies, and for feasibility check of magnetic immunoassay carried out in a live body, which was tested *in vitro* previously [1,2]. Detection of MP with attached mini-antibodies is promising for tumor diagnostics.

In our experiments, for the first time, the quantitative real-time study of MP concentration in mice tissues and blood stream of tail arteries have been performed. For these investigations a compact "BioMag" device [2] was used. After MP injections tails of mice were put through the coils of the device (Fig.2) for quantitative monitoring of MP dynamics in the blood flow. It was also shown that anesthesia can alter MP dynamics in blood. That emphasizes advantages of research methods which do not require anesthesia. The developed MP detection method and related room-temperature electronic devices are highly sensitive and very robust, they are not sensitive to linear dia- or paramagnetics which always surround MP in quantities nine-ten orders of magnitude higher than that of tested MP.

Fig.1. The coil system detects MP on the other side of the hand

Fig. 2. MP quantification in the tail of a living mouse, which rests in a special container

[1] P.I. Nikitin, P.M. Vetoshko, Patent RU 2166751 (2000), EP 1262766 publication (2001).
[2] P.I. Nikitin, P.M. Vetoshko, T.I. Ksenevich. Sensor Letters, 5 (2007) 296.
[3] M.P. Nikitin, M. Torno, H. Chen, A. Rosengart, P.I. Nikitin. *JAP*, 103, 07A304, 2008.

Digital Biomagnetism: Electrodeposited Multilayer Magnetic Tags

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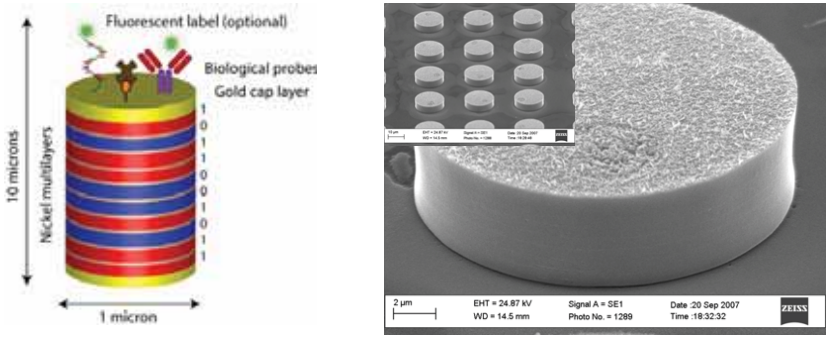
Microarrays and Suspension-based Assay Technologies (SATs) have recently become commonplace in biological laboratories for applications including genotyping, Single Nucleotide Polymorphism (SNP) analysis, proteomics, immunochemistry and even simple separations or purifications. Microarrays have the capacity to test for thousands of targets in a single assay, whilst SATs benefit from flexibility and increased reaction kinetics associated with being a solution-phase technique.

Recently there has been demand for technologies that can offer both the flexibility of SATs and the ability to multiplex, necessitating that the individual microcarriers (beads) must be distinguishable from one-another. Most techniques aimed at achieving this rely heavily on optical encoding/identification of the microcarrier, be it by fluorescence (e.g. Luminex, Quantum Dot) or using patterns or shape recognition. All of these have inherent disadvantages, such as limitations on the number of possible codes or the need for complex and bulky optical recognition equipment.

We are addressing these limitations by developing a magnetic-encoding technology, where multi-bit magnetic tags with magnetisations aligned in one of two distinct states allow for 2^N codes for an N bit tag. Using magnetic sensors, integrated into microfluidic channels to decode the tags offers a powerful lab-on-a-chip diagnostic tool.

We have studied the fabrication of 3D electrodeposited multilayer pillar structures deposited from a single electrolytic bath. The bath contains salts of both magnetic (Ni/Co/Fe) and non-magnetic (Cu) metals, which are deposited in alternating layers by varying the applied potential. By controlling the concentration of additives in the electrolyte and the height/composition of each layer it is possible to coercivity-tune individual layers. This means we can implement a 'global moment writing/reading strategy', whereby any of the 2^N code combinations can be encoded as magnetisation states by applying a monotonically decreasing magnetic field to the entire tag.

A bio-functionalisation scheme that exploits commercial microarray printing techniques to efficiently generate a vast library of probes will also be discussed. Including a novel method for releasing the tags based on an Al/Cu evaporated release layer that can be chemically dissolved without harming single-stranded DNA. This will allow tags functionalised with probe molecules by printing to be used within microfluidic systems for hybridisation, manipulation, and detection.



Schematic showing the pillar architecture, and SEM images showing an electrodeposited 8-bit multilayer tag with a gold cap layer as part of a homogeneous array of tags (inset).

Isolated Swine Heart Ventricle Perfusion Model for Implant Assisted-Magnetic Drug Targeting

Misael O. Avilés, Jan O. Mangual, Armin D. Ebner, and James A. Ritter

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Swearingen Engineering Center
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Columbia, SC 29208 USA

An isolated swine heart ventricle perfusion model was developed and used under physiologically relevant conditions to study implant assisted-magnetic drug targeting (IA-MDT). A stent coil was fabricated from a ferromagnetic SS 430 wire and used to capture 100 nm diameter magnetite particles that mimicked magnetic drug carrier particles (MDCPs). Four key cases were studied: 1) no stent and no magnet (control), 2) no magnet but with a stent, 3) no stent but with a magnet (traditional MDT), and 4) with a stent and a magnet (IA-MDT). When applied, the magnetic field was fixed at 0.125 T. The performance of the system was based on the capture efficiency (CE) of the magnetite nanoparticles. The experiments done in the absence of the magnetic field showed minimal retention of any nanoparticles whether the stent was present or not. The experiments done in the presence of the magnetic field showed a statistically significant increase in the retention of the nanoparticles, with a marked difference between the traditional and IA MDT cases. Compared to the control case, in one case there was nearly an eleven-fold increase in CE for the IA-MDT case compared to only a three-fold increase in CE for the traditional MDT case. This enhanced performance by the IA-MDT case was typical of all the experiments. Histology images of the cross-section of the coronary artery revealed that the nanoparticles were captured mainly in the vicinity of the stent. Overall, the IA-MDT results from this work with actual tissue were very encouraging and similar to those obtained from other non-tissue and theoretical studies; but, they did point to the need for further studies of IA-MDT.

Engineered Microparticles for MRI

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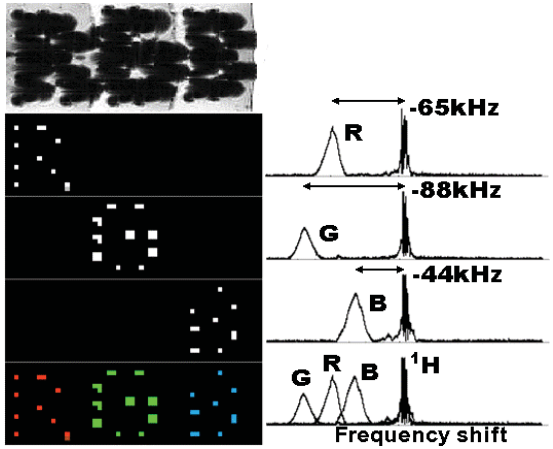
Magnetic particles are increasingly finding use as magnetic resonance imaging (MRI) contrast agents. With the use of ensembles of magnetic nanoparticles already well established, attention is increasingly turning towards larger, more stable, magnetic microparticles that could potentially be individually detected and used to label and track individual cells *in vivo*. Like most nanoparticles, however, these magnetic nano- and microparticles used in MRI are generally all chemically synthesized. Here we consider instead the potential of top-down microfabrication. We show how it is possible to use photolithographically defined microfabrication to construct contrast agents with purposely engineered features. Although a more complex and costly route than traditional chemical synthesis, the more direct control possible through a microfabrication approach allows MRI agents that have both increased functionality and sensitivity. In particular, we show how it is possible to engineer micromagnetic particles that can produce discrete spectral shifts in the MRI radio-frequency spectrum, enabling a new form of multi-spectral MRI. We also show how such agents can locally boost traditional MRI sensitivities by several orders of magnitude. As an example, the figure below shows actual MR images obtained from a set of microfabricated magnetic structures, demonstrating how their spectral content allows distinction between different agents.



The figure shows a regular gradient echo MR image (top) of a set of engineered magnetic particles showing the normal image contrast typical of magnetic microparticles. Their engineered spectral content becomes visible in the chemical-shift images below, improving spatial localization and enabling distinction between particle types. Fourier-transformed free-induction-decay signals (right) show the measured spectral shifts.

In vivo sensing, moving and heating of magnetic nanoparticles in the human body

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The emerging field of 'endomagnetics' – the sensing, moving and heating of magnetic nanoparticles in the human body for diagnostic and therapeutic purposes - will be reviewed. Examples will be given for each of the modalities:

Sensing: A high-T_c SQUID based sensor system, with a room temperature hand-held probe, designed for use in a hospital operating theatre to detect breast cancer sentinel lymph nodes (Figure 1). The system is currently being evaluated in patients, and has been used successfully in twelve operations to date .

Moving: A high field-gradient magnetic actuator designed to capture magnetic nanoparticle loaded haematopoetic stem cells for the treatment of atherosclerosis. Bench-top (Figure 2) and animal trials are under way to establish the efficacy of such a therapy, with promising results.

Heating: Magnetic field hyperthermia treatment for superficial and, as a long term goal, metastatic cancer, using antibody-targeted magnetic nanoparticles. Work is progressing on several fronts: the synthesis of improved magnetic particles for heat transduction (Figure 3), the engineering of new high-frequency drive circuits to produce rf fields in controlled geometries, and cell and animal studies of antibody-nanoparticle conjugation and tumour targeting.

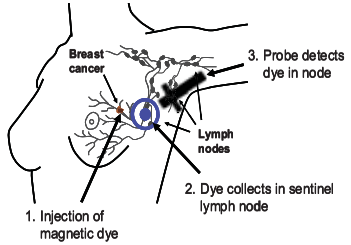

Figure 1: An intra-operative medical device for breast cancer surgery which uses a high-sensitivity, high-T_c SQUID based probe.

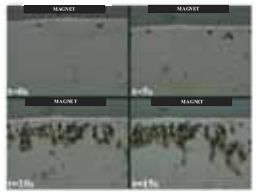

Figure 2: Actuation of magnetically labelled CD133 stem cells.

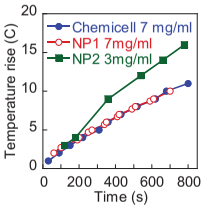

Figure 3: Heating of magnetite/maghemite nanoparticles in a 140 Oe field at 138 kHz.

Nucleic acid delivery to magnetically labelled cells and its potential to modify cell engraftments

Olga Mykhaylyk[1*], Hector Perea[2], Joachim Aigner[1], Andreas Steingoetter[3], Nataliya Dudchenko[4], René Botnar[3], Christian Plank[1]

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Magnetic labelling for cell tracking and targeting for cell therapy and tissue engineering are gaining increasing attention. In the present work we have studied magnetofection with non-viral vectors in adherent epithelial cells that had been pre-labelled with magnetic iron oxide nanoparticles. The nanoparticles were developed to achieve optimal magnetic properties of the labelled cells for magnetic seeding, gene expression and visualization of the cells by magnetic resonance imaging (MRI). The magnetic responsiveness of the labelled cells was evaluated using a magnetophoretic mobility assay. The efficiency of particle internalization was quantified using non-heme iron analysis in cell lysate. Customized two-dimensional cell culture assays and a three-dimensional model were established for transfection of magnetically labelled cells. A radial magnetic field was implemented to drive cells, labelled with magnetic particles, onto the luminal surface of a tubular scaffold. Magnetic cell labelling allows direct and rapid cell seeding combined with non-invasive imaging of the cell monolayer on the substrate using MRI relaxometry, thus providing a reliable tool to assess the quality of cell delivery and engraftment procedures. Optimized magnetic pre-labelling of cells does not interfere with or increases magnetofection efficiency both in two- and three-dimensional models in terms of reporter gene expression (luciferase gene) and the percentage of the transfected cells (eGFP and galactosidase gene) without causing cell toxicity.

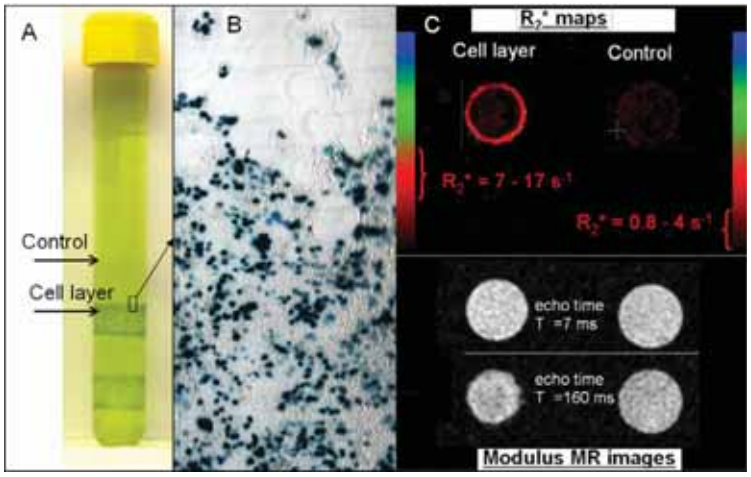

Figure. Imaging of the magnetically labelled H441 cell monolayer 48 h post-magnetofection of the β-galactosidase plasmid. (A) Visualization of the cell layer after cell engraftment at the surface of the tube in a radial magnetic field followed by magnetofection and staining for β-galactosidase (two blue bands), (B) microscopy and (C) MRI images of the cell monolayer.

Externally triggered on-demand drug delivery from polymer matrices induced by a magnetic field

Stefan A. Rovers, Richard Hoogenboom, Maartje F. Kemmere, Jos T.F. Keurentjes

Process Development Group, Department of Chemical Engineering and Chemistry, Eindhoven University of Technology, P.O. Box 513, 5600 MB Eindhoven, The Netherlands
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http://www.chem.tue.nl/spd

ABSTRACT

The pharmaceutical industry asks for prolonged and better control of drug administration to allow for more effective therapies. Highly potent drugs, e.g., as used in chemotherapy, require a certain amount of drug released in a specified time interval at a specific site in the human body. These potent drugs often have a very narrow therapeutic window, making it difficult to maintain an effective drug level for a prolonged time. A polymeric implant containing drugs can be used to create on-demand release using an external trigger. The focus of our work is to create a polymeric drug delivery system, which can be switched on and off by applying a magnetic field.

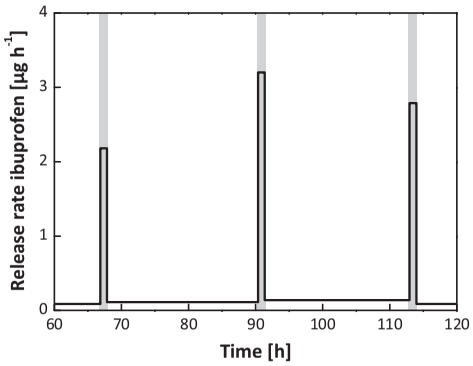

Figure 1: On-demand release triggered by an alternating magnetic field, gray bars indicate when magnetic field is applied

By heating the implant from below to above its glass transition temperature, the diffusion coefficient of the drug dissolved in the implant increases significantly. To induce drug release, superparamagnetic iron oxide nanoparticles have been embedded in the implant, which can be heated by an alternating magnetic field (2850 A m^{-1}, 745 kHz).

Commercially available nanoparticles are embedded into biocompatible polyacrylates by solvent casting and extrusion methods. The heating properties of nanoparticles, and the effect of their distribution in polymers, have been investigated in magnetic fields suitable for medical applications. Subsequently, these nanocomposites have been coated with a polymer with an appropriate T_g, containing ibuprofen as a model drug.

Results show the magnetic field induces a sufficient temperature increase to exceed the glass transition temperature of the polymer within seconds. Furthermore, the distribution of the iron oxide particles appears to have a clear effect on the heating of the polymer. Subsequent release experiments show a clear on/off release behavior from the implant, triggered by the external magnetic field. The ratio between on and off release depends on the iron oxide loading.

Application of Tat and Folate Mediated Magnetic Nanoparticles in the Therapy of Epilepsy

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Epilepsy is a chronic neurological disorder that is characterized by recurrent unprovoked seizures. Traditional antiepileptic drugs have two defects, i.e., they do not have target property and they can not pass the blood-brain barrier (BBB) efficiently. In this research, a novel magnetic nanoparticles consisting amphiphilic chitosan derivatized magnetic liposome, a peptide sequence from HIV-1 protein (Tat), folic acid (FA) and valproate (VPA) were fomulated using layer by layer assembly method. Characterized by FT-IR, 1H-NMR, TEM, Particle Size Analyzer, VSM and XPS, this magnetic drug delivery system (Tat-FA-VPA-LSMM) is about 100nm with well-shaped morphology and narrow size distribution, and has multiple functions including magnetic targeting, BBB crossing and therapeutic effect.

Rats suffering from epilepsy were used to investigate the distribution of the 99mTc-labeled Tat-FA-VPA-LSMM drug delivery system and the therapy effect in vivo. The results detected by single photon emission computed tomography (SPECT) showed that there were significantly more radio-aggregation areas in the brain than in other organs (e.g., the liver, spleen, kidney and bladders) of the rats. The therapy effect was studied using electroencephalography (EEG) as well as the brain slices analyzed by HE staining, nissl staining, glial fibrillary acid protein (GFAP) immunohistochemical staining and cell apoptosis detection after 7 days of injection of the Tat-FA-VPA-LSMM Nanoparticles. The results showed treatment group had significantly better therapeutic effects on epilepsy than control groups treated with saline and VPA alone (8 rats/group). The findings from this research indicate that the Tat and folate mediated magnetic drug delivery system has promising utility in treating neurological illnesses such as epilepsy.

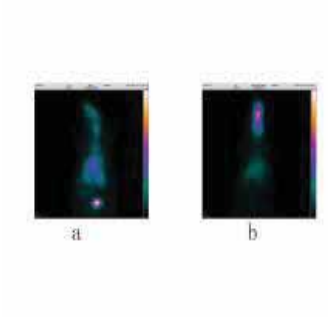

Fig.1 SPECT of ^{99m}Tc (a) and and ^{99m}Tc-Tat-FA-VPA-LSMM (b) in rats

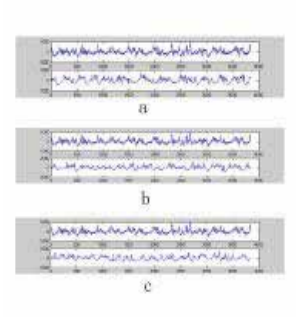

Fig.2. EEG of saline group (a) VPA group (b) Tat-FA-VPA-LSMM group (c)

Magnetically guided transport and deposition of anti-cancer drugs using nanostructured ferrocarbon particles – animal and clinical trials.

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We have been reporting our work on numerous clinical applications of nanostructured ferrocarbon micron-sized particles since the First International Conference on Scientific and Clinical Applications of Magnetic Carriers in1996. These particles have good magnetic properties, low toxicity, and unique adsorption capabilities. These properties can be fine-tuned by varying synthesis parameters, and make possible not only targeted delivery of anticancer drugs to the tumor, but also magnetically controlled embolization of the capillaries in the growth and creation of a local depot of the cytostatic medication slowly (up to 2 weeks) releasing into the tumor. Such particles are also useful for neutron-capture therapy, implant protection and for magnetic hemosorption. The particles can be produced either by the CVD method ("CeFeSorb") or by the plasmachemical technique ("CeFeSorb2"). The particles combined with anti-tumor drug carminomycin have passed all preclinical trials and were approved for clinical applications by Russian Pharmacological Committee. Two sterile pharmacological forms of the magnetically controlled preparation were developed and approved for clinical use in patients with advanced stages of cancer. Suspension of the particles loaded with the drug is injected locally through a catheter into an artery vascularizing the tumor under angiography control. Permanent magnetic field of 1-3 kOe, superimposed onto the tumor area for 30-40 min, causes the particles to agglomerate and block capillaries in the tumor. The drug is then slowly released from the particles, creating high local concentration for an extended period of time.

We report here new data on drug distribution throughout an organism after such injection performed on dogs (beagle breed). Suspension of CeFeSorb2 loaded with doxorubicin was injected through a catheter into the main artery of the left hind leg placed into ca. 1 kOe field. Tissue samples were collected and doxorubicin concentration was determined using HPLC (HP 1050). High concentration (>5 mg/ml) of the drug was measured in the muscles of the left leg, while in the general bloodstream, lungs, liver, kidneys and in the muscles from the right leg the doxorubicin concentration was low. 48 h later the injection zone still had large amount of the cytostatic.

Previous clinical trials have shown transfer of the particles to lymph nodes. This process was studied by injecting the CeFeSorb2 suspension into the abdominal cavity of mice. Histological studies have shown intensive phagocytosis of the particles by macrophages, while the macrophages remain viable.

We also report previously unpublished data on the clinical trials conducted in the early nineties at the City Hospital #30, St. Petersburg, Russia by Dr. A.A. Noskov's group. Chemotherapy of advanced stages of cancer using CeFeSorb with magnetically controlled transport was tested. The trials were conducted on 8 male and 3 female patients with 3rd or 4th stages of the disease. 7 patients had lung cancer, 4 patients had metastatic liver cancer. Each patient was extensively studied using a variety of clinical tests, and pharmacologically prepared before the procedure was performed. All patients responded well to the procedure: no complications typical for regular chemotherapy were observed. Only 2 patients had mild neurological sideeffects typical for angiography. Bloody cough stopped in all 6 patients who had it before. Fibrobronchoscopy showed reduction in edema and increase of openings of the bronchi. X-rays studies showed reduction of the tumor size and formation of more defined borders. Histological studies demonstrated numerous CeFeSorb particles in the tumor and aseptic necrosis of the oncological tissue. Two patients had surgery 3 weeks after the procedure. Tumor size reduction and change in its morphology was observed. Tumor tissue and lymph nodes were black because of the particle accumulation as confirmed by histology. Overall, all patients showed significant improvement in their condition after the procedure. More details will be presented. Clinical trials should be continued for other types of cancer, and the long-term effects of the procedure should be studied. Better success can be expected for earlier stages of the disease.

MAGNETIC NANOSPHERES FOR TARGETED THROMBOLYSIS: FABRICATION, DRUG RELEASE AND *IN VITRO* MAGNETIC TRAPPING

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A biodegradable, biocompatible carrier for the shielded transport of the clot lysis medication tPA (tissue plasminogen activator) which allows magnetic carrier guidance within the vasculature employing external magnetic fields as well as triggered drug burst release to achieve targeted, on-demand lysis at the clot site would be of great interest for many biomedical applications such as the treatment of heart attacks and strokes. Here we describe the synthesis and performance testing of the first tPA-encapsulated, magnetic nanospheres suitable for medical applications. Concentrated tPA aqueous solution (internal water phase (W1)) and DCM-dissolved poly(D,L-lactide-co-glycolide) and hydrophobic magnetite (oil phase (O)) were emulsified by probe sonication to form a W1/O primary emulsion. The primary emulsion was then added into an aqueous solution containing 0.5% poly(vinyl alcohol) (PVA) and further emulsified by homogenization. After rapid evaporation of the organic solvent, solid medicated magnetic nanospheres formed which were then characterized in terms of morphology, size, drug entrapment efficiency, *in vitro* ultrasound triggered release and magnetic trapping in a physiologically simulated model. The results identified that a) magnetic nanocarriers loaded tPA can be prepared by such a double emulsion technique; b) the tPA entrapment efficiency was 90%; c) particle size about 390~400nm in mean diameter; and d) the magnetization reached 25 emu/g. The tPA release from the carriers showed an initial burst release of 15% when placed into a fluid medium; however, brief ultrasound exposure trigged ~70% tPA release. The in vitro magnetic trapping efficiency was 65% in water at physiological flow rate employing a zero power magnetic of 0.3~0.4T. These results demonstrate for the first time the successful fabrication of magnetically- and ultrasound-responsive, biocompatible tPA nanocarriers.

Dynamic Control of Magnetic Fields to Focus Drug-Coated Nano-Particles to Deep Tissue Tumors

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[3]Department of Physics and Astronomy, University of Glasgow, United Kingdom
[4]Head, Cecilien-Klinik, Bad Lippspringe, Germany; [*]E-Mail: benshap@eng.umd.edu

The goal in magnetic drug delivery is to use external magnets to confine magnetically responsive particles to targets in the body, e.g., to focus chemotherapy to tumors. Significant research has gone into fabricating, improving, and functionalizing magnetic carriers, into testing their safety and efficacy in animals and humans, and into optimizing external magnets to hold particles with the strongest possible but still safe magnetic fields. Nevertheless, magnets attract magnetic carriers, and unless a magnetic material is implanted into a patient, statically held magnets can only focus carriers to targets near the skin surface (< 5 cm in our phase 1 clinical trials).

We have begun to develop methods, to control magnets one against the other *dynamically*, to focus particles between them to deep tissue targets, as shown in Figure 1a.

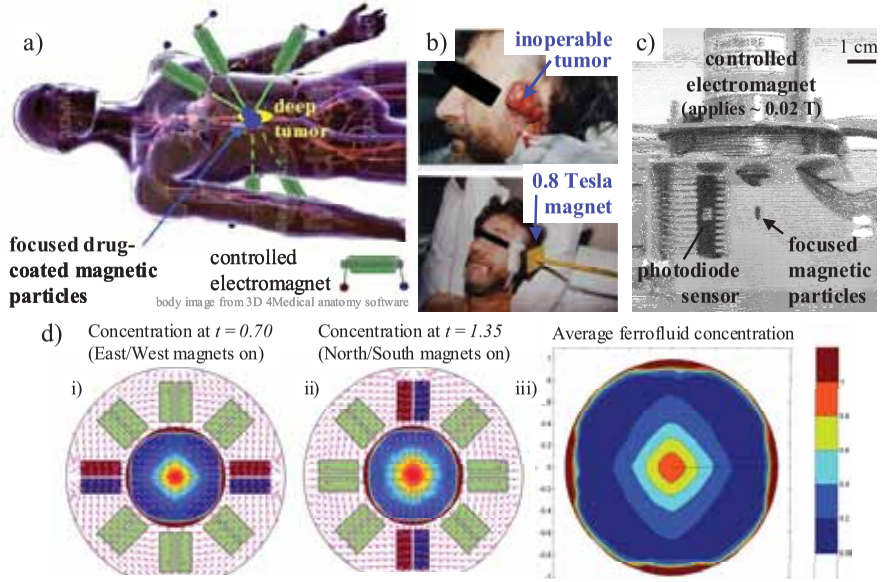

Figure 1: a) The goal is to dynamically control electromagnets to focus drug-coated nano-particles to deep tissue tumors, something that cannot be done today. b) In our phase 1 clinical trials, we have used strong stationary magnets, but these can only focus particles to < 5 cm below skin depth. Preliminary results. c) Holding a drop of ferrofluid at a distance from an electromagnet by dynamic control (the drop is held together by surface tension). d) Focusing of a distributed ferrofluid, not held together by surface tension, using dynamic control, in simulation.

Over the last 2 years, we have defined the *in-vivo* control problem, have created initial models that include magnetic fields, the resulting magnetic forces, and the drift and diffusion of particles in the blood flow, and have generated initial electromagnet control methodologies. Our calculations show that there is sufficient force, and that precise but robust controllers should be able to focus magnetic carriers to deep tissue targets.

Nanoparticle driven imatinib delivery into target cells

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Introduction

The targeted delivery of therapeutics into relevant cells and tissues is still of major concern. We present a simple straight forward strategy of binding a therapeutic drug onto magnetic nanoparticles. The magnetic core of the nanoparticles allows the precise localization of the nanoparticles and the release of the drug at the desired destination.

We choose imatinib (syn.: STI571) as a model substance, because it is well defined and shows a distinct molecular action by inhibiting the kinase activity of the bcr/abl fusion protein in chronic myeloic leukemia (CML). Therefore we used the CML cell line K562 as a model system. For preparing the delivering nanoparticles, iron oxide particles were coated with gold. As a control we used the basic iron oxide particles as well. Finally, the drug was adsorbed to both nanoparticles.

Results

The imatinib loaded nanoparticles have the same effect onto the leukemia cells as the pure drug itself. K562 cells exhibited a dramatic reduction in cell proliferation depending on the concentration of the nanoparticles and subsequently the transferred drug. Bcr/abl negative cells were not affected by the imatinib loaded nanoparticles. Cell cultures incubated with nanoparticle preparations without imatinib showed no effect and exerted a comparable proliferation as untreated K562 cells.

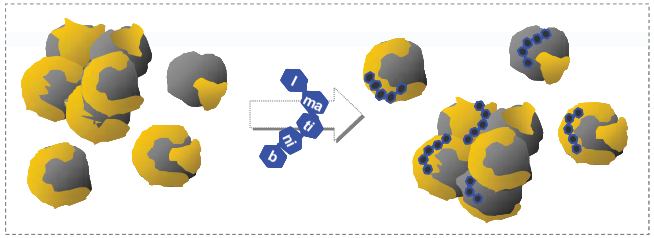
Intrestingly iron oxide particles with adsorbed imatinib but without gold coating showed the same proliferation inhibition as the particles with gold coating. The influence of buffer conditions and additives were more pronounced by using gold-coated particles.

Summary

In conclusion, we could prepare magnetic iron oxide nanoparticles with adsorption properties for imatinib. This approach allows the use of these imatinib loaded nanoparticles as a particle assisted delivery system to selected target cells. These results encourage us to use magnetic nanoparticles as vehicles for other drugs and as drug locating system.

Acknowledgments

This work was supported by DFG priority program 1104, grant CL 202/1-2



picture 1 schematic representation of imatinib loading onto gold-coated nanoparticles

Biodistribution, Clearance, and Biocompatibility of a Novel Iron-Based Magnetic Nanoparticle Drug Delivery Formulation

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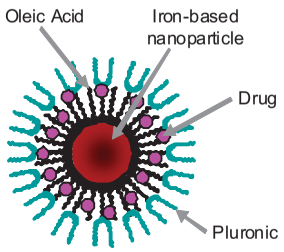
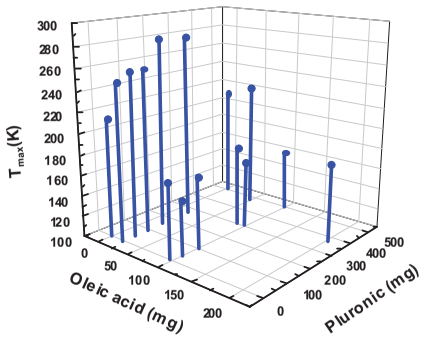
A novel water-dispersible magnetic nanoparticle formulation can be loaded with high doses of water-insoluble anticancer drugs. Chemically synthesized iron or iron-oxide nanoparticles of diameter ~10 nm are coated with a bilayer of oleic acid and Pluronic. The formulation is superparamagnetic, with the peak temperature T_{max} dependent on the OA and Pluronic® concentrations. Drug partitions into the OA shell surrounding the magnetic nanoparticles, while the Pluronic® that anchors at the OA-water interface confers aqueous dispersity to the formulation. The optimized nanoparticles demonstrate sustained intracellular drug retention relative to drug in solution and dose-dependent antiproliferative effect in breast and prostate cancer cell lines.

In addition to magnetically targeted drug delivery and magnetic resonance imaging applications, we have investigated the biodistribution, clearance, and biocompatibility of this formulation in rats. Changes in serum and tissue iron levels were analyzed over 3 weeks after intravenous administration of MNPs to rats. Serum alanine aminotransferase (AST), aspartate aminotransferase (ALT), alkaline phosphatase (AKP) levels, and total iron-binding capacity (TIBC) were also measured with time to assess the effect of MNPs on liver function. Selected tissues also were analyzed for oxidative stress and histologically studied to determine MNP biocompatibility. Serum iron levels gradually increased up to 1 week but slowly declined thereafter. Although there were increases in iron levels (especially in the liver and spleen), the magnetic nanoparticle formulation did not cause long-term changes in the liver enzyme levels or induce oxidative stress.





Exploiting the Electromagnetic Response of Polymer Composite Nanocarriers for Controlled Drug Release

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Remote, triggered release of therapeutic agents in novel treatment systems, such as drug-loaded nanocarriers, allows for minimal procedure invasiveness, effective drug delivery, and minimal patient side-effects. Nanocarriers injected systemically can deliver the payload to the target site. However, triggered release is essential for immediate therapeutic response at the site for many applications. On-demand release of drugs from nanocarriers is made possible through the controlled electromagnetic response of magnetic materials and the thermoresponse of the biodegradable polymer.

The nanocarriers tested in this study have been synthesized via a double emulsion method to encapsulate a hydrophilic clot lysis agent, tissue plasminogen activator or tPA, in the composite core. The nanocarrier shell is composed of a biodegradable polymer with magnetic nanoparticles dispersed throughout the polymer (Figure 1A). When subjected to a variable alternating current magnetic field, such as those employed in magnetic hyperthermia, the magnetic particles undergo local and selective heating (Figure 1B) and the resulting thermoresponse from the polymer matrix around the magnetic particles results in increased drug release (Figure 1C). Release kinetics of bioactive and total tPA from the nanoparticles have been determined as a function of magnetic induction. The electromagnetic response of the polymer composite greatly enhanced the tPA release and promises to improve clot lysis efficiency for therapeutic modalities.

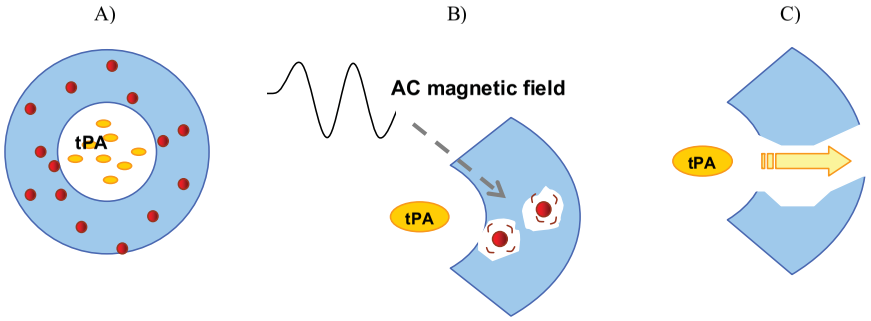

Figure 1. Electromagnetic response of nanocarrier polymer composite showing enhanced drug release. A) Cross-section of nanocarrier composed of biodegradable polymer (blue), magnetic nanoparticles (red) and hydrophilic drug encapsulated in core (yellow); B) magnetic particles undergoing localized and selective heating accompanied by thermoresponse of polymer; and C) leads to enhanced drug release from nanocarrier.

7th International Conference: Scientific and clinical Applications of Magnetic Carriers: Abstract

The in-flow capture of superparamagnetic nanoparticles in microcapillary film as a potential model for targeting of gene therapeutics

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Superparamagnetic magnetite nanoparticles have been synthesised and used for in-flow capture experiments *in vitro* to provide a better understanding of the physical principles that underlie magnetic directed gene therapy. Experimental observations and modeling work have enabled initial refinement of magnetic targeting strategies and superparamagnetic nanoparticle properties for different therapeutic targeting requirements. The initial nanoparticle capture experiments were performed in a constant flow rate regime in novel microcapillary films (MCF) extruded in-house. It has been discovered that 330 nm and 580 nm agglomerates of 10 nm magnetite cores can be captured with a 0.5 T magnet in flows of up to 0.35 ml•min^{-1} in 410 μm diameter microcapillaries. To better represent the blood flow in the cardiovascular system this *in vitro* model was further improved by splitting flow down multiple microcapillaries in an array introducing a constant pressure regime. Superparamagnetic nanoparticle capture was measured across the array under different conditions which together with magnetic field characterization provided useful data to improve the *in vitro* model. The aim of this work is to develop this approach to test magnetic directed therapies *in vitro*, enabling data to be provided for preclinical trials.

Targeted temperature sensitive magnetic liposomes for thermo-chemotherapy of cancer

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Targeted delivery of a liposome-encapsulated chemotherapeutic drug followed by its triggered release by hyperthermia within the tumor tissue would possibly be the best approach to achieve maximum therapeutic efficacy of both hyperthermia and chemotherapy with minimum side effects. To achieve this goal, a folate receptor targeted temperature sensitive magnetic liposome preparation (Fig. 1) was developed for thermo-chemotherapy of cancer. The optimized folate targeted temperature sensitive composition, DPPC Cholesterol DSPE-PEG_{2000} DSPE-PEG_{2000}-Folate at 80:20:4.5:0.5 molar ratio showed calcein release of about 70% in PBS and 50% in FBS (fetal bovine serum) at 43 °C and only less than 5% release at 37 °C following 1 hr incubation. Doxorubicin containing magnetic liposomes of the above composition ("MagFol2000Dox") was prepared by a modified ammonium sulphate gradient method encapsulating an aqueous suspension of superparamagnetic magnetite nanoparticles (mean size 10 nm) with encapsulation efficiency of doxorubicin and magnetic nanoparticles about 80% and 40% respectively. The magnetic liposomes showed satisfactory temperature-specific doxorubicin release (around 50%) in 50% FBS following 1 hr incubation at 43°C. The magnetic liposome showed higher magnetic responsiveness than the magnetic nanoparticles. In KB cells (had higher folate receptor expression), the amount of uptake of MagFol2000Dox (in terms of doxorubicin as determined by spectrofluorometry) following 2 hrs exposure including an initial 1 hr exposure of permanent magnetic field was about 1.5-fold higher than free doxorubicin, 5-fold higher than non-magnetic folate liposomes ("Fol2000 Dox") and 50-fold higher than Caelyx® (a commercially available liposomal doxorubicin preparation). In HeLa cells, the uptake under similar conditions was 4-fold higher than free doxorubicin, 5-fold higher than Fol2000 Dox and 118-fold higher than Caelyx. In KB cells, magnetic folate liposomes with 1 hr permanent magnetic field exposure showed about 9-fold and 3-fold decrease in IC50 values as compared to Caelyx® and non-magnetic folate liposomes, respectively. A similar trend but with higher IC50 values was observed in HeLa cells. The IC50 value of MagFol2000Dox was still 3-4 times higher than the one of free doxorubicin. However, we envisage that the IC50 value of this formulation can be further decreased by triggering release of the doxorubicin load inside cells using magnetic hyperthermia (by AC magnetic field).

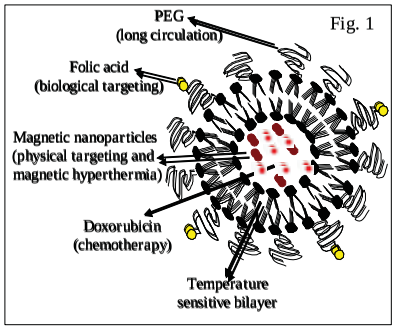

Fig. 1

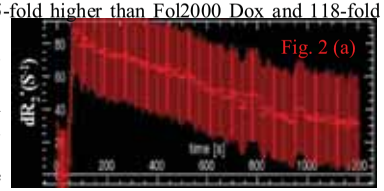

Fig. 2 (a)

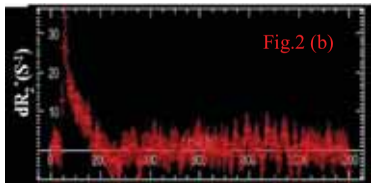

Fig.2 (b)

In addition, magnetic resonance imaging (MRI) studies showed higher r2 relaxivity for the MagFol2000Dox liposome than the free magnetic nanoparticles. T2* weighted image of MagFol2000Dox treated KB cells under 1 hr magnetic field exposure showed increased contrast compared with free magnetic particle treated cells and thus confirmed the higher uptake of magnetic nanoparticles in the liposomal form which might be facilitated both by magnetic field and folate receptor. In vivo real time MRI monitoring in mice confirmed significantly higher blood circulation time for MagFol2000Dox (Fig. 2a) compared with free magnetic particles (Fig. 2b). In summary, a temperature-sensitive folate-targeted magnetic liposome formulation has been developed for thermo-chemotherapy of cancer. The bio-distribution of this magnetic liposome can be monitored by MRI. Ongoing studies are aimed at characterizing and optimizing AC magnetic field heating and demonstrating the utility of the approach in a mouse tumor model.

IMPROVED MAGNETIC NANOPARTICLE-BASED GENE TRANSFECTION USING OSCILLATING MAGNET ARRAYS

Jon Dobson[1,2], Stuart C. McBain[1], Neil Farrow[1], Christopher D. Batich[2]

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Magnetic nanoparticle-based gene transfection has been shown to be effective both in combination with viral vectors and with non-viral agents[1-4]. In these systems, therapeutic or reporter genes are attached to magnetic nanoparticles which are then focused to the target site/cells via high-field/high-gradient magnets. The technique has been shown to be efficient and rapid for in vitro transfection and compares well with cationic lipid-based reagents, producing good overall transfection levels with lower doses and shorter transfection times.

In spite of its potential advantages (particularly for *in vivo* targeting), the overall transfection levels do not generally exceed those of other non-viral agents. In order to improve the overall transfection levels while maintaining the advantages inherent in this technique, we have developed a novel, oscillating magnet array system which adds lateral motion to the particle/gene complex in order to promote transfection. Experimental results indicate that the system significantly enhances overall in vitro transfection levels in several cell types compared to both static field techniques ($p<0.005$) and the cationic lipids ($p<0.001$) tested (Figure 1). In addition, it has the previously demostrated advantages of magnetic nanoparticle-based transfection – rapid transfection times and requiring lower levels of DNA than cationic lipid-based transfection agents. This method shows great potential for non-viral gene delivery both in vitro and in vivo.

References

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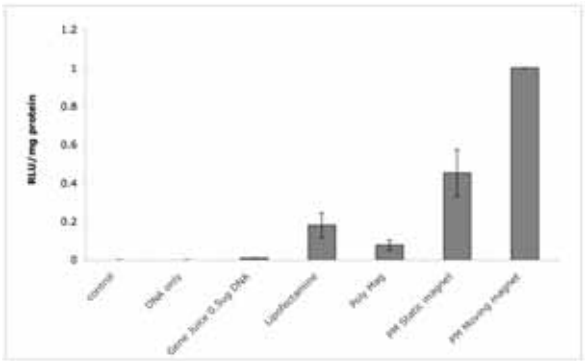

Figure 1: Luciferase activity in H292 human lung epithelial cells transfected with OzBiosciences Polymag particles coated with pciLux luciferase reporter construct in response to static and oscillating magnetic fields. All transfections were performed in serum free RPMI 1640 media as follows; Polymag 0.1ug DNA/0.1ul PolyMag per well and LF2000 0.1ug DNA/0.3ul LF2000). The plates were transferred to an incubator and exposed to static or oscillating (f=2Hz; amplitude 200μm) magnetic fields produced by pairs of 6x4mm NdFeB magnets per well for 2hr. At 2hr post transfection, the magnets were removed and at 48hr post transfection, the media was removed from each well and the cells lysed by the addition of 30μl of cell reporter lysis buffer (Promega CCLR). Samples were assayed for Luciferase activity using a Luciferase assay reagent (Promega) and the total protein concentration determined using a BCA assay reagent (Pierce, USA).

Talk Abstracts - Thursday, May 22, 2008

	Session 3a: Nanoparticle Synthesis and Analysis - *Chairs: Yali Cui (China) and Jian-Ping Wang (U.S.A.)*			
8:30	Athanassiou, Evagelos	Production of chemical functionalized carbon coated cobalt nanoparticles as a novel tool for magnetic separation technology	Switzerland	Talk 19
8:45	Dutz, Silvio	Ferrofluids of magnetic multicore nanoparticles for biomedical applications	Germany	Talk 20
9:00	Qin, Jian	Putting Smart Clothes on Nanoparticles	Sweden	Talk 21
9:15	Schaller, Vincent	Monte Carlo Simulation of Multi-Core Magnetic Nanoparticles	Sweden	Talk 22
9:30	Dilorio, Mark	Ultra-sensitive magnetic bioassays and the need for a better magnetic nanoparticle	U.S.A.	Talk 23
9:45	**Coffee break / poster session / exhibitors** - *coffee break sponsored by Integrated Engineering Solutions*			
10:25	Holschuh, Karl	Continuous Production of High Capacity Nanoparticles for Affinity Purification of Biomolecules	Germany	Talk 24
10:40	Huber, Dale	Optimization of Magnetic Nanoparticles for Biodetection Where Size Control is Critical	U.S.A.	Talk 25
10:55	Kennedy, Ian	Synthesis and characterization of multifunctional silica core-shell nanocomposites with magnetic and fluorescent functionalities	U.S.A.	Talk 26
11:10	Martinez-Boubeta, Carlos	Novel Route to synthesizing Core-Shell Fe-MgO Nanospheres: High magnetization particles for biomedicine	Spain	Talk 27
11:25	Meissner, Daniel	Tridentate ligands offer new ways for reliable functionalization of metallic nanoparticles	Germany	Talk 28
11:40	Nguyen, Thanh	Tunable shapes of FePt magnetic nanoparticles	U.K.	Talk 29
11:55	**Odenbach, Stefan**	**Ferrofluids**	**Germany**	**Invited talk 3**
12:35	**Lunch**			
14:05	**Hicks, Robin**	**Molecular Magnets**	**Canada**	**Invited talk 4**
	Session 3b: Nanoparticle Synthesis and Analysis - *Chairs: Yousef Haik (U.S.A.) and Dhirendra Bahadur (India)*			
14:45	Begin-Colin, Sylvie	Study of magnetite phosphatation in view of improving grafting	France	Talk 30
15:00	Riffle, Judy	Dispersion properties of magnetite-polymer complexes in physiological media	U.S.A.	Talk 31
15:15	Salaklang, Jatuporn	Fixed bed micro-reactor for surface derivatization of Superparamagnetic Iron Oxide nanoparticles	Switzerland	Talk 32
15:30	Grass, Robert	Are carbon coated metal nanoparticles a high magnetization alternative to oxide based beads?	Switzerland	Talk 33
15:45	Tanaka, Toshiyuki	Preparation of spherical and monodisperse ferrite nanoparticles with diameters between 50-150 nm for biomedical applications	Japan	Talk 34
16:00	Lalatonne, Yoann	Novel magnetic labels for bioassays	France	Talk 35
16:15	Lim, JitKang	Synthesis and Stabilization of Iron Oxide-Core, Gold-Shell Nanoparticles for Biosensing	U.S.A.	Talk 36
16:30	Gu, Hong-chen	Preparation of mono-dispersed nanobeads by miniemulsion polymerization and application in lateral flow immunoassay	China	Talk 37
16:45	Prozorov, Tanya	Novel Bio-Inspired Route to Complex Ferrite Nanomaterials	U.S.A.	Talk 38
17:00	**Poster session with beer and pretzels - PLEASE RATE POSTERS** - *sponsored by Magnisense*			
18:30	**End of session / bus to harbour and boat trip including dinner; will be back around 23:00 (11 PM)**			

Today's Social Program

Starting at 9:00 (9 AM), we will have a full day complimentary tour for spouses. It will include the visit of some parts of the city as well as of the scenic surroundings of Vancouver. Please tell us on the previous day if your spouse would like to take part in this old tradition at our meetings!

After the talks, the poster session will begin around 17:00 (5:00 PM) upstairs in the SUB. Beer and pretzels have been graciously sponsored by **Magnisense**.

After the poster session, we will be picked up by bus punctually at 18:30 (6:30 PM) in front of the Gage Towers. We will board the M.V. Brittania for our traditional dinner cruise at the North foot of Denman Street downtown. We will be back at UBC around 23:00 (11 PM).

Production of chemical functionalized carbon coated cobalt nanoparticles as a novel tool for magnetic separation technology.

E.K. Athanassiou[1*], R.N. Grass[1], W.J. Stark[1]

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Core-shell nanomaterials combine the beneficial properties of a core material with the surface properties of a second, shell material. We demonstrate how a metal/carbon core-shell material can significantly improve the magnetic properties of currently used magnetic nanoparticles.

Such increased magnetic properties can be achieved by the use of metallic compounds (Fe, Co, Ni) with an up to 3-fold increased mass magnetization. However, if the size of the metal is reduced to the nano-size range these materials usually become highly pyrophoric, impeding their application. A way out of this problem is to coat the metallic nanoparticles with an inert shell material (Figure 1). We present the one-step fabrication of 1-2 nm carbon coated cobalt nanomaterial. Besides the protection of the metallic core at a wide pH and temperature range, the carbon layer offers the possibility to covalently functionalize the surface of the nanomagnets (Scheme 1). This not only allows influencing the dispersion properties of the material but also allows designing a material for the specific binding of ligands. These materials can now be used for the rapid magnetic separation of target compounds from liquid mixtures and offer a platform for new separation technologies in organic chemistry and biotechnology.

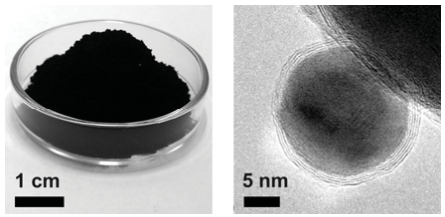
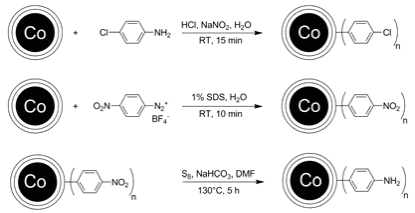


Figure 1: Photograph of as-prepared carbon coated cobalt nanoparticles (~5 g, left), and a transmission electron micrograph of an individual particle showing the metallic cobalt core, which is protected by several layers of graphene carbon.



Scheme 1: The carbon coated nanoparticles can be chemically functionalized with different chemical groups acting as linkers for magnetic separation.

References:

[1] R. N. Grass, E. K. Athanassiou, W. J. Stark, *Angew. Chem. Int. Ed.* **2007**, 46, 4909.

[2] R. N. Grass, W. J. Stark, *J. Mater. Chem.* **2006**, *16*, 1825.

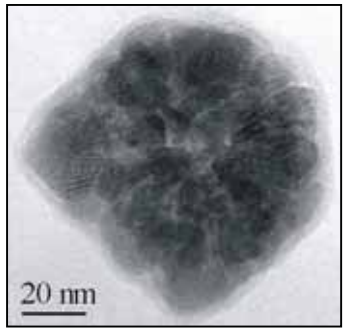
Ferrofluids of magnetic multicore nanoparticles for biomedical applications

S. Dutz [1], D. Eberbeck [2], T. Gelbrich [3], R. Hergt [1], R. Müller [1], M. Zeisberger [1]

[1] *Institute of Photonic Technology, Jena, Germany*
[2] *Physikalisch-Technische Bundesanstalt, Berlin, Germany*
[3] *Institute of Organic Chemistry and Macromolecular Chemistry, Heinrich Heine University, Düsseldorf, Germany*

For a variety of magnetically based biomedical applications (e.g. hyperthermia, cell separation, and drug targeting) it is advantageous to use aqueous suspensions of relatively large (d > 20 nm) magnetic nanoparticles (MNP). Unfortunately, MNP of this size show the trend to form agglomerates which results in a sedimentation of the ferrofluid.

To solve this problem, a novel type of MNP – so called multicore nanoparticles (MCNP) – was synthesized by a modified alkaline precipitation method. Water based suspensions of these particles were prepared by coating of the MCNP with a carboxymethyldextran (CMD) shell. The resulting fluids were structurally characterized by TEM, XRD, PCS, and magneto-optical relaxation of ferrofluids (MORFF) as well as magnetically by VSM, SQUID based magneto relaxometry (MRX), and magnetic field calorimetry (MFC).



Typical TEM image of a prepared multi-core nanoparticle.

It was found, that during the precipitation primary particles with a mean diameter of 13 to 15 nm (XRD) were formed which build clusters of 40 to 80 nm (TEM) with a common CMD shell. The hydrodynamic diameters of these clusters were determined by PCS, MORFF, and MRX in the range from 100 to 120 nm. Due to the distribution of the magnetization directions of the single cores in absence of a magnetic field the resulting magnetic moment of a single cluster is relatively weak. This fact causes a low agglomeration rate and therefore a good stability against sedimentation. The investigated fluids showed no significant changes of their properties after strong dilution by pure water and BSA buffer. VSM measurements at immobilized particles provided high hysteresis losses and by MRX strong Brownian relaxation losses were found. Corresponding to these results a comparatively high specific heating power of up to 160 W/g (at f = 400 kHz and H = 10 kA/m) was determined by MFC measurements. This value was increased up to 225 W/g by a simple fractionation method, based on centrifugation.

The prepared MCNP are promising materials for a lot of magnetic biomedical applications due to their magnetic properties and the good stability against sedimentation. At present, the test of the suitability of these novel particles for drug targeting, hyperthermia, magnetofection, and cell separation takes place in cooperation with clinical partners.

Putting Smart Clothes on Nanoparticles

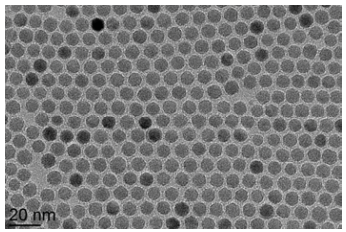
Jian Qin*, Mamoun Muhammed

Division of Functional Materials, School of Information and Communication Technology, Royal Institute of Technology, Stockholm, Sweden, Email: jianq@kth.se

Recent development of monodisperse nanoparticles has dramatically advanced nanotechnology. One of the widely adopted methodologies relies on reactions at elevated temperature in non-aqueous solutions, yielding nanoparticles with high crystallinity. However, a major disadvantage is that those particles cannot be dispersed in aqueous solutions, due to the presence of hydrophobic ligands. This greatly limits their use in several interesting applications of biomedical field. Therefore intensive research is directed to improve the dispersion of such nanoparticles in aqueous fluids by means of phase transfer process.

We have synthesized a thermosensitive amphiphilic polymer as a phase transfer agent, which can be applied, generally, for all the organic ligand coated monodisperse nanoparticles. Superparamagnetic iron oxide nanoparticles (SPION) coated with oleic acid is taken as an example in this study. A typical TEM image of iron oxide nanoparticles is shown in the Figure below. Poly(maleic anhydride-*alt*-1-octadecene) (PMAO) is selected as the polymeric backbone. Thermosensitve polymers with an amino end group poly(*N*-isopropylacrylamide) (PNIPAAm-NH_2) have been synthesized with different molecular weight. The anhydride groups on PMAO chains readily react with amino groups on the end of PNIPAAm chains, resulting in a thermosensitive amphiphilic copolymer PMAO-PNIPAAm. When the temperature is below the lower critical solution temperature (LCST), PNIPAAm block is hydrophilic. The hydrophobic 1-octadecene intercalates into the oleic acid layer on the surface of SPION, while the PNIPAAm chains render the particles with favorable hydrophilicity. When the temperature is elevated above the LCST, PNIPAAm blocks undergo phase transition and tend to be hydrophobic, inducing agglomeration of particles which used to be dispersible in aqueous solution at low temperature. The transition of solubility is reversible that is when the particles are cooled down the water dispersibility is thus restored.

Therefore, the thermosensitive amphiphilic copolymer PMAO-PNIPAAm provides a very simple way to generate temperature-responsive nanoparticulate system which is promising for many potential applications such as colorimetric diagnosis, temperature-induced drug release and etc.



Work supported by European Commission 6th Framework Program (NanoEar NMP4-CT-2006-026556)

Monte Carlo Simulation of Multi-Core Magnetic Nanoparticles

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Magnetic nanoparticles containing a number of single-domains, and referred to as multi-core particles, are very promising for use in bio-medical applications due to their higher particle magnetic moment compared to single-core particles containing only one or a few single-domains. These bio-medical applications require, however, particles with well-characterized magnetic properties.

In this paper we present a numerical approach to investigate the magnetization process of multi-core magnetic nanoparticles in a liquid. These particles typically have a hydrodynamic diameter of 50-200 nm, and contain a magnetic core consisting of a cluster of magnetite (Fe_3O_4) or maghemite (γ-Fe_2O_3) single-domains with a diameter of 10-15 nm each, surrounded by a polymer coating (e.g. dextran).

Our numerical model consists of two steps; monodispersed spherical domains are first aggregated into a three-dimensional (3D) cluster representing the magnetic core of the particle. This clustering algorithm is based on a set of criteria that we have established from the examination of TEM images of multi-core nanoparticles. Secondly, the mean value of the particle magnetic moment is simulated by a Monte Carlo algorithm which includes the following energy contributions: (1) dipole-field interaction between the single-domains and the applied magnetic field, (2) dipolar interactions between the single-domains in the particle, and (3) magnetic anisotropy of the single-domains. The minimization of the particle energy due to particle rotation in the liquid is also taken into account. We simulate the magnetization process of the particle in thermal equilibrium in a static, homogeneous field, as a function of a variety of parameters such as microstructure (number and size of the domains), magnetic material (anisotropy constant K_a and saturation magnetization M_s), temperature T, and applied magnetic field B.

We observe that the magnetization of a multi-core particle cannot be fully described by the Langevin function. Both inter-domain dipolar interactions and domain anisotropy contribute to the deviation from the Langevin values for all field amplitudes. The simulated values approach the Langevin function in the limits of high and low fields; however, the discrepancy is significantly larger in the intermediate field region. Our numerical data will be compared with magnetization measurements.

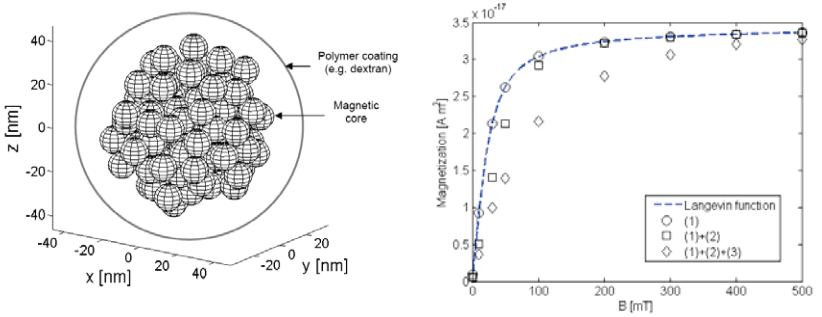

Fig. 1. (Left) Simulated 3D cluster of 101 monodispersed spherical single-domains of diameter 12.3 nm representing the magnetic multi-core of a fluidMAG-D100 nanoparticle from Chemicell GmbH; (Right) Simulated magnetization curve including (1) dipole-field interaction, (2) dipolar interactions between single-domains, and (3) magnetic anisotropy of the single-domains.

The support of the project by the European Commission Sixth Framework Programm under contract No. NMP4-CT-2005-017002 is acknowledged.

Ultra-sensitive magnetic bioassays and the need for a better magnetic nanoparticle

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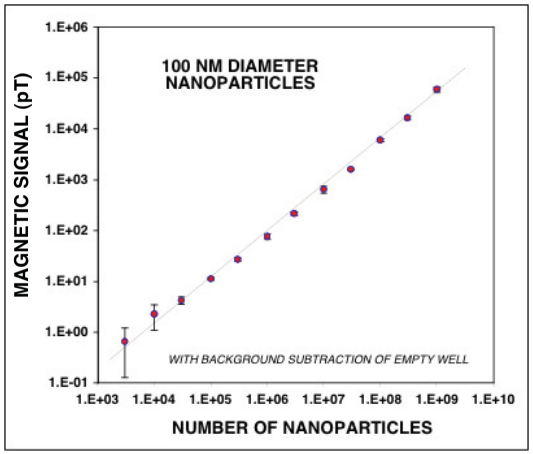
MagnesSensors, Inc. San Diego CA email: markd@magnes.com

We have developed high temperature SQUID based instruments for ultra-sensitive magnetic bioassays, with a primary focus on diagnostics. A large number of proof-of-concept magnetic assays have been performed over the past five years including: homogeneous (mix and measure) sandwich immunoassays detecting 4 amoles (1 pg/ml) IL-6, cell surface receptor assays detecting <40 cells, and assays for E. *coli* and S. *aureus* detecting <2,000 CFU/ml. Our primary focus is on highly sensitive mix and measure assays in blood and serum.

Our present instrument can rapidly detect below 3,000 magnetic nanoparticles (Fe_3O_4) in a microplate well format as shown in the figure below. While the magnetic sensor is cooled, the samples are measured at room temperature. A next generation, high throughput, bench top instrument that uses a small cryocooler is also now in development.

While we have examined nearly 150 different magnetic nanoparticle preparations provided by many companies and laboratories from around the world, the ideal magnetic nanoparticle for diagnostics is still lacking. Part of the problem stems from the fact that the primary commercial applications for magnetic nanoparticles to date, such as cell separation, do not share the more stringent requirements as for diagnostics. Additionally, production of "ideal" magnetic nanoparticles with tailored biofunctional coatings requires highly interdisciplinary skills.

This talk will focus on the characteristics desired in an "ideal" magnetic nanoparticle (for magnetic assays), along with how we assess many of these properties with our magnetic measurement system. Some of the more readily obvious characteristics include high magnetic moment, size uniformity, and stable coatings. Minimizing nanoparticle aggregation is critical, particularly in complex matrices such as blood and serum. Our SQUID instrument is particularly well suited in evaluating the amount of nanoparticle aggregation at the relatively low levels that impact the background in sensitive magnetic assays. Note that the ideal size for magnetic nanoparticles depends on the application, as intracellular assay on live cells would benefit from smaller size nanoparticles compared to applications centered on cell surface receptor assays.



Work supported by NIH and DARPA

Continuous Production of High Capacity Magnetic Nanoparticles for Affinity Purification of Biomolecules

Karl Holschuh[1], Achim Schwaemmle[2]

[1, 2] PLS R&D, Merck KGaA, Frankfurter Straße 250, 64293 Darmstadt, Germany
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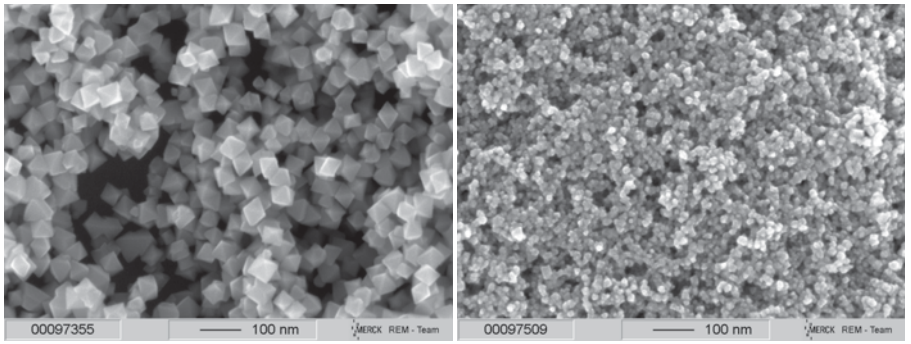
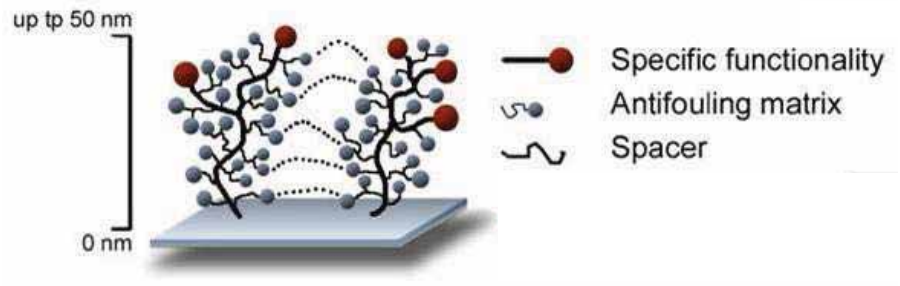
We have developed a procedure for the continuous production of almost monodispers magnetite crystals in the range of 10 – 100 nm with a surface area of about 20 - 200 sqm per gram. The particles are currently synthesized on a kilogram scale with unlimited upscaling potential by increasing the throughput and/or running the process in parallel.



Electron micrographs of different sized magnetite crystals

To further extend the surface capacity the particles are encapsulated in batch with a carboxylated tentacle polymer for the covalent immobilization of affinity ligands like protein A, streptavidin or specific antibodies. The optimum size to magnetically separate the beads in a manual or automated procedure within a reasonable time of a few seconds is in the range of 20 – 25 nm with a surface area of about 80 sqm per gram. This enables a binding capacity of up to 250µg IgG per mg of protein A coated particles and > 200µg biotinylated IgG per mg streptavidin magnetic beads.



Scheme of tentacle modified particle surface

Optimization of Magnetic Nanoparticles for Biodetection Where Size Control is Critical

Dale L. Huber[1*], Todd C. Monson[1], Danielle L. Fegan[2], Natalie L. Adophi[2], H. C. Bryant[2], Eugene L. Venturini[1], Edward R. Flynn[2]

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[2] Senior Scientific, 11109 Country Club NE, Albuquerque, NM 87111, USA

Biodetection using SQUID (Superconducting Quantum Interference Device) detected magnetic remanence decay is a very promising and exquisitely sensitive technique, owing to the complete lack of background noise in biological systems. The technique is based upon the observation of the Néel relaxations of magnetized superparamagnetic nanoparticles as a function of time. Due to instrumental design requirements, the relaxation window that can be conveniently measured is from approximately 50 milliseconds to several seconds. This sets up severe constraints upon the nanoparticles to be used for detection. The relaxation time of superparamagnetic particles, being a thermally activated process, follows Arrhenius kinetics where the activation energy term depends directly upon the particle volume. The result is that the relaxation time has an extraordinarily strong dependence upon the particle radius, as r^3 is in the exponent.

The design criteria we have developed for these nanoparticles include: sub-nanometer size control, narrow polydispersity, low magnetocrystalline anisotropy, low shape anisotropy, high magnetic moment, biocompatibility, and the ability to functionalize with antibodies. The best mix of properties comes from magnetic iron oxides, and we have chosen to synthesize them using high temperature decompositions in organic solvents because of the size and shape control that are achievable (see Fig. 1). We have evaluated particles that we have synthesized and a variety of commercially available particles, and to date have not observed a sample with more than a few percent of particles that respond in the relaxation time probed. Details of these experiments will be discussed, as will a new technique to synthesize magnetic nanoparticles that includes a novel size-selection process within the synthesis itself.

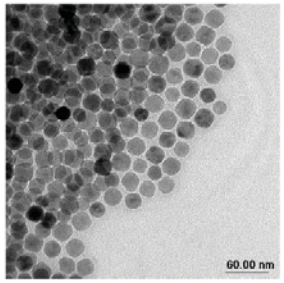


Figure 1. Transmission electron micrograph of approximately 25 nm magnetite nanoparticles synthesized as part of this study.

This work was performed, in part, at the Center for Integrated Nanotechnologies, a U.S. Department of Energy, Office of Basic Energy Sciences user facility. Sandia National Laboratories is a multi-program laboratory operated by Sandia Corporation, a Lockheed-Martin Company, for the U. S. Department of Energy under Contract No. DE-AC04-94AL85000. We also acknowledge financial support from the National Institutes of Health.

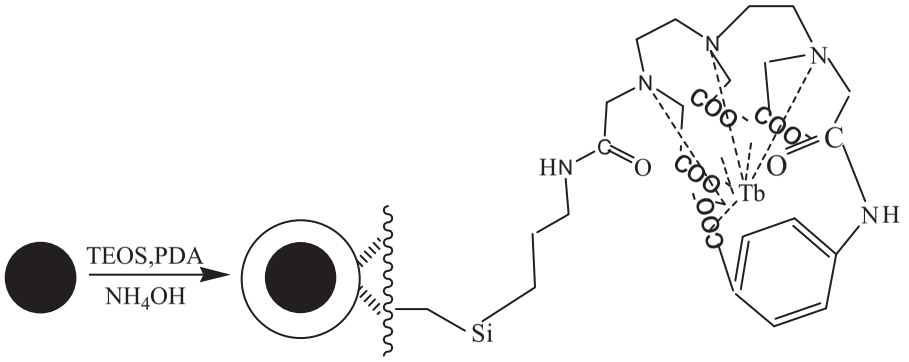
Synthesis and characterization of multifunctional silica core-shell nanocomposites with magnetic and fluorescent functionalities

Z. Y. Ma[1], D. Dosev[1], M. Nichkova[2], S. J. Gee[2], B. D. Hammock[2], I. M. Kennedy[1*]

[1] Department of Mechanical and Aeronautical Engineering, [2] Department of Entomology, University of California Davis, CA 95616 *Email: imkennedy@ucdavis.edu

The multifunctional nanostructures combined with magnetic and fluorescent properties have recently been attracting increased interests for biological and biomedical applications. Lanthanide related compounds have great potential for application in bioassays and in biolabeling due to their unique luminescence properties, such as sharp absorption and emission lines, long lifetimes, superior photo-stability and effective elimination of short-lived background.

In this study, multifunctional core-shell nanocomposites with a magnetic core and a silica shell doped with lanthanide chelate have been prepared by a simple method. First, citric acid-modified magnetite (Fe_3O_4) nanoparticles were synthesized by a chemical co-precipitation method. Then the Fe_3O_4 nanoparticles were coated with silica shells doped with terbium (Tb^{3+}) complex by a modified Stöber method based on hydrolyzing and condensation of tetraethyl orthosilicate (TEOS) and a silane precursor (PABA-DTPA-APTS, PDA), which was prepared by covalent coupling of an organic chromophore (*p*-aminobenzoic acid, PABA) and a chelator (diethylenetriaminepentaacetic acid, DTPA) with a silane (3-aminopropyltriethyloxysilane, APTS). These multifunctional nanocomposites are potentially useful in a variety of biological areas such as bioimaging, biolabeling and bioassays because they can be simultaneously manipulated with an external magnetic field and exhibit unique phosphorescence properties.



Novel Route to synthesizing Core-Shell Fe-MgO Nanospheres: High magnetization particles for biomedicine

C. Martinez-Boubeta[1*], Ll. Balcells[1], R. Cristòfol[2], C. Sanfeliu[2], Ll. Casas[3], J. Santiso[4], C. Monty[5], B. Martínez[1]
1. ICMAB, Bellaterra, Barcelona, Spain. * e-Mail : cboubeta@icmab.es
2. IIBB, CSIC-IDIBAPS, Barcelona, Spain
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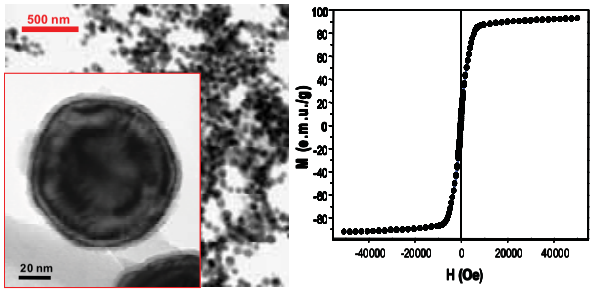
5. CNRS/Procédés, Matériaux et Energie Solaire (PROMES), Font Romeu, France.

In spite of encouraging progress in recent years, the development of magnetic nanoparticles that can be used as drug delivery vectors remains challenging. Among the multiple hurdles that must be overcome are the provision of a sufficiently high magnetic response and an adequate degree of biocompatibility. Therefore, our work has been directed towards the synthesis and characterization of new materials suitable to act as contrast agents for magnetic resonance imaging and further use in hyperthermia studies.

We have developed a novel route for the synthesis of magnetic core-shell nanoparticles by means of their particular self-assembly signature due to the difference in surface energies of Fe and MgO. In the first step Fe particles were produced using a solar vapour-phase condensation oven. However, we also proved the method universality by laser ablation synthesis in liquid solution. The particles produced can exhibit a size ranging from 2 to 200 nm, which depends mainly on the gas pressure during evaporation and/or the laser fluence.

X-Ray Diffraction, Mössbauer spectroscopy and electron microscopy studies were used to study the crystal structure and particle morphology. Nearly spherical crystals with negligible shape anisotropy were obtained [Fig. 1(a)]. MgO forms a continuous and epitaxial shell over the Fe islands, providing exceptional advantages such as environment stability, controlled interparticle interactions, enhanced magnetic moments and non-toxic hydroxyl surface groups that allows surface attachment of drugs or biomolecules. Hysteresis loops show a much stronger magnetic response (220 emu/g_{Fe}) than any composite material produced up to now involving magnetic nanoparticles encapsulated in inorganic matrices [Fig. 1(b)]. Magnetic measurements, Mössbauer spectroscopy and TEM analysis were repeated on some of the samples in order to examine any aging effects, but no diminish of the properties was detected over several months' storage under ambience conditions.

In this report we discuss the use of these core-shell formations for biological applications. We have analyzed the biosafety level of the nanocrystals in cell cultures as the first step. Preliminary results in 3T3 fibroblast cells showed no cell death after 1 or 3-day incubation with nanocrystal solutions up to 100 µM total-metal molar concentration, whereas a slight reduction of cell proliferation is under study. Exhaustive magnetic and cell culture characterization will be presented.



Tridentate ligands offer new ways for reliable functionalization of metallic nanoparticles

Daniel Meißner[1*], Klaus Wojczykowsky[1], Sarah Sahm[1], Inga Ennen[2], Britta Vogel[2], Peter Jutzi[1], Andreas Hütten[2]

[1] Department of Chemistry - inorganic chemistry III, Bielefeld University; [2] Department of Physics - thin films and nanostructures, Bielefeld University, Universitätsstraße 25, Bielefeld, Germany;
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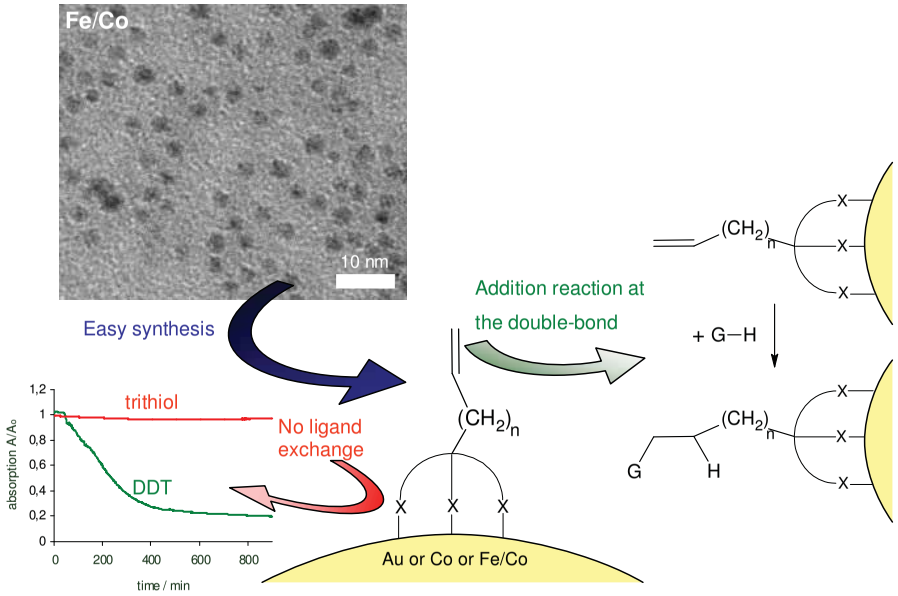
The bond between the surfactant and the nanoparticles' surface atoms is a weak point of the functionalization of nanoparticles. This bond has to be strong in terms of the thermodynamic and kinetic stability.

We have synthesized new ligands suitable for many metallic and oxidic magnetic nanoparticles, which bind strongly to the particles' surface and can hardly be displaced by competing molecules. In addition, the ligands offer reactive positions at their ends, which allow a further functionalization.

The strongly decreased rate of ligand exchange reactions is shown by NMR- and UV-Vis Spectroscopy. Furthermore some addition-reactions to the ligands are shown, leading to carboxy- or hydroxy-terminated nanoparticles, for example.



X = -SH, COOH; n = 7 - 20; G = COOH, OH…

Tunable shapes of FePt magnetic nanoparticles

D. Ung[†], L.D. Tung[§], Le T. Lu [†], Ian. Robinson [†], G. Caruntu∞, D. Delapostas[¥], Y. Alexandrous[¥], D.G. Fernig[±], I. Prior[‡], N.T.K. Thanh[†±]*

[†]Department of Chemistry, [§]Department of Physics, [¥]Department of Electrical Engineering and Electronics, [±]School of Biological Sciences, [‡]Physiological Laboratory, Liverpool Institute of Nanoscale Science Engineering and Technology (LINSET), University of Liverpool, Crown Street, L69 7ZB.

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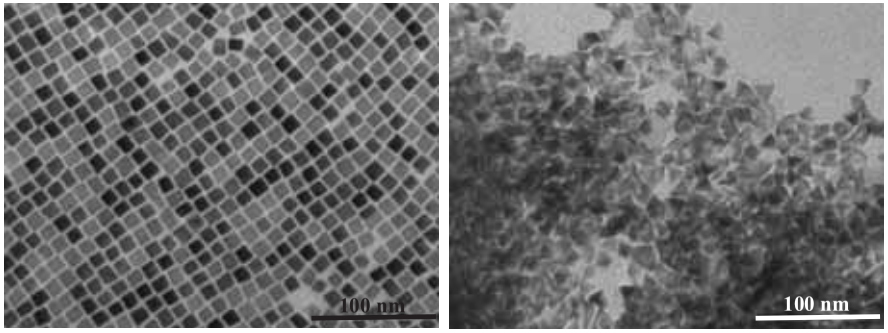
We report the synthesis of nanoparticles in the FePt system of varying shapes, including spheres, cubes, octopod cubes, stars, rods and bilobars. Here, the kinetics of the particle growth process in different directions induce the formation of nanocrystals having different shapes, which are influenced by the synthetic conditions such as the nature and concentration of the surfactants and precursors, as well as the reaction time, temperature and atmosphere. These parameters affect the (supersaturated) concentration of Fe and Pt metallic atoms in the synthetic solution, upon which the growth of the nanoparticles is primarily dependent.

Characterisations have been performed to examine the structure by HRTEM, XRD and composition of the anisotropic shapes was determined by AAS. The magnetic properties were also investigated using SQUID magnetometer and heating capacity of these materials was also measured.



Ferrofluids

S. Odenbach

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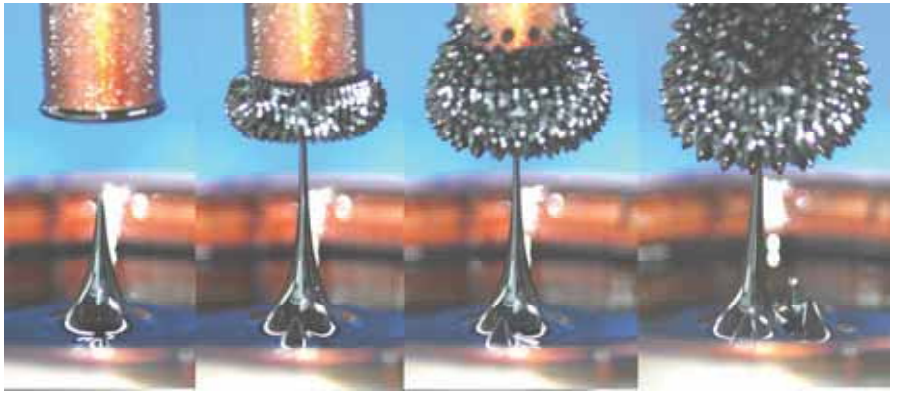
Suspensions of magnetic nanoparticles provide the possibility to use magnetic fields to change their thermophysical properties as well as to influence and control their flow. The strong magnetic response of these fluids results from the fact, that the particles are small enough to represent only a single magnetic domain resulting in an enormous magnetic moment interacting with an applied magnetic field. This interaction leads even for small magnetic field strength to a force comparable with the gravitational force density acting on the fluid being thus able to provide a dominant magnetic control of the fluid's behaviour as it is shown in the figure at the bottom of this page.

For technical applications such suspensions have been produced in a way that they are stable for more than a decade leading to the possibility to employ them in standard devices like loudspeakers or hard disk drives. In contrast ferrofluids prepared for biomedical use suffer often from stability problems limiting their range of application. Despite the fact that the fluids synthesized for technical applications will never be suitable for biomedical use, basic research on these fluids can provide valuable data about their behaviour in magnetic fields which can be used for the preparation of clinical applications too.

Beside a basic introduction of ferrofluids and their technical applications the talk will concentrate on interaction phenomena between the particles and the resulting changes of their thermophysical properties – especially their viscosity – in magnetic fields. The results obtained shed a light on problems that may appear if ferrofluids are used for treatments like magnetic drug targeting.



A ferrofluid being attracted by a small electromagnet, flowing against gravitational acceleration and forming a characteristic spike pattern

Molecule-based Magnets: What, why, and how?

Robin G. Hicks

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The world of magnetic materials has conventionally been dominated by inorganic-based species – metals, alloys, or simply binary or ternary phases. However, there has been also been interest in expanding the repertoire of magnetically responsive materials, particularly by designing and creating magnets made from molecules instead of "just" metal atoms. Thus, a major goal of contemporary research in magnetism is to discover new kinds of *molecule-based magnets* with properties that rival conventional magnets, or offer new kinds of properties.

For over two decades, chemists have pursued new kinds of magnets constructed in whole or in part from molecules. It has been claimed that these molecule-based magnets could have many features (lightweight, flexible, processible, tunable magnetic or other properties, etc.) that would fundamentally distinguish them from conventional atom-based magnets. However, the challenges of making molecular materials magnetic have proven to be complex and formidable.

In this presentation I will offer a critical overview of chemical strategies for making magnets, and will present results from my research group based on our goals of overcoming these challenges.

Study of magnetite phosphatation in view of improving grafting of therapeutic molecules and higher suspension stability

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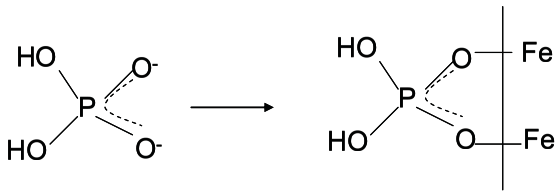
Functionalization of magnetic oxide nanoparticles by organic molecules gives rise to intensive research in particular for biomedical applications such as magnetic drug targeting, cancer treatment, enzyme immobilisation or magnetic resonance imaging. For *in vivo* applications, the functionalization step appears to be of fundamental importance to ensure suspension stability and to bring bioactive functions. A covalent bonding between the molecule and the oxide is therefore a good answer. So, we have chosen to develop molecular grafting through a phosphonate coupling agent.

To investigate the grafting and its effect on the magnetite surface structure and on the magnetic properties, we have first studied phosphatation of magnetite in orthophosphoric acid[1]. Phosphatation occurs rapidly, by interaction with both positively charged groups and hydroxyl. It is reversible and inhibits the dissolution of magnetite in acidic media. Scanning electron microscopy and high resolution transmission microscopy coupled to an EDX analysis have shown that both the morphology and the crystallographic structure of nanoparticles have not been modified and that the phosphate is distributed on the surface. The temperature of magnetite to maghemite transformation is shifted towards higher temperatures. The magnetite properties are preserved, in particular the Verwey transition at 120K. Both FTIR and XPS have shown that the phosphate is linked to octahedral Fe^{3+} of the most dense (111) plane through a binuclear complex. ^{57}Fe Mössbauer spectrometry has clearly shown that the nanoparticles have a maghemite-magnetite core-shell structure. The maghemite shell thickness has slightly increased after grafting phosphate entity. Furthermore, a quadrupolar doublet characteristic of paramagnetic Fe^{3+} in octahedral sites appears, in agreement with the surface iron-complex described below.



Scheme of phosphate and magnetite surface interaction.

[1] Phosphate adsorption properties of magnetite based-nanoparticles T.J. Daou et al. Chem Mater. 2007, 19, 4494

DISPERSION PROPERTIES OF MAGNETITE-POLYMER COMPLEXES IN PHYSIOLOGICAL MEDIA

J. S. Riffle, J. D. Goff, P. P. Huffstetler, W. C. Miles, C. Reinholz and R. M. Davis, Macromolecules and Interfaces Institute, Virginia Tech, Blacksburg, VA

Magnetic nanoparticles that display high saturation magnetization and high magnetic susceptibility are desirable for many applications in biotechnology. Examples under current investigation include cell and other molecular separations, contrast agents for MRI, field-induced tumor hyperthermia, target nanoparticles for magnetic biochip sensors, and in our case, hydrophobic magnetic fluids for treating retinal detachment. For all of these applications, it is desirable to understand how to control dispersion and aggregation of magnetic nanoparticles by applying magnetic fields. This lecture will describe our current findings regarding the synthesis, dispersion and actuation of magnetic nanoparticles in water and phosphate buffered saline (to simulate physiological media). Superparamagnetic nanoparticles ranging from 3-10 nm in diameter can be readily dispersed in hydrophilic media by coating the nanoparticles with an appropriate macromolecular steric dispersant. Our approach to learning how to control magnetic nanoparticle dispersions is to combine experimental measurements with colloidal theories.

Aqueous dispersions. Hydrophilic poly(ethylene oxide) and amphiphilic poly(ethylene oxide-*b*-propylene oxide) have been prepared with one to three functional groups on one end. The types of functional groups have been varied to examine amines, carboxylic acids, phosphates and phosphonates. The isoelectric point of magnetite is pH 6.8, so at pH 7.4 both negatively and positively charged groups are present. At pH 7, it is reasoned that negative carboxylate, phosphate or phosphonate groups adsorb onto cationic surface sites. By contrast, positive ammonium ions on the polymer endgroups can adsorb onto negative sites on the nanoparticles. The copolymers have been coated onto magnetite nanoparticles via adsorption of the functional endgroups and the relative stabilities of the adsorbed polymers on the magnetite have been investigated in water and in phosphate buffered saline. At pH 7 in water, all of the polymers and copolymers remain on the magnetite, but the situation is dramatically different in the presence of added phosphate salts (i.e., in phosphate buffered saline). In phosphate buffered saline, polymers that are adsorbed through carboxylate ions are displaced quickly and the resultant complexes tend to sediment. Polymers adsorbed through terminal ammonium ions are displaced more slowly and complexes containing phosphates remain stably on the magnetite.

Colloid stability is being modeled using the extended DLVO theory modified to account for steric stabilization forces due to terminally attached polymer chains, and for magnetic dipole forces. The total interparticle potential energy V_t between pairs of particles is given by $V_t = V_a + V_e + V_s + V_m$, where V_a is the attractive van der Waals energy, V_e is the electrostatic energy (typically repulsive), V_s is the repulsive steric energy, and V_m is the attractive magnetic energy. The solution sizes of the complexes have been predicted and compared to sizes measured by dynamic light scattering, with good agreement. Methods for predicting the formation of small controlled clusters are currently under investigation.

Fixed bed micro-reactor for surface derivatization of Superparamagnetic Iron Oxide nanoparticles

Jatuporn Salaklang, Benedikt Steitz, Heinrich Hofmann, Alke Petri-Fink

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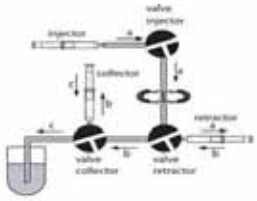
The surface of nanoparticles plays a major role in cellular interaction/uptake, biodistribution, clearance and cytotoxicity. Surface functionalization of nanoparticles still remains a difficult task and represents not only a chemical challenge but constitutes a basic requirement for future scientific investigations. Alteration of surface charges and/or stabilization by the addition of bi- / multi-functional molecules, such as differently charged proteins or plasmids, frequently leads to particle flocculation and rapid sedimentation. The biological functionality, in such cases, is achieved by covalent binding of bio-active molecules on a preexisting single surface coating.

The yield and quality of the resulting coated and derivatized super paramagnetic iron oxide nanoparticles (SPIONs) can be significantly improved and reduction in reaction times can be obtained by using solid phase synthesis strategy. We have developed a fixed bed micro-reactor with a quadrupole repulsive arrangement of permanent magnets to allow for magnetic immobilization of the particles in order to perform the derivatization step(s) on the immobilized magnetic particles. In this way, pH changes across the isoelectric point, washing steps or even solvent exchanges are easily tolerated and the problems of colloidal instability during the derivatization steps can be overcome for SPION surface derivatization.

Several surface derivatizations were carried out exemplarily and compared to conventional liquid phase coupling chemistries. It could be shown that the surface functionalization of SPIONs using a magnetic fixed bed reactor was superior to the liquid phase reaction in terms of reaction yield, particle size distribution, colloidal stability and scalability.

In particular we show the synthesis of organelle targeting peptide derivatized SPIONs. The combination of functionalized SPIONs and their ability to be recovered using a magnetic column coupled with biomolecular mass spectrometry has allowed us to explore a complex intracellular pathway using a peptide that is known to target mitochondria. Here we demonstrated the concept of biomolecular interaction network elucidation with an organelle-targeting peptide, but the concept would also be applicable with more specific biomolecular, rather than organelle, targeting. Other applications of this technology include organelle-specific drug delivery, study of complex cellular signaling pathways and metabolism, and imaging specific pathologies such as malignant neoplasia.



Schematic representation of magnetic micro-reactor

Are carbon coated metal nanoparticles a high magnetization alternative to oxide based beads?

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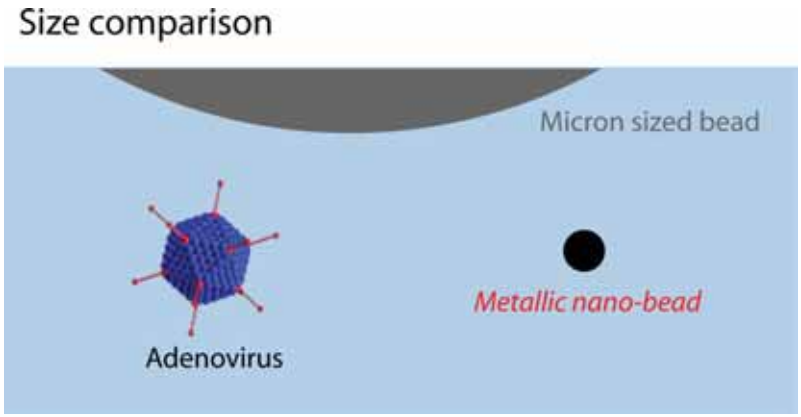
Ferrite particles and polymers loaded with iron oxides have been used in chemical and biological separation tasks for more than 50 years. Although metals (iron, nickel, cobalt & alloys) usually show highly enhanced magnetic properties, their use as magnetic micro- or even nano- particle has been hampered by their lack of oxidation-stability under most operation conditions. This limitation has however been circumvented by coating metallic nanoparticles with carbon coatings only 1-2 nm thick. [1]

We present advantages, possibilities and challenges of carbon coated metallic nanoparticles, which have recently been commercialized by TurboBeads Llc [2]. The high functional loading of these structures in combination with very responsive magnetic properties proposes applications in magnetic polymers, water purification, immunoprecipitation and chemical catalysis. These applications will be discussed in terms of chemical reactivity, dispersion stability and time of operation. We will use this discussion to highlight the opportunities as well as possible pitfalls of this novel technology in comparison with ferrite nanoparticles.



References:

[1] R. N. Grass, E. K. Athanassiou, W. J. Stark, *Angew. Chem. Int. Ed.* **2007**, 46, 4909.

[2] R. N. Grass, W. J. Stark, PCT Patent Application, Nov 2006.

Preparation of spherical and highly monodisperse ferrite nanoparticles with diameters between 50–150 nm for biomedical applications

Toshiyuki Tanaka [1,2,*], Ryuichi Shimazu [1], Hironori Nagai [1], Masaru Tada [1], Takashi Nakagawa [1], Adarsh Sandhu [3,4], Hiroshi Handa [4,5], Masanori Abe [1,4]

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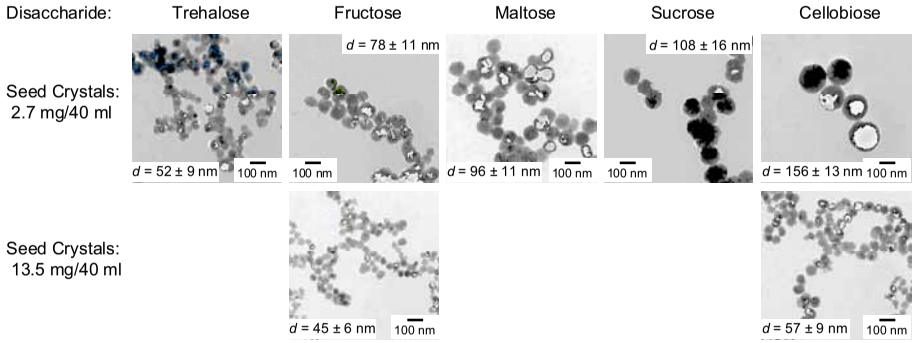
Spherical and monodisperse ferrite nanoparticles play a critical role as magnetic carriers in bio-screening, drug delivery systems, magnetic hyperthermia and related biomedical applications. The spherical shape with non-dominant crystal planes enables the high dispersion in water and the uniform immobilization of bioactive molecules on the surfaces. The monodisperse properties endow the particles with uniform hydrodynamic and magnetic properties, advantageous for magnetic separation of the particles from the suspension. To the best of our knowledge, however, spherical and monodisperse iron ferrite nanoparticles prepared by chemical synthesis have been limited to smaller than 30 nm or larger than 200 nm in size.

We will report that spherical and highly monodisperse ferrite particles with crystalline structures intermediate between Fe_3O_4 and γ-Fe_2O_3 and diameters of 50–150 nm were synthesized by adding one out of five specific disaccharides and seed ferrite crystals (3–8 nm in size) into an aqueous reaction solution [1]. The spherical shapes and monodisperse properties of the particles were as a result of adding the disaccharide and seed crystals, respectively. The average size of the particles was controlled by choosing an appropriate disaccharide and by changing the quantity of the seed crystals as shown in the figure. The particles had a saturation magnetization of 75 emu/g, which is similar to that of bulk samples of Fe_3O_4–γ-Fe_2O_3. When coated with citrate, the particles, even those with diameters larger than 100 nm, formed a suspension in water that was stable for over two days. The physical properties of these novel particles will find applications as magnetic carriers in biomedical applications.

[1] M. Abe, H. Handa, T. Nakagawa, M. Tada, R. Shimazu, and T. Tanaka; Patent No. PCT/JP2007/075246 (2007).



TEM images for ferrite nanoparticles synthesized with addition of a disaccharide and seed crystals. Particle diameter reduced as seed crystal concentration increased.

Novel magnetic labels for bioassays

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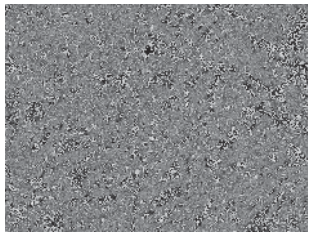
Magnetic beads (MBs) are commonly used for contrast enhancement in magnetic resonance imaging, as magnetic carriers in automated immunoassays instruments, or for cell concentration at the bench. More recently have MBs arisen as candidates for new labels in magnetic immunoassays in lieu of commonly used enzymatic, fluorescent, or radio-isotopic labels. A number of detection methods candidate for biosensing applications, including multi-frequency non-linear measurement, giant magnetoresistive (GMR) sensors and spin valves, inductive sensors, superconducting quantum interference devices (SQUIDs). But so far, scientific efforts have been focused on the magnetic detection itself and have totally left aside the optimization of intrinsic MBs magnetic features. Only readily available MBs, based on iron ferrites, have been used.

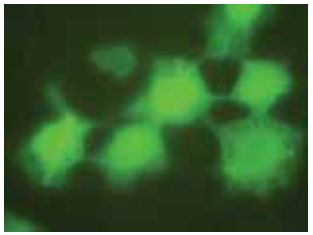
Hence we identified promising superparamagnetic particles in order to dramatically improve sensitivity and to allow for multiparametric testing. More specifically, magnetic nanoparticles as γ-Fe_2O_3; α-Fe_2O_3 or $CoFe_2O_4$, differing by their size, shape or composition have been synthetized. The nanoparticle will have two essential roles: to act as a probe owing to its specific magnetic properties and to carry on its surface precursor groups for the covalent coupling of biological recognition molecules, such as antibodies, nucleic acids. We want to develop several families of such magnetic labels to use them for multiparametric testing, based on their differing magnetic properties.

The magnetic evaluation of these nanocrystals exhibit different magnetic signatures depending on their shape, composition or size. The magnetic behaviour of these systems is also modified with dipolar interactions between particles depending on their coating. Hence, the different magnetic signature of these nanoparticles could be used as labels for bioassays.

The nanocrystal passivation is performed using bifunctionnal molecules that are hydrophilic on one end and bind to nanocrystal surface with the other end via a bisphosphonate moiety. The hydrophilic function will be used to link biological macromolecules. The faisability of such bioconjugation has been made using amino fluorescein.



γ-Fe_2O_3 nanowires



Magnetic-fluorescent nanoparticles incorporated within breast cancer cells

Synthesis and Stabilization of Iron Oxide-Core, Gold-Shell Nanoparticles and Nanorods for Biosensing

JitKang Lim[a], Xi Tan[b], Robert D. Tilton[a,c], Frederick Lanni[d], Sara A. Majetich[b,1]

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d. Biological Sciences, Carnegie Mellon University

Abstract

We describe the development of iron oxide-core, gold-shell nanoparticle probes for eventual use as magnetically guided biosensor particles. The particles combine magnetic control of motion and surface plasmon resonance for single particle optical imaging. Monodisperse, eighteen nanometer diameter magnetic spheres are first synthesized in organic solvents, then transferred into water and coated with gold. The particles are then coated with biocompatible polymers and the dispersion stability in phosphate buffered saline solution is monitored by dynamic light scattering (DLS).

The completion of the gold shell produces an intensification and pronounced shift of the surface plasmon resonance peak, in quantitative agreement with Mie scattering theory. DLS analysis shows that at least 85% of these particles are singlet core-shell, with the remainder mostly doublets or triplets. Magnetometry measurements indicate that these particles are superparamagnetic at room temperature. The specific magnetization of these particles at 10 K is approximately 54 emu/g of Fe_3O_4. Suspensions of magnetic particles are readily and reversibly collected by a permanent magnet. Darkfield optical microscopy, with supporting random walk statistical analysis, demonstrates the feasibility of detecting single nanoparticles undergoing Brownian motion.

We also discuss the synthesis and gold coating of iron oxide nanorods, and differences that arise due to shape anisotropy. The asymmetry of the rods leads to anisotropy in the rotational and translational diffusions, which is measured using angle-dependent DLS. Au nanorods have both transverse and longitudinal modes in the surface plasmon spectra. The implications of rod hydrodynamics for biosensing (with a gold coating) and for hyperthermia (without coating) are discussed.

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Preparation of mono-dispersed magnetic polymer nanobeads via the modified miniemulsion polymerization and application in quantitative lateral flow immunoassay

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Immunoassay based on magnetic beads as carriers or signal marker plays an important role in clinical examination field now. In this paper, firstly the carboxylated magnetic polymer nano-scale beads with high and uniform magnetite content and the mono-dispersed carboxylated magnetic polymer nano-scale beads with core-shell structure were synthesized via modified miniemulsion polymerzation by of acrylic acid, styrene and divinyl benzene in the presence of oleic acid stabilized Fe3O4 nanoparticles[1,2]. The effects of various polymerization parameters, such as the different surface modifications of Fe3O4 nanoparticles, magnetite weight ratio to styrene, and acrylic acid weight ratio to styrene, on the properties of the composite nanospheres were analyzed in detail. Extensive characterization by transmission electron microscopy (TEM) shown as Figure 1, thermogravimetric analysis (TGA), fourier transform infrared spectrometer(FTIR) and physical property meaturement system (PPMS) showed that the superparamagnetic polymer nanospheres, with carboxyl groups on the surface whose density could be adjusted from 0.2mmol/g to 0.5mmol/g according to application demand. The magnetite nanoparticles content of obtained magnetic nanobeads could be adjusted from 50% to 80% by weight, and the diameter could be controlled from 60nm to 200nm. The size polydispersed index detected by high-performance particle sizer (HPPS) is less than 0.100. And then, the obtained magnetic nanobeads were used as signal maker substitute for colloidal gold to perform quantitative lateral flow immunoassay based on sandwich principle[3]. hCG as model protein was quantitative tested by MAR measurement. The sensitivity of detection is less than 5mMIU/ml shown as figure 2 and CV of T line and C line are lower than 10%, the detection time is only 12min due to the nano scale beads were used as signal label, which faster than conventional ELISA method. The results further indicate that as-synthesized material is a promising candidate for application in immunoassay, especial for point-of-care field.

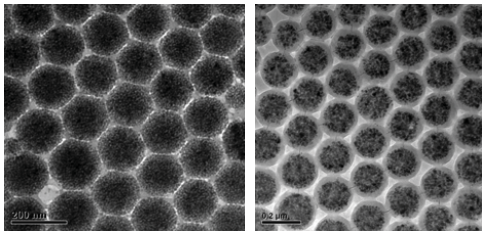

Figure 1 TEM of obtained magnetic polymer nanobeads (left is high magnetization beads, right is beads with core-shell strcture)

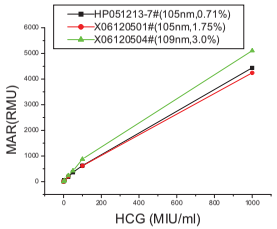

Figure 2 The linear relationship between hCC concentration and MAR value

Acknowledgements: This work was supported by Hi-tech Research and Development Program of China (Project 2006AA03Z3591)and shanghai nano project (0652nm012)

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[2]. Hong Xu, Longlan Cu, et al., J. AM. CHEM. SOC. 2006, 128, 15582-15583,CN
[3]. Gerd Rundstrom, Ann Jonsson, Ola Martensson, et al. Clin Chem.2007,53:342-348

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† These authors contributed equally to this work.

Novel Bio-Inspired Route to Complex Ferrite Nanomaterials

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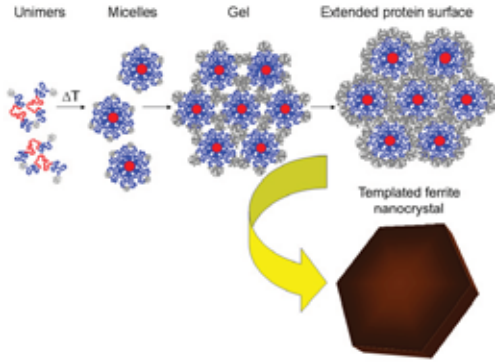
There is increasing interest in using biomolecules to develop the next generation of advanced materials. Biomimetic synthetic routes offer the opportunity of controlling size, shape, crystal structure, orientation, and organization of nanoscale matter. Magnetic nanoparticles obtained via biomimetic routes find applications in numerous fields.

Magnetotactic bacteria produce ordered chains of uniform magnetite and greigite crystals with various morphologies. Using a new bio-inspired route involving the novel acidic protein Mms6 found in magnetotactic bacteria, we were able to replicate shape-specific magnetic nanocrystals closely resembling bacterial magnetite. This bio-inspired approach was extended to synthesize a variety of nanostructured magnetic materials, many of which are not known to occur in Nature in living organisms. We successfully synthesized cobalt ferrite, $CoFe_2O_4$, which does not occur in bacteria. We have covalently attached full-length mms6 and associated C-terminal of Mms6 to self-assembling polymers that form thermoreversible gels in order to prevent nanoparticle aggregation and to enable templated hierarchical architectures resembling those observed in Nature.

This new methodology enables facile room temperature synthesis of complex magnetic nanomaterials with uniform and well-defined shapes, sizes and crystal structures, with nanocrystals in the range of 40-100 nm that are difficult to produce using conventional techniques. These nanocomposites exhibit unique magnetic and other properties not realizable via conventional synthesis methods. Overall, this bio-inspired strategy facilitates a bottom-up approach to design and synthesis of tailored nanostructured materials.



Schematic representation of hexagonal nanocrystals templated by the Pluronic®-conjugated protein on hexagonally packed micelles.

Talk Abstracts - Friday, May 23, 2008

	Session 4: Hyperthermia - *Chairs: Stefan Odenbach (Germany)*			
8:30	Goya, Gerardo	Cell Internalization of Magnetite Nanoparticles by Dendritic Cells for Intracellular Magnetic Hyperthermia	Spain	Talk 39
8:45	Haik, Yousef	Multifunctional Nanoparticles for Imaging-Guided Interventions	U.S.A.	Talk 40
9:00	Kallumadil, Mathew	Suitability of Commercial Magnetic Colloids for Magnetic Hyperthermia	U.K.	Talk 41
9:15	Tedesco, Antonio Claudio	Evaluation of Magnetohyperthermia and Photodynamic therapy in Erlich Ascitic Tumor bearing animals	Brazil	Talk 42
9:30	Innocenti, Claudia	Influence of structural and magnetic properties of cobalt ferrite and magnetite nanoparticles on hyperthermal effectiveness	Italy	Talk 43
9:45	Dennis, Cindi	The Influence of Collective Behavior on the Magnetic and Heating Properties of Iron Oxide Nanoparticles	U.S.A.	Talk 44
10:00	**Coffee break / poster session / exhibitors**			
10:40	**Session 5: Analytical Methods** - *Chairs: Sylvain Martel (Canada)*			
10:45	Kriz, Dario	Magnetic Permeability Based Diagnostic Test for Determination of Canine C-Reactive Protein Concentration in Whole Blood	Sweden	Talk 45
11:00	Lee, Gil	Non-linear Magnetophoretic Separation Using Micro-Magnet Arrays	U.S.A.	Talk 46
11:15	Mistlberger, Günter	Magnetic optical sensor particles: A versatile tool for life science	Austria	Talk 47
11:30	Ludwig, Frank	Characterization of nanoparticles by magnetorelaxometry, ac susceptibility, TEM and photon correlation spectroscopy – a comparative study	Germany	Talk 48
11:45	St. Pierre, Tim	Apparent dependence of the interfacial energy of suspended ferrofluid droplets on magnetic field strength	Australia	Talk 49
12:00	**Martel, Sylvain**	**Automatic transport of magnetic particles in the blood vessels using a clinical MRI system**	**Canada**	**Invited talk 5**
12:40	**Lunch**			
13:55	**POSTER PRIZE** - *Cordula Gruettner*			
14:00	**Session 6: Imaging** - *Chair: Tim St. Pierre (Australia)*			
14:05	Insin, Numpon	Silica Microspheres and Silica Nanoparticles Containing Magnetic Nanoparticles and Semiconductor Quantum Dots	U.S.A.	Talk 50
14:20	Horák, Daniel	The Effect of Magnetic Nanoparticle Coating on the Efficiency of Stem Cell Labeling	Czech Republic	Talk 51
14:35	Wiekhorst, Frank	A stand-alone multi-channel magnetorelaxometry device for quantitative and spatial resolved MNP detection	Germany	Talk 52
14:50	Markov, Denis	Magnetic Particle Imaging: quantitative assessment of tracer performance	The Netherlands	Talk 53
15:05	Zenke, Martin	Magnetic Nanoparticles for Tracking of Stem Cells and Dendritic Cells	Germany	Talk 54
15:20	Roca, Alejandro G.	Understanding the relaxometric properties of high quality MR contrast agents based on magnetite nanoparticles	Spain	Talk 55
15:35	Surguladze, Besiki	Ultrasound Imaging of Sentinel Lymph Nodes in The Patients With Breast Cancer by Using The Preparation “Unimag”	Georgia	Talk 56
15:50	**Coffee break / poster session / exhibitors**			
16:30	**Session 7: Magnetic Separation** - *Chairs: Maciej Zborowski and Jeff Chalmers (U.S.A.)*			
16:35	Earhart, Chris	Microfabricated Sifter for High-throughput and High-gradient Magnetic Separation	U.S.A.	Talk 57
16:50	Faraudo, Jordi	Experimental and theoretical study of a novel and fast magnetophoretic separation technique using low magnetic gradients	Spain	Talk 58
17:05	Wilson, Robert	Cell Separations Using Magnetic Chains and Grids	U.S.A.	Talk 59
17:20	Prina-Mello, Adriele	High content screening of nanomaterial toxicological response to proliferating MC3T3-E1 osteoblast cells: a time course study	Ireland	Talk 60
17:35	Abe, Masanori	High-throughput bioscreening utilizing high-performance affinity magnetic carriers exhibiting minimal non-specific protein binding	Japan	Talk 61
17:50	Sinha, Ashok	Characterizing magnetic separation systems for µ-TAS	U.S.A.	Talk 62
18:05	Pamme, Nicole	Magnetic microparticles as manoeuvrable solid supports for multi-step (bio)reactions in continuous flow.	U.K.	Talk 63
18:20	Carpino, Francesca	Characterization of magnetic nanoparticles using quadrupole magnetic field-flow fractionation	U.S.A.	Talk 64
18:35	**End of session / Vancouver city tour on yellow school buses**			

Today’s Social Program

On this evening, we will take a tour of the city and Stanley Park in yellow school buses. We will end up on the beautiful English Bay beach, on the South bottom of Denman Street, a great area for food and night life. And not too far for a stroll on Davie, Denman and Robson to other parts of downtown.

Cell Internalization of Magnetite Nanoparticles by Dendritic Cells for Intracellular Magnetic Hyperthermia.

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Magnetic nanoparticles (MNPs) are being increasingly used for targeting eukaryotic cells as a way for labeling,[1], assessment of cytotoxicity [2] or DNA transfection protocols. [3] Targeting specific tumor sites with drug-loaded MNPs is also a promising strategy to fight proliferation of metastatic cells, but it is now recognized that the reticulo-endothelial system (RES) is a major obstacle since it detects and phagocytes NPs, preventing their therapeutic function. Dendritic cells (DCs) obtained from myelomonocytic progenitors, and primed with tumor antigens have shown antitumoral activity when inoculated in animal models [4] and humans [5].The injection of MNP-charged DCs into the blood system appears as a valuable MNP-delivery strategy for tumor targeting, since the cargo could be delivered inside a 'biological' unit from the same organism and therefore no RES action against these carriers is expected. The MNPs vectorized in this way could be used as heating agents for magnetic hyperthermia (MHT) therapy, and efficiently kill tumors to which a sufficient numbers of MNPs have been delivered. We present experiments on the internalization of magnetite-based MNPs into dendritic cells in order to assess a) the final location of the particles; b) the viability of the cultured cells from their mononuclear cells progenitors, and c) the effectiveness of alternating magnetic fields as source of intracellular heat for MHT *in vitro* protocols. We found that magnetite nanoparticles of 20-40 nm were efficiently incorporated by DCs, showing minimum effects on the DCs viability, i.e., the fraction of viable cells on days 0,1,2,3 and 4 after NPs incorporation was not significantly affected by the internalization of Fe_3O_4 NPs. After separating the MNPs/loaded cells by centrifugation in density gradient, the resulting material was analyzed by electron microscopy and magnetic measurements. It was found that NPs are internalized in lysosomes, providing a large magnetic signal. A simple method using room temperature magnetometry was applied to quantify the incorporation of MNPs into DCs. Using Fe_3O_4 nanoparticles of 35 nm, we found that mature DCs are able to incorporate magnetic material in the range of 1-10 pg/cell after 24 h of incubation, depending on particle surface. Applying 30 minutes of AC magnetic field (260 kHz, 16 mT) on magnetite-charged DCs cells resulted in 40 % of cell death, as reflected by flow cytometry (anexine-propidium iodide staining) analysis. Our results suggest that loading DCs with proper number of MNPs could be a promising strategy for improved vectorization and hyperthermia therapy in cancer treatment.

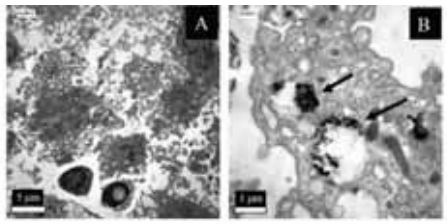

Figure 1 *Electron microphotographs of DCs without (A) and with (B) MNPs. Intra-lysosomal NPs aggregates inside lysosomes are observed (black arrows). Without NPs, the content of lysosomes is seen as homogeneously white.*

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Multifunctional Nanoparticles for Imaging-Guided Interventions

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Cancer remains the second leading cause of mortality following cardiovascular diseases in the world. These statistics point to the fact that there is a need to develop new approaches for early cancer detection and cancer therapy. The ideal therapeutic approach will target multiple facets of the cancerous process while respecting the normal cells; while a cocktail of drugs will achieve this goal it must be administered in a targeted and localized fashion to ensure optimal drug levels in the tumor while preventing an increase in its side effects. However, multiplexing the diagnosis, targeted delivery, and monitoring remains a major technical challenge for cancer research. We have developed an effective and minimally invasive intervention protocol that is applied at the tumor site as part of tumor management. We have designed *multifunctional magnetic nanoparticles (MNPs)* that is encapsulated in *thermosensitive* drug-bearing shells and delivered to the tumor site by genetically modified and *non-pathogenic strains of bacteria* with known affinity for tumors. The physical and chemical properties of the MNPs are specifically dictated by the synthesis process so that the MNPs serve as a *non-invasive imaging agent (Figure 1)*, *heating agent (Figure 2)*, as well as a *thermometry monitoring agent (Figure 1)*.

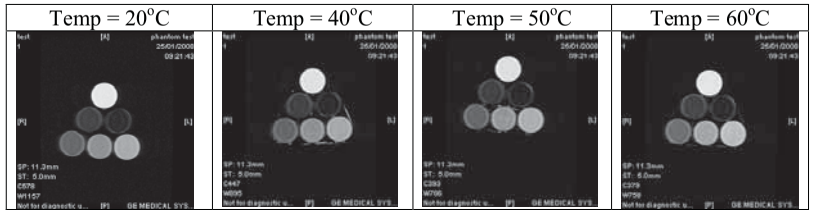

Figure 1: Contrastability and Thermometry of the particles. Six concentrations (0-0.3 mmol/kg) of nanoparticles were prepared in viscous cellulose solutions to maintain them in suspension. The concentrations were chosen to match current practice of contrast agent usage in human. The solutions were heated up to temperatures blow and above the particles Curie temperature (42.8°C). The suspensions were imaged in 1.5 tesla human MRI machine.

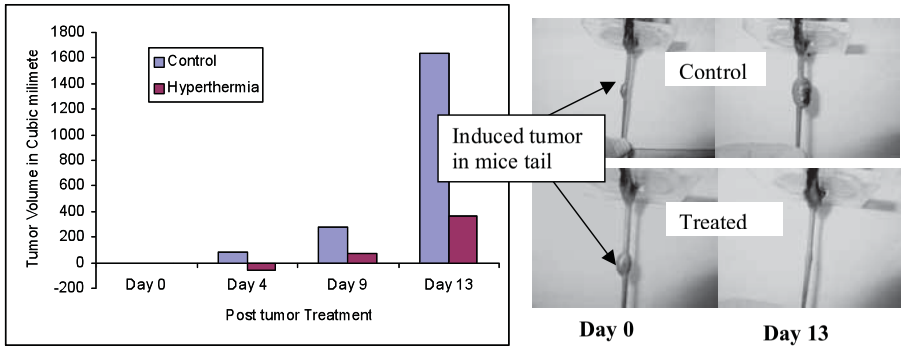

Figure 2. Tumor volume for control and magnetic hyperthermia treated mice. The figure on the left demonstrates the effectiveness of the treatment. The control shows continued growth while the treatment shows a substantial reduction in tumor size.

Suitability of Commercial Magnetic Colloids for Magnetic Hyperthermia

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In recent years, magnetic hyperthermia as a novel form of cancer therapy has aroused an increasing interest across the globe. The concept of remotely inducing heat into a carcinogenic site using magnetic nanoparticles (MNPs) has resulted in growing research into the physical, chemical, medical and engineering aspects of this novel technology. The ferrofluidic compound can be injected directly or otherwise (e.g. antibody-antigen bonding) targeted to the tumour site and non-invasively 'excited' to heat using an alternating magnetic field (AMF).

- Our group has now extended earlier comparative studies of a set of commercially available particles (Figure 1) to characterise a wider range of colloids and to expose them to our most powerful hyperthermia system to date. The samples were subjected to an AC field of 160 Oe, at a frequency of more than 1 MHz. The experiments were designed to simulate the temperature environment of the human body by using an airflow-controlled setup. Commercial ferrofluids that were investigated in this study including samples supplied by Chemicell GmbH, Micromod GmbH, Spherotech Inc, Ferrotec Corp, Ferrolabs Inc, Guerbet Group and TurboBeads GmbH.
- MNPs were additionally suspended in agarose and immobilised whereby significant heating of the particles was still observed (Figure 2). This shows the relative contributions of the Neel and Brownian relaxation mechanisms to the total heating of the MNPs.
- Further experiments investigated the relationship between the specific absorption rate (SAR) and the volume of ferrofluid in a sample tube. The results suggest that the relative ferrofluid volume significantly affects the SAR and that standards need to be established for comparing SAR of different ferrofluids in hyperthermia systems in the future.

The different colloids are compared in terms of their potential for therapeutic use in hyperthermia. This research lays groundwork for investigating the parameters that dominate the heating rate and for developing optimised nanoparticles for hyperthermic cancer therapy.

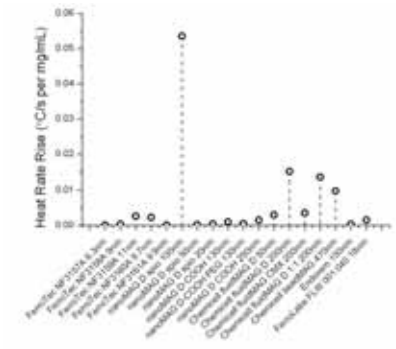

Figure 1 Previous study showing a comparison of heat rise rate

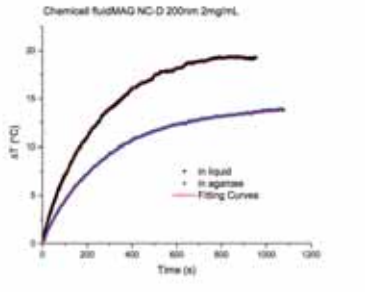

Figure 2 Ferrofluid heating rate in liquid and agarose suspension exposed in the new hyperthermia system

Evaluation of Magnetohyperthermia and Photodynamic Therapy in Erlich Ascitic Tumor bearing Animals

L. S. Barbosa-Braun[1], N. Sadeghiani[1], J. P. Coelho[1], L. L. C. Estevanato[1], D. L. Guedes[1], F. A. Portilho[1], A. Brugin[1], I. C. Silva[1], R. B. Azevedo[1], D. C. Zancanela[2], A. C. Tedesco[2], P. C. Morais[3], Z. G. M. Lacava[1]

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Magnetohyperthermia (MHT) and Photodynamic therapy (TFD) are promising therapies for the cancer treatment by the possibilities of collateral effects reduction. With the aim to better know the biological and therapeutic effects related to the simultaneous use of both therapies in the treatment of tumors, it was developed a new sample: furtive (PEG) liposomes containing the photosensitizer zinc-phthalocyanine (ZnP) and citrate coated cobalt-ferrite magnetic nanoparticles (NPMs). The sample was named ML_{PEG}-ZnP (4.8×10^{14} particle/mL). As control, several ML_{PEG}-ZnP sub-components samples were also synthesized: liposome (L_{PEG}), magnetoliposome (ML_{PEG}), and photosensitized liposome, L_{PEG}-ZnP. The investigation was performed in 6 steps. In the first one was determined the better MHT experimental conditions. In the steps 2 to 5 it was performed an evaluation of the ML_{PEG}-ZnP sample biocompatibility in healthy animals submitted or not to the Ac magnetic field (MHT) and/or to the laser (TFD). As a rule, tests showed not severe and temporary inflammatory activity (blood and peritoneum cytometry) that was increased by the AC magnetic field, small reduction of cellular viability, total absence of genotoxicity (micronucleus test) and inflammatory process only in the lungs (morphology analysis of the liver, lungs and spleen). The sixth step had the aim of verifying the possible tumor remission in Erlich Ascitic Tumor (TAE) bearing animals after injection of ML_{PEG}-ZnP and subsequent AC field and/or laser submission. It was observed (by AgNOR tests, cellular viability, ascite volume and hemorrhagic pattern, cytometry, clear/dark cells, animal weight and survival) that in the used experimental conditions, no treatment caused the total tumor remission. Nevertheless, it was observed a significant anti-proliferative activity, mainly after the ML_{PEG}-ZnP treatment and subsequent laser exposition, evidencing that this system is more adequate to the TFD performance than to the MHT process. The results evidences that the treatment mediated through MHT and TFD is viable and effective, and may confer advantages to the cancer treatment.

Work supported by CAPES, CNPq/MCT, FINATEC, FAP-DF, and CNANO

Key words: Nanobiotechnology, Magnetohyperthermia, Photodynamic therapy, Magnetoliposome, Cobalt ferrite, Zinc-Phthalocyanine, Biocompatibility, Tumor remission, Ehrlich Ascite Tumor.

Influence of structural and magnetic properties of cobalt ferrite and magnetite nanoparticles on hyperthermal effectiveness

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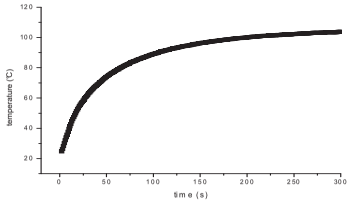
One of the most appealing properties of magnetic nanoparticles is their ability of self heating when irradiated by electromagnetic radiations (magnetic fluid hyperthermia - MFH)[1] oscillating in the frequency range of 100-500 kHz. Magnetic Fluid Hyperthermia by biocompatible nanodevices is one of the most promising technological application forecasted for nanomedicine as the heating effect can act as a therapeutic agent by itself or can induce specific drug local release.

Early results obtained by Jordan[2] show that this approach is feasible and could lead to innovative therapies of cancer. Despite these interesting preliminary results, many aspects are still to be investigated. In order to reduce any toxicity, it is essential to achieve the suitable temperature enhancement with as low as possible amount of magnetic nanoparticles, employing magnetic nanoparticles with the highest Specific Absorption Rate (SAR). Up to now the research attention has been essentially restricted to iron oxide-based materials, mainly because they are easily prepared, biodegradable and biocompatible and can thus be recycled by cells using natural biochemical pathways for iron metabolism. The use of different materials with larger magnetic anisotropy and larger magnetic moment, however, is strongly envisaged since it can allow a significant improvement of the material efficiency, particularly as concerns magnetic hyperthermia and MRI applications. Cobalt ferrite ($CoFe_2O_4$), for instance, is a magnetic material with almost the same saturation magnetisation as magnetite but with a crystalline anisotropy one order of magnitude larger. Even though it has already been proposed as MFH material, its hyperthermic behaviour has not been studied in depth.

In this contribution we present our recent investigation on the synthesis of cobalt ferrite nanoparticles and on the existing correlation between their structure, magnetic and hyperthermic properties, in comparison with those of magnetite nanoparticles synthesized using the same experimental protocol. Recently we have proven that cobalt ferrite can be chemically stabilized using proper ligand molecules[3] and how this stabilization drastically decreases nanoparticles toxicity.

A deep study on magnetic hyperthermic effect of both materials with different sizes and organic coating, dispersed in a solution and in a solid matrix has been carried out together with a complete magnetic (static and dynamic) and physico-chemical characterizations (XRD, TEM, DLS) of the samples[4]. This approach is necessary in order to understand which material could be the best candidate for biomedical applications as hyperthermic agent.

Electromagnetic Field Generator



Temperature vs time course for 7.4 nm $CoFe_2O_4$ particles (3.0% w/w in glycol)

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The Influence of Collective Behavior on the Magnetic and Heating Properties of Iron Oxide Nanoparticles

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Magnetic nanoparticles are being developed for a wide range of biomedical applications. In particular, hyperthermia involves heating the magnetic nanoparticles through exposure to an alternating magnetic field. These materials offer the potential to selectively treat cancer by heating cancer tissue locally and at the cellular level. This promises to be a successful method if there are enough particles in the tumor possessing a sufficiently high specific absorption rate (SAR) to deposit heat quickly while minimizing thermal damage to surrounding tissue. High SAR magnetic nanoparticles have been developed and used in mouse models of cancer. The magnetic nanoparticles are comprised of magnetic iron oxide cores surrounded by a dextran layer (shell) for colloidal stability. The average diameter of a single particle (core plus dextran) is varied between 100 nm and 30 nm, as measured by photon correlation spectroscopy. Small Angle Neutron Scattering (SANS) and Ultra-SANS (USANS) measurements performed at NCNR under several H2O/D2O contrast conditions and at varying concentrations of iron oxide nanoparticles have revealed three length scales in this system. The first corresponds to the diameter of the magnetic core, and details a parallelepiped construction. The second, roughly double the particle diameter, corresponds to a mean interparticle distance between the magnetic cores. The third corresponds to the formation of collective structures, possibly held together by magnetic interactions. The existence of these three length scales has also been confirmed in magnetometry, and is consistent with transmission electron microscopy. The long range collective magnetic behavior appears to play a major role in enhancing the SAR. In mouse models of cancer, these high SAR nanoparticles show promise as an effective cancer treatment.

Magnetic Permeability Based Diagnostic Test for Determination of Canine C-Reactive Protein Concentration in Undiluted Whole Blood

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Like human C-reactive protein (CRP), the resembling canine CRP has been identified as a useful acute phase marker for diagnosis and monitoring of inflammatory disease in dogs. Commercially available Dog CRP assays such as ELISA, turbidimetric assays, slide/capillary reverse passive latex agglutination tests require time consuming steps, dilution of sample, give false positive results and are limited to plasma or serum samples. Automated, rapid and reliable diagnostic tests are needed for routine evaluation of CRP concentration in veterinary clinics.

We describe a one-step magnetic permeability based sandwich-immunoassay utilizing polyclonal anti-CRP antibody conjugated dextran iron oxide nanoparticles as superparamagnetic labels and polyclonal anti-CRP conjugated silica microparticles as carriers. The inductance based LifeAssays® Reader is used to detect the target analyte, CRP in 10 µl whole blood, by magnetic permeability increase of the silica microparticle sediment due to the amount of bound superparamagnetic nanoparticles.

Initial measurements show a measuring range of 0.4 – 30 mg/L CRP for 10 µl canine whole blood sample. The one-step Canine CRP diagnostic test has a measuring time of 11 minutes. Measurements performed on 16 whole blood samples from mixed breeds showed good correlation (y=0.952x+4.022, R^2=0.960) with a commercially available ELISA assay (Figure 1).

The one-step, rapid and accurate magnetic permeability immunoassay provides a new method for dog CRP diagnosis and is suitable for veterinary clinic usage. The LifeAsssays® Reader also has the advantage of measuring a wide range of analytes, among them human CRP, hsCRP and albumin, by simply changing the reagents provided.

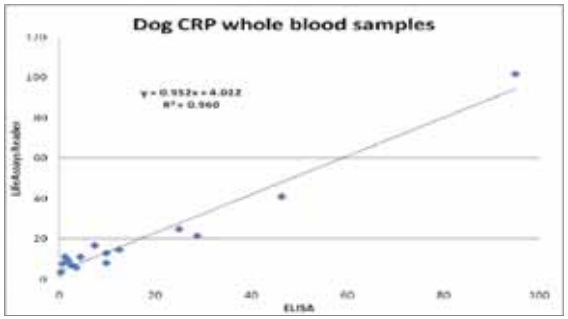

Figure 1. Correlation plot (y=0.952x+4.022, R^2=0.960, n=16) for measurements performed with the LifeAssays® Reader and a commercially available ELISA kit.

Non-linear magnetophoretic separation using micro-magnet arrays

G.U Lee [1,2], B.B. Yellen[3], R. Erb[3], H. Son[3], R. Hewlin[3], and H. Shang[2]

[1] Presenting author
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[1] Department of Mechanical Engineering and Materials Science, Pratt School of Engineering, Duke University, Durham, NC 27708

Our ability to understand and work with living systems is based on separation – bioseparation is the basis for determining the molecular state of a single cell (or whole organism) and defines our capacity to utilize biologically derived molecules. Magnetophoresis utilizes receptor-coated superparamagnetic beads to perform affinity separation of specific biological materials from complex mixtures of cells and cell lysates. Here we describe a significant advance in the field of bioseparations, in which we use dynamic non-linearities in magnetic particle transport to extract biological materials from complex fluids based on only variations in size or magnetic moment. A traveling magnetic field wave is created by applying rotating magnetic field to an array of micro-magnets patterned on a substrate. At low frequencies, magnetic beads are shuttled between adjacent magnets with a speed that is proportional to the frequency of rotation of the field. At higher frequencies, we observed the onset of non-linearities in the bead's transport behavior, leading to the identification of certain critical frequencies above which a specific population of beads no longer moves. This critical frequency was found to be proportional to the bead's magnetic moment and inversely proportional to its hydrodynamic drag factor. By exploiting this frequency dependence, we have demonstrated highly sensitive separation of magnetic beads based on fractional differences in bead diameter or the specific attachment to *B. globigii* or *S. cerevisiae*, and we demonstrate the possibility of tuning the external driving frequency to cause the migration velocities for different bead types to differ by orders of magnitude. In principle, the non-linear effect can be applied to other modes of separation, such as electrophoresis.

Magnetic optical sensor particles: A versatile tool for life science.

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Real-time monitoring of biologically important parameters, such as oxygen, pH or certain metabolites is of great interest to different areas of research as well as to industry. Knowing the exact level of these parameters can, e.g., elucidate bottle necks in metabolic pathways, teach scientists how to "push" an organism in the desired direction, or it can also be useful for improving large scale bioprocesses. In general, optical sensors can be applied either as fiber optical sensors, wall-incorporated sensing spots or dispersed micro- or nanoparticles.

Recently, the use of magnetic optical sensor particles for *in situ* sensor spot formation was published [1–3]. This novel concept together with specially designed magnetic separators [4] allows sensing at ultra low indicator concentrations, because the actual sensor beads are quantitatively separated from the media [5]. The magnetic particles are used as vehicles for bringing the sensor dyes to the desired position in a vessel. For real time monitoring of oxygenation during fermentations, we developed special magnetic separators which collect the sensor particles right in front of an optical fiber. The design with radially magnetized adapters improved every aspect of a magnetic separator: The attachment was stronger, the signals higher and the collection more efficient. In addition, magnetic sensor particles open a whole new field of applications. Such sensor particles can be trapped and released in micro fluidic devices, or used for high throughput screening techniques and real time monitoring of chemical parameters such as oxygen or pH in cultivations ranging from microtiter plates up to large scale fermentations. Currently, we are capable of preparing versatile magnetic sensor particles using different techniques, such as sol-gel bulk and emulsion polymerization, spray drying and precipitation. We are constantly evaluating new methods for the production of even smaller magnetic optical sensor particles for imaging applications in order to investigate biological materials such as biofilms.

At the moment we develop stimuli responsive magnetic optical sensor particles for oxygen and pH responding to different stimuli (e.g. temperature, light and pH). Such particles are suitable for drug delivery to a certain point of a biological material while simultaneously measuring the effects of the delivered drug on the surrounding (e.g. changing pH or oxygen concentration). Metaphorically speaking, our magnetic vehicles should act like a postman who is delivering a package and interviewing the recipient at the same time.

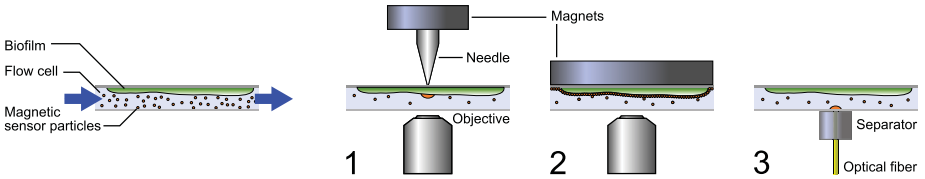

Figure 1: Possible applications of magnetic sensor particles in a flow cell: (1) Focused particle spot on a distinct region of the sample (very low sensor amount required), (2) imaging of parameters close to the surface and (3) monitoring parameters in the medium with an optical fibre.

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Characterization of magnetic core-shell nanoparticles by magnetorelaxometry, ac susceptibility, transmission electron microscopy and photon correlation spectroscopy – a comparative study

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Magnetic nanoparticles (MNPs) find wide application in medicine and bioanalytics. For the various applications, the requirements on the particles are quite different. In addition, for in-vivo applications one has to guarantee that the MNPs do not aggregate in the given medium. For applications, such as the magnetic relaxation immunoassay (MARIA), only specific bindings of the functionalized MNPs may occur. Consequently, there is a strong need for a fast, reliable and inexpensive technique for the MNP characterization which is important for both manufacturers and users of core-shell MNPs.

As has been demonstrated in [1-3], the measurement of the magnetorelaxometry (MRX) in combination with the analysis of the experimental curves with the moment superposition model (MSM) is a quick and powerful tool for the estimation of structure parameters like core and hydrodynamic size distributions as well as anisotropy constant. Based on the MRX technique and the MSM, we are currently developing a compact, fluxgate-based MRX measurement system placed in a 19'' housing (Fig. 1). To verify the structure parameters of magnetic core-shell MNPs as determined from fluxgate MRX, we compared them with those estimated from ac susceptibility, transmission electron microscopy and photon correlation spectroscopy measurements.

To determine the MNP core parameters, one generally immobilizes the MNPs, e.g., by freeze-drying. Consequently, Brownian relaxation is inhibited, so that the relaxation behavior is determined by the Néel mechanism reflecting the MNP core properties. The core size distribution estimated for superparamagnetic Fe_3O_4 MNPs from chemicell GmbH agrees well with that from TEM measurements.

To analyze the MRX curves on MNP suspensions, one generally has to consider distributions of core and of hydrodynamic diameters. In order to reduce the number of fit parameters, it is recommended to first estimate the core parameters on a freeze-dried reference sample. The estimated distribution of hydrodynamic diameters is compared to that obtained from photon correlation spectroscopy (PCS) and ac susceptibility measurements. Again good agreement is found. Note that the ac susceptibility data were analyzed with a refined model that similarly to the MSM accounts for both Brownian and Néel relaxation.

This work was financially supported by the DFG via SFB 578 and by the BMBF under contract number 13N9174.

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Fig. 1: Photo of prototype of magnetic nanoparticle analyzer.

Apparent Dependence of the Interfacial Energy of Suspended Ferrofluid Droplets on Magnetic Field Strength

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As part of an ongoing project to develop biocompatible ferrofluids for use in retinal detachment therapy [1,2], we have developed instrumentation to enable the study of the static and dynamic response to magnetic fields of ferrofluid droplets suspended in diamagnetic fluids. The ultimate objective is to be able to predict the change in shape of ferrofluid droplets and their movement through the vitreous gel of the eye under the influence of applied magnetic fields so that they can be guided to sites of retinal tears after being injected into the eye.

Several previous studies of suspended ferrofluid droplets have shown how the shape of the droplets changes as a uniform magnetic field is varied [3,4]. Droplets tend to elongate along the direction of the applied field as shown in Figure 1. The changes in shape are explained in terms of equilibrium configurations that balance surface energies and self-demagnetizing energies. Previous approaches have assumed a constant value for the interfacial energy between the droplet and the medium in which it is suspended. By combining independent measurements of the field-dependent magnetic susceptibility of the ferrofluid with measurements of droplet shape change as a function of field strength, we observe an apparent increase in the interfacial energy with increasing field. Quantification of this dependence will be critical to the complete understanding of the field-induced motion of ferrofluid droplets through immiscible viscous media.

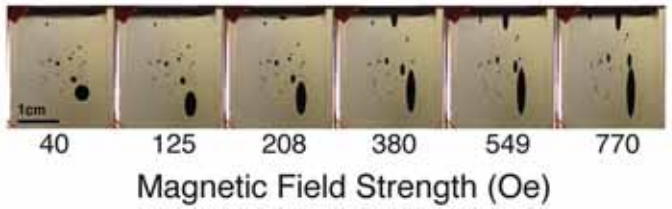

Figure 1: Magnetic field dependence of ferrofluid droplet shape. System comprises PDMS coated magnetite nanoparticle ferrofluid suspended in glycerol.

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Automatic Transport of Magnetic Particles in the Blood Vessels Using a Clinical MRI System

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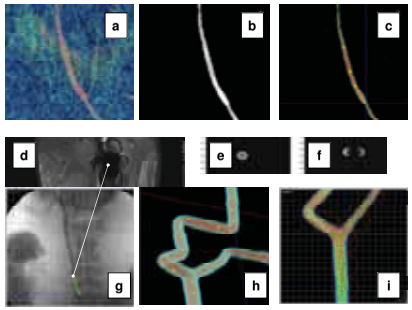
Present implementations for targeting tumors rely on an external permanent or an electro-magnet located near and above the tumor. A catheter is typically used to release the magnetic nanoparticles as close as possible to the target. But because of higher field intensity towards the external magnet, targeting is mostly restricted to tumors near the skin with significant reduction of targeting efficacy as the targets get deeper in the body. Furthermore, since the approach relies on trapping the particles without navigation or trajectory control over pre-planned paths, the distance between the releasing site and the tumor significantly affects targeting effectiveness. Indeed, the reachable limits of catheterization combined with complex microvasculature networks contribute to lower significantly targeting performance further. Hence, as minimum requirements, an efficient interventional platform for tumor targeting or for other operations in the human microvasculature must not only be able to manipulate magnetic carriers in a 3D volume but also provide an integrated imaging modality allowing effective navigation along pre-planned paths through real-time closed-loop control from the catheterization boundaries to specific targets such as tumoral lesions.

Here, a novel medical interventional platform based on a clinical Magnetic Resonance Imaging (MRI) system used not only for feeding back information to a controller responsible for the real-time control and navigation of magnetic particles along pre-planned paths in the blood vessels but also for propulsion and steering, is presented.

Unlike known magnetic targeting methods, the present platform when upgraded with additional gradient coils will allow us to reach locations deep in the human body while enhancing targeting efficacy using real-time navigational or trajectory control.



(a) and (b) unfiltered and filtered MR-image of the carotid artery of the living animal; (c) the same artery with waypoints being plotted to provide trajectory control commands to the computer; (d) distorted MR-image created by the 1.5mm chrome steel sphere; (e) and (f) MR-image of the ferromagnetic sphere using a special algorithm as seen in the transversal and sagittal plane respectively; (g) the same ferromagnetic sphere being tracked over a pre-acquired x-ray image while being controlled at 24 Hz in the carotid artery of the living swine along the waypoints; (h) shows the waypoints plotted over the MR-image of a phantom mimicking human vascular conditions and being showed through a dedicated imaging software with (i) the respective real-time displacement of the sphere being displayed.

Silica Microspheres and Silica Nanoparticles Containing Magnetic Nanoparticles and Semiconductor Quantum Dots

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Silica nanoparticles and microspheres with superparamagnetic and fluorescent properties were synthesized using a sol-gel process. Monodispersed particles of silica that are magnetic and fluorescent were constructed in two different ways. The first method consists of incorporating both iron oxide magnetic nanoparticles (MPs) and CdSe/CdZnS quantum dots (QDs) simultaneously into a growing shell of silica onto pre-made silica cores (Figure 1). In the second method, we used silica-coated iron oxide nanoparticles as a core and grew QD-containing silica shell onto their surfaces (Figure 2). These particles were characterized using TEM, SEM, optical microscopy, Scanning Transmission Electron Microscopy (STEM), and SQUID magnetometry. We demonstrated the manipulation of these microspheres using an external magnetic field with real-time monitoring under a fluorescent light microscope. These superparamagnetic, tunably fluorescent, and photo-stable particles can be useful for applications that required biocompatible and functionalizable surfaces.

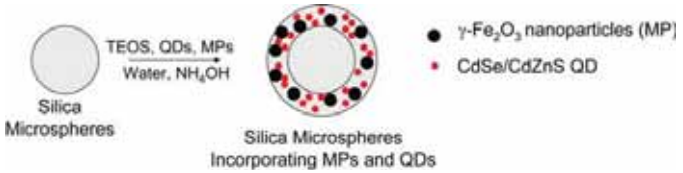

Figure 1. Reaction scheme for incorporating MPs and QDs into the shell of silica microspheres

Figure 2. Reaction scheme for growing a QD-containing silica shell onto the MP

The Effect of Magnetic Nanoparticle Coating on The Efficiency of Stem Cell Labeling

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Using stem cells in human medicine remains a distant goal because of the still-considerable risk of tumor formation or differentiation into an improper phenotype. Non-invasive cellular imaging allows the real-time tracking of grafted cells as well as the monitoring of their migration. Various coatings have been used to optimize the delivery of magnetic nanoparticles into cells.

We have developed three coatings of maghemite nanoparticles prepared by the alkaline coprecipitation of iron (II) and iron (III) precursors in an aqueous solution. Coating was achieved by a one-step *in situ* coprecipitation in D-mannose solution, a two-step post-synthesis coating with poly(L-lysine) (PLL), or by the solution radical polymerization of *N,N*-dimethylacrylamide (DMAAm) in the presence of γ-Fe_2O_3. The synthesized nanoparticles were characterized by dynamic light scattering, transmission electron microscopy, FT infrared spectrometry and magnetic resonance (MR) relaxometry in terms of morphology, size, polydispersity, surface composition and relaxivity. The mesenchymal stem cell labeling efficiency of the developed nanoparticles was compared with that of a commercially available contrast agent, Endorem®, used as a control. The mechanism responsible for the cellular internalization of the nanoparticles differed depending on their coating. While the intracellular uptake of *ca.* 10 nm D-mannose-coated nanoparticles is thought to be receptor-mediated endocytosis through the cell membrane, the mechanism of PLL-mediated and PDMAAm-coated iron oxide uptake by the cells is assumed to be based on endocytosis and/or diffusion through the cell membranes. A MR image (see the Figure) taken 3 days after the implantation of cells labeled with PLL-coated nanoparticles clearly shows a hypointense signal in the left hemisphere; such a signal is not visible in the right one, where Endorem®-labeled cells were implanted.

Cell labeling using the developed nanoparticles opens new possibilities for the non-invasive *in vivo* monitoring of cell manipulation, migration, proliferation and differentiation.

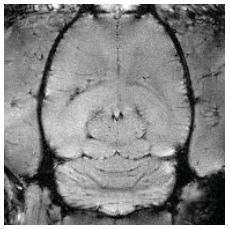

Figure. Coronal MR image of a rat brain with 1000 cells labeled with PLL-modified iron oxide implanted into the left hemisphere and 1000 Endorem®-labeled cells implanted into the right hemisphere.

A stand-alone multi-channel magnetorelaxometry device for quantitative and spatial resolved MNP detection: Characterization and first measurements.

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Safe and effective application of magnetic nanoparticles in procedures like Magnetic Drug Targeting or Magnetic Hyperthermia requires detailed knowledge about the biodistribution of the nanoparticles in the organism. Magnetorelaxometry (MRX) is an effective and reliable technique for the quantitative and spatial resolved detection of magnetic nanoparticles in biological systems.

In these applications magnetoelaxometry signals usually are found to be in the order of a few tens of a pico tesla. This requires the use of Low-Tc-SQUID sensors operated inside a magnetically shielded room, which suppresses environmental magnetic interferences.

Here, we developed a stand-alone multi-channel magnetorelaxometry device for in-vivo quantification of magnetic nanoparticle distributions in small animals up to rabbit size (diameter < 11 cm), which circumvents the use of a bulky and expensive mu-metal shielded room, An integrated superconducting Niobium shield against external magnetic interference allows the operation of the system in conventional laboratory environment. Up to 18 SQUID sensors each connected to a rectangular pick-up coil of variable size up to 1.5 cm x 3 cm arranged regularly around the circumference of the sample are used for spatially resolved detection of the relaxation signals.

We present the results of the basic physical characterization of the MRX-device focussing on the behavior of the integrated superconducting shield. We emphasize that the shielding against external magnetic fields turned out to be better than 10^5 for radial and 10^7 for axial external magnetic fields. Furthermore, residual magnetic fields in the sensor area were found to be smaller than 100 nT frozen in during the transition of the shield into its superconducting state during cool down of the device. This together with the mechanical robustness of the device against mechanical vibrations results in a total system noise level $S_B^{-1/2}$of only 2 to 3 fT Hz $^{-1}$, which is in the range of state-of-the-art magnetic SQUID measurement systems.

In addition to MRX measurements on magnetic nanoparticle assemblies, in-vivo measurements of the magnetic heart signals of a rabbit were performed. Signal amplitudes of up to 4 pT and even fine structure (P-wave) of the hearts activity could be resolved in the raw data, demonstrating the excellent performance of the system in a conventional laboratory environment.

The PTB multi-channel magnetorelaxometry device for in-vivo quantification of magnetic nanoparticle distributions in small animals.

Magnetic Particle Imaging: quantitative assessment of tracer performance

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Recently Magnetic Particle Imaging (MPI), a novel imaging modality, was proposed to directly visualize a local concentration of a magnetic nanoparticle tracer.[1] It capitalizes on the nonlinear response of magnetic material to an oscillating magnetic field in a dedicated static field arrangement. Due to this arrangement, a tracer is in saturation throughout the whole examination zone except for one point, the field-free point, where the static field is zero. Scanning the field-free point over an examination zone results in a local remagnetization of the magnetic tracer, which generates a nonlinear magnetic response indicative for the tracer spatial distribution after reconstruction. [2]

MPI combines high resolution and sensitivity in 3D imaging with fast acquisition. Besides challenges in instrumentation, this imposes a number of requirements onto a tracer material. A complete understanding of the chemical and physical mechanisms responsible for MPI signal generation is key to improve efficacy of the tracer.

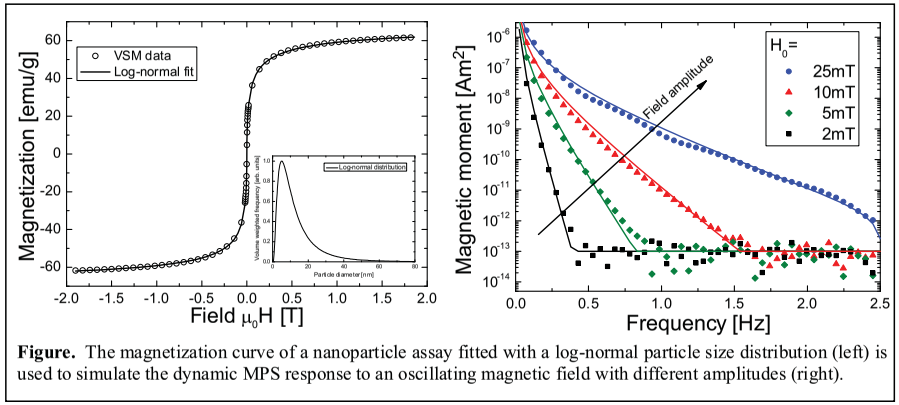

Figure. The magnetization curve of a nanoparticle assay fitted with a log-normal particle size distribution (left) is used to simulate the dynamic MPS response to an oscillating magnetic field with different amplitudes (right).

In the presentation we will introduce magnetic particle spectroscopy (MPS), equivalent to 0-D MPI signal acquisition, which is used for advanced characterization of magnetic nanoparticle performance in MPI. We further derive a semi-empirical tracer model that is based on a detailed chemical and physical analysis of a magnetic nanoparticle assay. The model parameters are independently determined using a number of techniques. The Figure shows the magnetization curve (left) of a commercially available MRI contrast agent that, in combination with an iron content analysis, is used to derive the magnetic particle size distribution. The latter is one of the input parameters used in our model that accurately describes the field amplitude (right) and frequency dependence of a ferrofluid MPS response using a characteristic anisotropy value as single fit parameter. It will be further detailed that, and how, particle size distribution, saturation magnetization and magnetic anisotropy, all set by the synthesis protocol, affect the MPS response of ferrofluids.

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Magnetic Nanoparticles for Tracking of Stem Cells and Dendritic Cells

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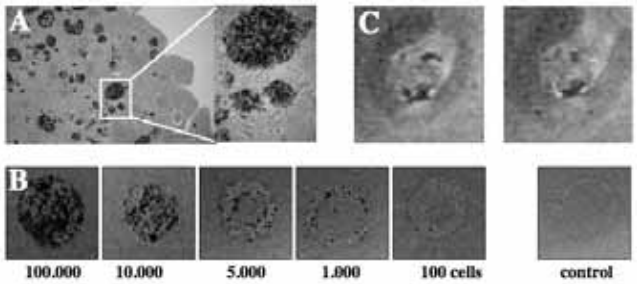
Magnetic nanoparticles (MNPs) exhibit unique properties that are currently being explored for cell tracking *in vivo*, including tracking of stem cells and antigen presenting dendritic cells (DC). We previously reported on 'smart' contrast agents for simultaneously monitoring cell position and function by magnetic resonance imaging MRI (1).

MNP size, surface charge and chemistry are critical for interaction with cells and detection of MNP-loaded cells *in vivo* by MRI. Here we have analyzed MNPs for their interaction with and uptake into cells, with a particular focus on DC that represent the principal antigen-uptake and presenting cells of our body (2, 3). To this end we synthesized a series of MNPs containing (i) a phospholipid bilayer shell which can be considered an artificial cell membrane (4) or (ii) a biopolymeric shell of polyelectrolytes generated by layer-by-layer (LbL) technology (5) and (iii) also prepared bacterial MNPs of the magnetotatic bacterium *M. gryphiswaldense* MSR-1, referred to as magnetosome. MNPs were then analyzed for (i) their interaction with DC using flow cytometry and RT-PCR analysis for determining DC activation, cytokine release and gene activation, (ii) MNP uptake into and localization in DC by immunomagnetic cell separation, laser scanning fluorescence and electron microscopy and (iii) detection of MNP-loaded DC *in vitro* in agarose phantoms and *in vivo* in animal models by 11.7 Tesla high resolution MRI.

It was found that lipid shell MNPs were readily taken up into DC (see Figure A) without overt DC stimulation. Bacterial MSR-1 MNPs stimulated DC activation, cytokine release and immune function, presumably because they contain bacterial proteins and/or lipids that are recognized by DC. MNPs generated by LbL were used to establish a relationship of physicochemical MNP properties on DC activation and uptake, including MNP size, charge and surface chemistry. MNP-loaded DC were then detected by MRI in phantoms *in vitro* (detection limit 100 cells, Figure B) and following transfer into mice *in vivo* (MNP-loaded DC in lymph node, Figure C). These results will be discussed.



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Understanding the relaxometric properties of high quality MR contrast agents based on magnetite nanoparticles

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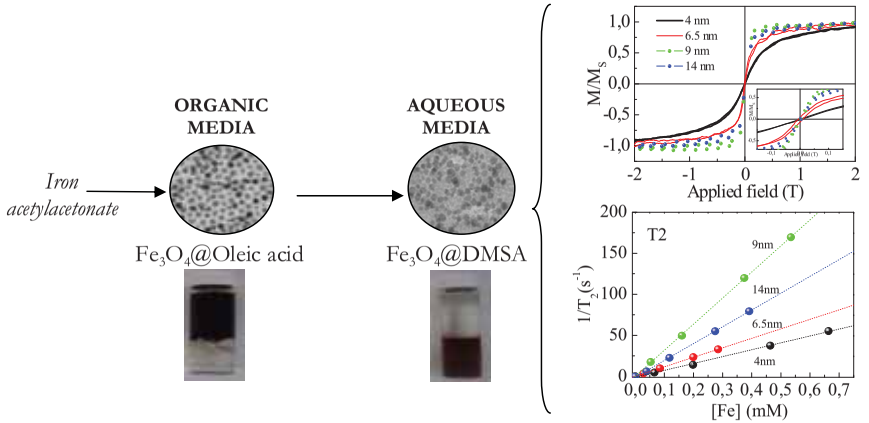
During the last decades, Magnetic Resonance Imaging (MRI) has become a powerful technique in medicine because of its capacity to detect cancer diseases. Nowadays one of the main challenges in this field is the design of contrast agents based on superparamagnetic iron oxide nanoparticles to make them more effective and specific. This can be done by tuning not only the particle size to enhance the contrast but also the surface coating to improve biodistribucion and blood half-time life.

In this work we have studied the key parameters that control the water proton relaxivity rates produced by the presence of iron oxide nanoparticles with different size. The correlation between structural, magnetic, colloidal and relaxometric properties of aqueous suspensions of these superparamagnetic iron oxide nanoparticles has been analyzed. The aqueous suspensions were prepared from nanoparticles obtained by decomposition of iron acetylacetonate in different organic solvents in the presence of oleic acid at high temperatures and the subsequent ligand exchange reaction with dimercaptosuccinic acid (DMSA).

X-Ray Diffraction and Transmission Electron microscopy studies show that the suspensions consist of high-crystalline and uniform nanoparticles of different sizes, 4, 6, 9 and 14 nm (polydispersity lower than 20%). As expected, saturation magnetization (M_S) increases from the smallest to the largest particles reaching M_S values near the bulk, being these values not affected by the DMSA ligand exchange. By contrast, magnetic parameters for the suspensions in agar showed a different trend. Thus, 9nm core suspension saturated its magnetization at lower applied magnetic field than the 14nm suspension. This result fit with the T2 proton relaxation measurements. The T2 relaxation rate (known as r_2) increases from 84 $mmol^{-1}\cdot s^{-1}$ for the 4nm suspension to 317 $mmol^{-1}\cdot s^{-1}$ for the 9nm suspension, and 204 $mmol^{-1}\cdot s^{-1}$ for the 14 nm suspension. These results can only be explained by the bigger hydrodynamic size (70 nm) of 9nm suspension compared to the 14nm suspension (50nm). It can be concluded that T2 proton relaxation rate not only depends on magnetic nanoparticle crystallinity and core size but also on the colloidal properties of the suspensions. These results are particularly relevant for the design of small SPIO contrast agents, colloids with extremely small aggregate size which may need to be produced from nanoparticles with improved magnetic properties, i.e. bigger and more crystalline nanoparticles.



Ultrasound Imaging of Sentinel Lymph Nodes in The Patients With Breast Cancer by Using The Preparation "Unimag"

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Numerous studies have shown that the histological result of sentinel node is very important for the planning of operation in patients with breast cancer, but in spite of numerous proposed methods sentinel node detection in the pre operative period for the performing of biopsy steel is problematic. We have developed novel original decision of this problem by the using of new original preparation "Unimag" (registered by Drag Agency of Republic of Georgia; Cert.: DA Nr-000142; 08. 04.2005). Preparation "Unimag" represents stabile suspension of magnetite nano particles, magnetic fluid.

After the peri-tumoral injection of "Unimag", magnetite nano-particles will be absorbed by macrophages, which deliver them trough the lymphatic capillaries to regional lymph nods. This mechanism of magnetite nano-particles transport underlies to the new method of sentinel lymph node detection.

Diversely to the other dyestuff agents used in this direction, magnetite nano-particles, besides of intra-operative indication of lymphatic nodes (actively filled them and colored in a boldly black), "Unimag" gives the possibility of ultrasound imaging of sentinel lymph nodes in the pre-operative period and performing of it's biopsy. This assuredly helps in a better planning of the operation and considerably improves its radicalism.

Even in the case of ideal surgical intervention it's impossible to get out all the microscopic lymphatic vessels and nods, which could be damaged by the tumor micro-metastasis. In the field of high frequency electro-magnetic waves the magnetite nano-particles give us possibilities of local, distance hyperthermia on micro-metastatic lesions in postoperative period. This will minimize the potentiality of development of postoperative recurrence and secondary metastatic processes without any radioactive and chemotherapeutic means.

Sentinel lymph node detection by using of preparation "Unimag" is a safe and accurate method of screening of nodes in preoperative period, discovering of them during the operation and affecting on them in postoperative period in women with breast cancer.

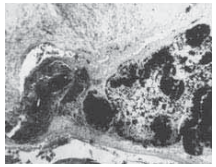

Fig. 1 Magnetite nano particles in lymph node. H&E, x300

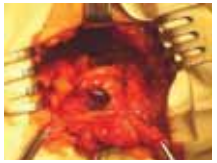

Fig. 2 Lymph node colored by magnetite nano particles

Fig. 3 Unimag enabled to view lymphatic nodes on ultrasound

Microfabricated Sifter for High-throughput and High-gradient Magnetic Separation

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Magnetic nanoparticles (MNP's) conjugated with biomolecules or recognition moieties are finding many biomedical applications, including sensing and detection. In this context, we are developing a microfabricated magnetic sifter for biological sample preparation applications.

The microfabricated sifter consists of arrays of micron-sized slits etched through a silicon wafer, onto which a soft magnetic film is deposited (Fig. 1). In the presence of an external field, the magnetic film produces high magnetic field gradients, comparable in magnitude to gradients found in microfluidic separation devices. As the solution flows through the sifter, MNP's are captured by the magnetic material surrounding the slits. The large number of slits (>100,000/sifter) allows for processing of large volumes of liquid.

In our separation experiments, the sifter is placed in a custom holder with fluid inlets and outlets. During the capture step, the sifter is magnetized by a pair of permanent magnets. Fluid containing 50 nm diameter MACS CD 138-labelled MNP's, with a concentration of ~10^{12} particles/ml, is pumped through the sifter at a rate of 1 ml/hr. The captured particles are released by removing the external field and flushing the sifter with water. The magnetic content of the fluids is measured with alternating gradient magnetometry. Separation efficiencies for one pass ranged from 34-37%. Higher efficiencies, up to 60%, have been obtained for smaller fluid volumes. After separation, more than 70% of captured nanoparticles are eluted during the washing step. Separation efficiencies can be improved by placing multiple sifters in series. The high magnetic field gradients and capacity for large volume flow of the sifter are promising for biomedical applications requiring high throughput capture of MNP's.

This work is supported by US National Cancer Institute through Center for Cancer Nanotechnology Excellence focused on Therapeutic Responses (CCNE-TR).

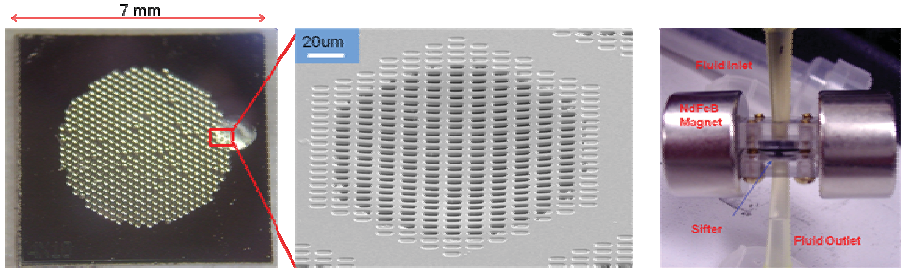

Fig. 1: (Left) Microfabricated magnetic sifter & SEM image of a slot array. (Right) Separation assembly.

Experimental and theoretical study of a novel and fast magnetophoretic separation technique using low magnetic gradients

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An essential step in the development of scientific and clinical applications of magnetic carriers is the control of particle behaviour. Control of particle trajectory in drug delivery applications or extraction from dispersion in immunoassay applications using externally applied magnetic gradients are typical examples. However, the magnetic gradients required to produce a significant magnetophoretic drift in a magnetic carrier are very high, even in the case of superparamagnetic particles. For example, gradients of the order of 10^4 T/m could be found in typical separation columns using a packed bed of magnetically susceptible wires (diameter 50μm) placed inside an electromagnet.

Here, we present new experimental and theoretical results showing that low gradient magnetic separation (LGMS) is also possible. The key step in this LGMS process is the reversible aggregation of magnetic carriers induced by the magnetic field. We report fast separation (a few minutes for a 25 mL sample) of dispersions of superparamagnetic particles with different concentrations (0.01 g/L to 10 g/L) using a 30 T/m magnetic gradient (the employed particles are commercial Estapor$^{(R)}$ superparamagnetic microspheres and the magnetic separator is commercially available from Sepmag Tecnologies). The interplay between the different factors determining low gradient magnetophoresis (magnetization of particles, size, ...) is consistently described by a magnetic analogous to the Bjerrum length concept [1]. We formulate a simple criterion predicting the onset of low gradient magnetophoresis separation as a function of the sample properties (e.g., minimum particle radius). The separation times of samples with different concentrations and different particles can be described with a unique curve depending on the ratio between the magnetic Bjerrum length and the particle separation (depending on concentration).

References

[1] G. De Las Cuevas, J. Faraudo and J. Camacho, J. Phys. Chem. C 2008, **112**, 945-950

Cell Separations Using Magnetic Chains and Grids

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Magnetic bead methods for cell separations utilize diverse approaches to isolate rare cells and to enrich heterogeneous samples for further characterization such as single cell mRNA expression profiling. In this work we present optical observations of the motion and separation of magnetically labeled HUVEC cells in the presence of high concentrations of unbound magnetic nanoparticles or on micron scale magnetic grids.

Magnetic nanoparticles can often be made to form chains which magnetically bond to immuno-magnetically labeled cells. The attachments are sufficiently strong that the assembly is nearly rigid, so that the attached chains greatly increase magnetic forces on attached cells. Cell velocities on the order of 1 mm/min can be generated by modest field gradients or rotationally induced translation. Fig 1 shows HUVEC cells labeled using a modified protocol with a PlusCellect kit targeting PECAM1 surface markers. When a 1 kOe field is applied, the labeled cells are drawn to the surface and magnetic chains of the free magnetic nanoparticles form. After some minutes, these chains link to the cells and allow control of magnetically directed cell transport. When the field is removed, the chains break up, and the cells are restored to their immuno-magnetically labeled state.

Patterned magnetic structures are well known sources of high field gradients. We have fabricated a slotted, flow through magnetic grid structure (Fig. 2) using silicon micromachining and observed strong interactions between magnetically labeled cells and these smooth grid structures. We find that applying a field can draw weakly labeled cells onto patterned regions where their magnetic interactions effect cell transport during flow. We are evaluating such structures for optimizing capture and release of cells, and for reducing losses which are often reported for high gradient columns. This work is supported by US National Cancer Institute.

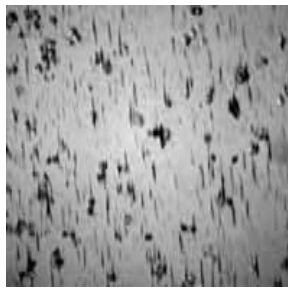
Fig. 1: Images of cells decorated by magnetic nanoparticle chains.

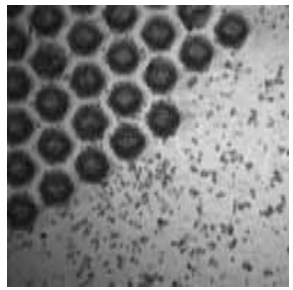
Fig. 2: Cells on magnetic grids.

High content screening investigation of nanomaterial toxicological response to proliferating MC3T3-E1 osteoblast cells: a time course study

Adriele Prina-Mello[1,3*], Fiona Byrne[1,2,3], John Michael David Coey[1,2], Bashir M. Mohamed[2], Anthony Davies[2], Yuri Volkov[1,2]

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Qualitatively we have previously reported on the cell responses to magnetic nanowires[1]. Alignment, internalisation and cell viability have been studied on the model of MC3T3-E1 osteoblast cells[2]. Magnetic nanowires are receiving an increased interest among the scientific community due to their anisotropic magnetisation and potential applications where their orderly alignment is required. In this paper we used nickel nanowires because of their large magnetic moment and remnant magnetisation. Nonetheless, nickel as bulk material has been associated with a strong cytotoxic response when solubilised. Therefore it is vital to evaluate the interaction between proliferating cells and putative non-soluble nickel-based magnetic materials for future clinical applications.

The cytotoxic response and interactions between magnetic nanowires and human cells have been a subject of several recent studies. Conversely to the more widely investigated cell types (e.g., lung, kidney and macrophage cells), osteoblast cells have not been investigated thoroughly. Therefore in this paper we focussed on the multi-parametric time-course evaluation of the osteoblastic MC3T3 cell line response to the manufactured magnetic nickel nanowires.

Magnetic nickel nanowires were synthesised by electrochemical deposition and fully characterised by SQUID, XRD, VSM and SEM as previously described by Prina-Mello *et al.* (2006). Time course analysis was then carried out implementing the High Content Analysis (HCA) approach on the Cellomics KSR device (Thermo Fisher Scientific, USA) to measure the toxic effects of nickel nanowires on proliferating MC3T3-E1 osteoblasts. The 72 hour-observations were successfully carried out from a starting cell concentration of 2000 cell/well cultured with different nanowires concentrations. Positive and negative controls were included at each time point readout.

The multiparametric analysis in a series of experiments enabled to identify and evaluate the critical time points of the MC3T3-E1 cellular response to the engineered magnetic nanowire carriers. The full content analysis included automated counting of the nuclei, evaluation of the nuclear size, measuring the cell membrane permeability, lysosomal mass and pH.

The results presented here are in agreement with the previously reported cell viability studies where osteoblasts showed a very robust response to the nickel nanowires. Nonetheless, the high content analysis revealed that the cytotoxic response of the osteoblast cells was dependent on the different nanomaterial concentrations (figure 1).

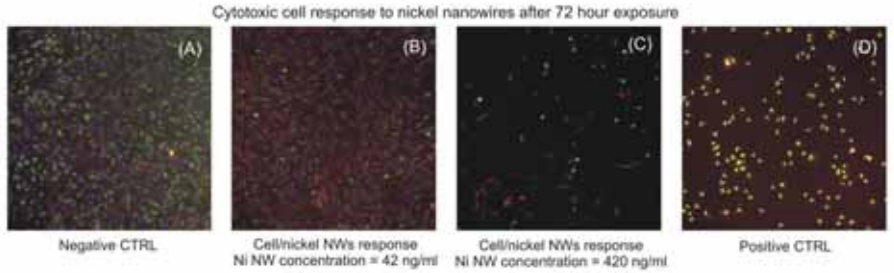

Figure 1: Cytotoxic response of MC3T3 osteoblasts cell after 72 hours exposure to different concentration of nickel nanowires: high content screening images. (A) Negative control of healthy cells (green stain = cell permeability, normal proliferation); (B) Cells exposed to low concentration of nickel nanowires (predominance of green permeability stain versus stress response lysosomal red stain). (C) Cells exposed to high concentration of nickel nanowires (predominance of lysosomal stress response, red stain, and reduce cell viability). (D) Positive control of stressed cells to nickel nanowires chemical precursor (yellow staining = nuclear penetration of permeability stain = all cells highly stressed).

At same time, there is a lot of ambiguity over the adoption of magnetic nanomaterials in biomedical studies due to the potential cell health impact. Therefore, there is a continuous need to broaden the understanding of the specificity of interactions between different human cell types and novel magnetic nanocarries to better regulate their research and clinical applications.

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[2] Prina-Mello A, Diao Z. and Coey JMD, *Journal of Nanobiotechnology* 4:9, 2006.

ACKNOWLEDGEMENTS

CRANN CSET and PI grant, Science Foundation Ireland for the financial support.

High-throughput bioscreening system utilizing high-performance affinity magnetic carriers exhibiting minimal non-specific protein binding

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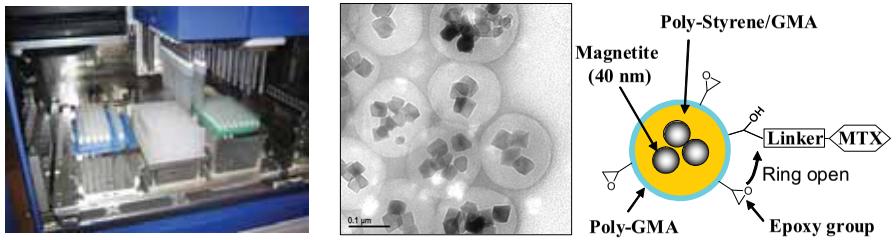
We describe our new high-throughput, automated bioscreening system (left figure). It utilizes novel, high performance magnetic carriers for high efficiency affinity purification of target biomolecules without non-specific binding of proteins, as compared to conventional commercial magnetic carriers.

Our novel magnetic carriers were core-shell structured spheres, highly monodisperse with 184 ± 9 nm diameter, as observed by TEM (middle figure). Several magnetite particles, 40 nm in diameter, were encapsulated in the core made of a copolymer of styrene and glycidyl methacrylate (GMA), which was further encapsulated in the shell of a polymerized GMA (poly-GMA; right figure). We refer to our magnetic carriers as "FG beads," after ferrite and GMA. FG beads had a saturation magnetization of 24.1 emu/g, and were highly dispersible in aqueous and organic solvents. With a magnet they were separated from suspensions in a few minutes and re-dispersed by stirring the suspensions when the magnet was removed.

FG beads not only exhibited minimal non-specific binding of proteins but also had high stability in water and several organic solvents. This enabled affinity purification of target proteins that make specific bindings to probe molecules immobilized on the surfaces of FG beads from protein libraries. We successfully performed affinity purification of target proteins against drugs for various diseases (cancer, rheumatism, osteoporosis, inflammation, etc.), vitamin K, porphyrins, environment hormones, etc. FG beads allowed 10,000 times concentration of these biomolecules by single step affinity purification, greatly exceeding conventional affinity columns which allow only 5-10 times concentration.

[1] K.Nishio et al., to appear in Colloids Surfaces B: Biointerfaces.



Automated bioscreening system (left), TEM images for FG beads (middle), and core/shell structure of FG beads immobilized with MTX (anticancer drug) as ligand (right). We immobilized MTX via the linker and the ring-opened epoxy of poly-GMA.

Characterizing magnetic separation systems for μ-TAS

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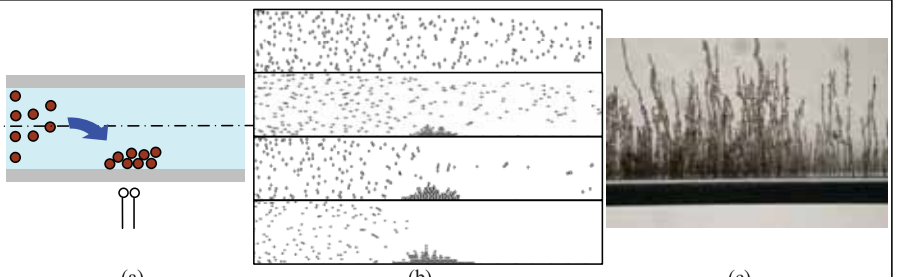
Magnetic microspheres are micron sized polystyrene beads embedded with superparamagnetic nanoparticles. These particles can be readily and selectively functionalized to bind with target species or molecules, and also be manipulated precisely using magnetic fields. Thus they can be used as mobile substrates in micro-total-analysis systems (μTAS) where magnetic separation is an important process. It is therefore important to characterize the transport of magnetic microspheres in microfluidic environment.

Although the motion of magnetic microspheres has been well characterized in the literature for dilute suspensions many microfluidic applications involve higher concentrations, when dipolar interactions become important. This dipolar interaction is weak and limited to approximately 10 × (particle radius) and forms ordered structures.

Here, a force model for particle interaction is proposed for concentrated magnetic bead suspensions. A point dipole models the imposed magnetic field produced by a microelectromagnet. A Lagrangian particle tracking algorithm is followed assuming that the principal forces acting on the particles are viscous drag and magnetic (both due to the imposed field, and due to particle interaction). The model is validated through the similarity in the field-induced aggregate shape between experiments and theory as seen in fig. 1.

The microstructure evolution of the aggregate is a result of the competing effects between fluid shear force (destructive) and magnetic force (constructive). For on-chip separation systems, collection efficiency (Φ) is an important parameter, which denotes the number fraction of the beads that are captured in the device (Φ=1 implies complete capture). When particle dipole interactions are considered in the model, capture efficiency is found to be aided due to the formation of self-organized structures of the captured beads near the region of the highest field strength. A parametric study provides the functional relation between capture efficiency and a dimensionless group composed of viscosity, magnetic susceptibility, fluid velocity and particle size and concentration. The results help in designing chip-based magnetophoretic separation devices for bio-MEMS applications especially including other non-magnetic species.



(a) (b) (c)

Figure 1: (a) Schematic of a typical separation process in a microchannel. (b) Simulation snapshots for various Φ ranging from 0 (top) to 1(bottom). (c) An enlarged view of the microsphere aggregates on the channel wall.

Magnetic microparticles as manoeuvrable solid supports for multi-step (bio)reactions in continuous flow.

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Magnetic microparticles are a popular choice for solid supports in microfluidic applications because of their large surface to volume ratio and ease of manipulation using an external magnetic field.[1] Most commonly particles are trapped in flow to form particle beds or plugs and reagents are consecutively pumped over their surface in a stop-start procedure that can be both labour intensive and time consuming.

Here we demonstrate a microfluidic device in which magnetic microparticles act as a manoeuvrable solid support for multi-step bioreactions in continuous flow. Inside the microfluidic device a series of parallel laminar reagent streams are generated along a flow chamber. Functionalised magnetic particles are then pulled across the different reagent streams using an external magnetic field. As they traverse these streams, multiple reaction and washing steps can be performed on their surface followed by particle isolation in a single operation in continuous flow. (figure 1)

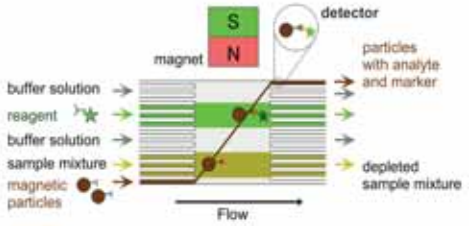

Figure 1. Principle of multi-step reactions on the surface of magnetic particles in continuous flow.

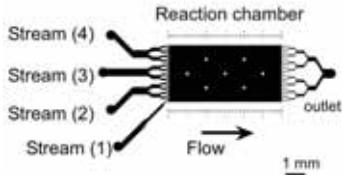

Figure 2. A schematic of the microchip design with four inlets and a reaction chamber.

Two types of bioassays have been successfully demonstrated using this system. Firstly, a streptavidin – biotin binding assay was performed in which streptavidin coated magnetic particles were introduced via stream 1 (figure 2) and deflected through a stream of fluorescently labelled biotin introduced through stream 3. Streams 2 and 4 contained washing buffer solutions. Particles were observed to fluoresce after passing out of the biotin stream.[2] Secondly, an immuno-recognition assay was performed in which mouse IgG immobilised to the surface of magnetic particles was deflected through a stream of fluorescently labelled anti-mouse IgG. Again, particles were observed to fluoresce brightly after traversing the anti-mouse IgG stream, indicating successful binding between complementary antibodies. Both assay types were performed in under 60 seconds in a single operation, a fraction of the time required for on-chip batch methods. A new microchip design incorporating five inlets is currently being investigated and a bioassay containing two reaction steps in the same operation is just a short step away.

[1] Gijs, M.A.M. *Microfluidics and Nanofluidics*, **2004**, *1*, 22 - 40.

[2] Peyman, S.A. ; Iles, A. ; Pamme, N. *Chem Comm*, **2008**, 1220 - 1222

Characterization of magnetic nanoparticles using quadrupole magnetic field-flow fractionation.

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Dept. of Biomedical Engineering, Cleveland Clinic, 9500 Euclid Avenue, Cleveland, Ohio 44195, carpinof@ccf.org

Magnetic nanoparticles have been employed in bioseparation, drug delivery, hyperthermia, and other areas of application. Their characterization is important for the optimization and quality control of particle preparations as well as for modeling magnetic cell separation, drug targeting, and hyperthermia.

Magnetic Field-Flow Fractionation (MgFFF) is a relatively new form of FFF that has the potential of quantifying the distribution of magnetic material in a nano-sized particulate sample. This elution technique is similar to liquid chromatography. Differently from chromatography the separation of the sample components is achieved within the mobile phase alone by applying a magnetic field gradient perpendicularly to the flow of mobile phase flowing inside the separation device (channel). The mobile phase velocity profile across the channel thickness is parabolic, or near-parabolic, with highest fluid velocity near the channel centre and zero velocity at the walls. Particles interact differently with the field gradient depending on their magnetite content. Particles that interact more strongly with the field gradient are confined to slowly moving fluid streams and take a longer time to elute than particles that are less influenced by the field. The relationship between sample properties and retention time can be so rigorously predicted that the measurement of retention times can yield quantitative properties for a fractionated sample. The retention time t_r for a given particle can be obtained from the elution profile of the sample and it depends on the retention parameter λ:

$$t_r \approx \frac{t^0}{6\lambda\left(1-2\lambda\right)} \qquad \lambda = \frac{kT}{F_m w}$$

Where t^0 is the elution time for non retained material, k is the Boltzmann constant, T is the absolute temperature, F_m is the force on a single particle due to the magnetic field, and w is the channel thickness.

The force F_m on a particle due to its interaction with the magnetic field gradient is given by:

$$F_m = V_m M \nabla B$$

By knowing F_m we can determine the value of the particle core diameter d:

$$d = \sqrt[3]{\frac{6V_m}{\pi}}$$

The applied magnetic field is generated by a quadrupole electromagnet. This allows for the elution of broadly polydispersed samples by varying the field strength with time. We propose to show MgFFF as a method for separation and characterization of magnetic nanoparticles, such as the immunospecific nanoparticles used for labeling biological cells for magnetic separation. Samples of magnetic nanoparticles were kindly supplied by BD Biosciences Pharmingen and Columbus NanoWorks, and were analyzed by MgFFF.

Talk Abstracts - Saturday, May 24, 2008

	Session 8: Biological Applications - *Chairs: Hans Bäumler and Christoph Alexiou (Germany)*			
8:30	Crecea, Vasilica	Measuring Biomechanical Properties of Tissue Phantoms with Magnetomotive Optical Coherence Elastography	U.S.A.	Talk 65
8:45	Hajdú, Angela	Predictive stability test for salt tolerance of water based magnetic fluids	Hungary	Talk 66
9:00	Tresilwised, Nittaya	Oncolytic adenovirus magnetic complexes: characteristics and biological efficacy in vitro	Thailand	Talk 67
9:15	Erb, Randall	Kinetics of Nonmagnetic Particle Chain Growth in Biocompatible Ferrofluid	U.S.A.	Talk 68
9:30	Kose, Ayse	Rapid and effective cellular manipulation and separation	U.S.A.	Talk 69
9:45	Dragnea, Bogdan	On the encapsulation of superparamagnetic nanoparticles in icosahedral virus capsids	U.S.A.	Talk 70
10:00	Cui, Yali	Applications of GoldMag Particles in Molecular Biology	China	Talk 71
10:15	**Coffee break / exhibitors**			
10:45	Ménager, Christine	Vesicles encapsulating quantum dots and magnetic nanoparticles for multimodal imaging	France	Talk 72
11:00	Saatchi, Kathy	Regulation of Cardiac Metabolism with Magnetic Particles	Canada	Talk 73
11:15	Safarik, Ivo	Hydrogen Peroxide Removal with Magnetically Responsive Saccharomyces cerevisiae Cells	Czech Republic	Talk 74
11:30	Xie, Xiaomao	Application of Magnetic Nano Particles to Full-automated Chemiluminescent Enzyme Immunoassay (CLEIA)	Japan	Talk 75
11:45	Aref, Amirreza	Monitor the kinetics of stem cells differentiation on nanosurfaces using optical waveguides	U.K.	Talk 76
12:00	**Bäumler, Hans**	**Red blood cells as carriers for magnetically targeted delivery of drugs**	**Germany**	**Invited talk 6**
12:40	**Lunch - Salmon Barbecue** - *sponsored by Integrated Magnetics*			
13:55	**Session 9: Biosensors** - *Chair: Ed Flynn (U.S.A.)*			
14:00	Ghionea, Simon	Lab On A Chip Detection of Biomolecules Using Magnetic Bead Labels	U.S.A.	Talk 77
14:15	Schotter, Joerg	Development of a magnetic lab-on-a-chip for point-of-care diagnosis	Austria	Talk 78
14:30	Stakenborg, Tim	Magnetic beads on biosensor platforms for protein, DNA and cell detection	Belgium	Talk 79
14:45	Aurich, Konstanze	Affinity Analysis for Biomolecular Interactions Based on Magneto-Optical Relaxation Measurements	Germany	Talk 80
15:00	Eberbeck, Dietmar	Measurement of the specific binding of functionalised MNP in whole blood	Germany	Talk 81
15:15	Tondra, Mark	Lateral Flow Immunoassay Using Magnetoresistive Sensors	U.S.A.	Talk 82
15:30	Lenglet, Luc	Multiparametric Magnetic Immunoassay	France	Talk 83
15:45	**Closing Comments and Announcement of the NEXT MEETING: Urs Hafeli / Maciej Zborowski / Wolfgang Schuett**			
16:00	**End of the scientific part of the meeting**			

Today's Social Program

The meeting will end at 4 PM. Please take the opportunity to explore beautiful British Columbia on your own! You might also be interested in a whale watching tour, exploring the wilderness surrounding the city or visiting one of the many museums in Vancouver.

Measuring Biomechanical Properties of Tissue Phantoms with Magnetomotive Optical Coherence Elastography

Vasilica Crecea[1,2*], Amy L. Oldenburg[2,3], Xing Liang[2,3], Tyler S. Ralston[4], Stephen A. Boppart[2,3]

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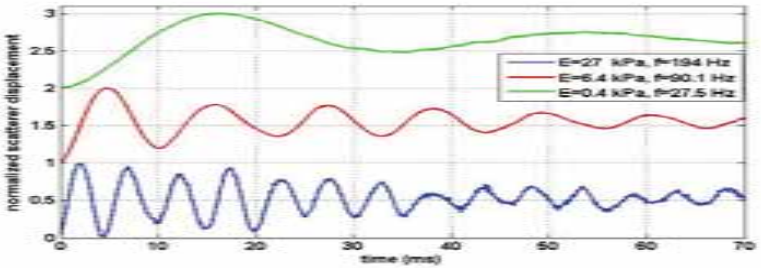
Mechanical properties of biological tissues, measured from the centimeter to the micron scale, are often indicative of the developmental stage, function, and health or disease of the tissue. In the biomedical field there is a great interest in assessing biomechanical tissue properties non-invasively and *in vivo*.

We present a new method for measuring elastic properties of tissue-like phantoms, which employs magnetite (Fe_3O_4) nanoparticles as contrast agents in a technique called magnetomotive optical coherence elastography (MM-OCE). Polydimethylsiloxane (PDMS)-based tissue phantoms with optical and mechanical properties similar to those of soft biological tissue, with elastic moduli in the range 0.4-120 kPa, were prepared with titanium dioxide (TiO_2) microparticles (MPs) to serve as scatterers and magnetic nanoparticles (MNPs) to serve as perturbative agents. Both types of particles were distributed homogenously within the tissue phantoms. The MNPs are displaced upon probing with an external magnetic field aligned with the axis of optical imaging, engaging the MNPs and adjacent MPs in axial motion. The scatterers in the samples were imaged with a spectral-domain optical coherence tomography (SD-OCT) system and shown to undergo underdamped relaxation oscillations when the magnetic field was removed, releasing the MNPs. We extracted the natural frequencies of oscillation (30-400 Hz) from the time-resolved displacement traces and showed that they correlate linearly with the square root of the elastic moduli of the tissue phantoms, which were measured and validated with a commercial indentation apparatus.

This novel, real-time, non-invasive technique affords the potential for *in vivo* tissue studies, while promising to enable molecular-level probing by functionalizing the MNPs to target specific molecules at the cellular level.



Normalized average relaxation displacement of scatterers in tissue-like phantoms with different elastic moduli, which were doped with magnetic nanoparticles and probed with a magnetic field gradient.

Predictive stability test for salt tolerance of water based magnetic fluids

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Most of the biomedical applications of magnetic fluids (MFs) require magnetic nanoparticles to be non-toxic, chemically stable, uniform in size and well-dispersed in aqueous media. Besides the several unique problems of particular applications, the colloidal stability of magnetic fluids is critical in general, to stabilize the magnetic particles under physiological condition, i.e., blood pH~7.4 and salt concentration ~0.15 M in high magnetic field gradient is of great practical importance [1-2].

In the present work, magnetite nanoparticles were synthesized by alkaline hydrolysis of iron (II)- and iron (III)-salts and stabilized in aqueous medium. Citric acid (CA) as a well-known complexant of ≡Fe-OH surface sites, and sodium oleate (NaO) were used for coating magnetite nanoparticles in order to be dispersed them in water. The adsorption and overcharging effect were quantified, and the salt tolerance of stabilized systems was studied. While only ~0.15 mmol/g CA can be adsorbed in monolayer on the surface of magnetite, the adsorption isotherm of Na-oleate proved the double layer formation with 2 mmol/g saturation value. These coated magnetite particles become anionic and carry great amount of negative charges independently of the pH of aqueous medium. Especially large electrophoretic mobility values were measured in the negative region with increasing oleate loading. Such huge negative mobility values were never reached in the presence of citrate, even if it was added in high excess, because CA is adsorbed only in monolayer forming mono- or bidentate complexes on iron oxide surface. The electrolyte tolerance was tested in coagulation kinetic measurements using dynamic light scattering (DLS). The colloidal stability depends sensitively on the surfactant dose, the pH and the salt concentration [3]. At neutral pH, the salt tolerance of citrate stabilized MFs was ~0.08 M NaCl, so remained much below the concentration 0.15 M of physiological salt solution, while that of oleate stabilized MFs was 0.2 M. The latter can be used in biological systems. We suggest this colloidal stability test to use for predicting the aggregation behavior of MFs under physiological condition.

Different aggregation behaviors of CA and NaO stabilized MFs were predicted in the coagulation kinetic measurements. The cytotoxic effects of both magnetic fluids were investigated by means of the MTT assay using a human cell line (HeLa) [4]. Phagocytosis was experienced *in vitro*, tremendous nanoparticles were able to pass cell membrane. The ferrofluids did not dispose significant cytotoxic effect, but the aggregation behaviors were the same as predicted in the simple laboratory test.

Acknowledgement: Grant NKTH-OTKA (A7-69109/2007) supported this work.

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Oncolytic adenovirus magnetic complexes: characteristics and biological efficacy *in vitro*

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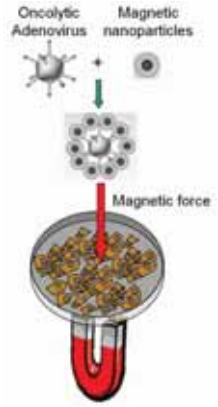
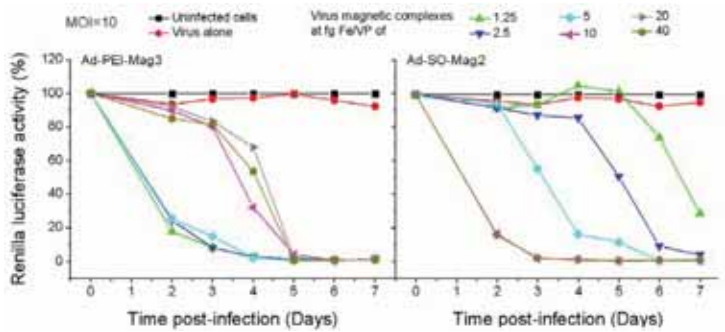


YB-1-dependent oncolytic adenovirus Ad520 was developed to replicate in multidrug resistant (MDR) tumour cells but not in normal cells due to the nuclear localization of the human transcription factor YB-1 in MDR cells and therefore is able to specifically kill tumour cells. Adenoviral infection in many tumour cell types is limited by lacking expression of the coxsackie and adenovirus receptor (CAR). We have previously shown that this limitation can be overcome by magnetofection. We have synthesized a set of polycation-coated iron oxide magnetic nanoparticles and characterized their association with Ad520 adenovirus in terms of binding capacity of the magnetic particles and size and magnetic responsiveness of the resulting complexes. Subsequently we established dose-response relationships in terms of oncolytic activity in vitro in daunorubicin-resistant human pancreatic cancer 181RDB cells stably expressing Renilla luciferase, which are CAR deficient. We found that the native virus required high virus concentration and long incubation times at high concentration to mediate an oncolytic effect. In contrast, the association of the virus with magnetic particles and forcing the magnetic virus complex on or into the target cells by magnetic force under magnetofection conditions greatly increased its oncolytic potency and significantly improved kinetics of oncolysis. Using non-replicating adenovirus as a control, we demonstrated that this effect is entirely due to increased virus replication and not to unspecific toxic effects of the magnetic complexes. We also found that to ensure optimal oncolytic potential and kinetics of oncolysis, magnetic particle-to-virus ratio (shown in the figure below in terms of iron-to-virus particles ratio *fg Fe/VP* for virus complexes with PEI-Mag3 and SO-Mag2 nanoparticles) has to be optimized for each nanomaterial. Ongoing studies will demonstrate whether the observed magnetic boosting of oncolytic adenovirus can be translated to *in vivo* therapy in a CAR-deficient multidrug resistant mouse tumour model.



Funding by the German Academic Exchange Service (DAAD), financial support from the Thailand Research Fund (TRF) through the Royal Golden Jubilee Ph.D. Program to N.T. (Grant No. PHD/0002/2548) and by the German Excellence Initiative via the Nanosystems Initiative Munich (NIM) are gratefully acknowledged.

Kinetics of Nonmagnetic Particle Chain Growth in Biocompatible Ferrofluid

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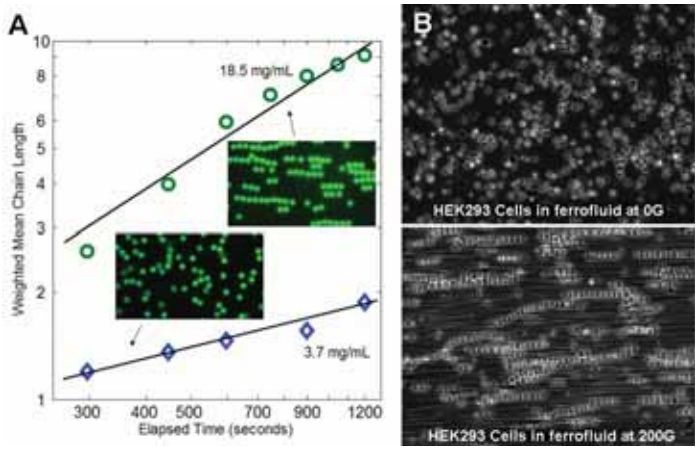
"Inverse magnetophoresis" refers to the manipulation of nonmagnetic materials (e.g., polymer beads, cells, bacteria) inside magnetizable fluids, known as ferrofluid. In essence, the nonmagnetic particles behave like nonmagnetic cavities, which allow them to interact with field gradients produced by other particles as well as magnetic separation systems. Previous work [1] has focused on ferrofluids with high volume fractions which have the advantage of maximizing magnetic potential energies to promote the assembly of long particle chains. However, suspensions with high volume fractions of magnetic nanoparticles are often toxic when used with cells and other biologics. Recently, Rotello et al. have synthesized a new low toxicity ferrofluid [2] passivated with bovine serum albumin (BSA), which has been shown to be highly compatible with cells over long time scales at concentrations around 15 mg/mL.

In this work, we report on the kinetics of chain growth for 10-um sized nonmagnetic particles suspended inside biocompatible ferrofluid. We focus these studies on the low magnetic particle concentration regime, which minimizes issues with cell toxicity and colloidal stability in cell culture medium. We discovered that standard dynamic scaling theory does not correctly describe the chain growth for particles of this size, which assumes a constant dynamic scaling exponent of approximately 0.4. Instead, we found that the scaling exponent strongly depended on the interaction energy, which was adjusted through the ferrofluid concentration, as shown in Figure A. In low ferrofluid concentration of 0.2% vol. fraction, the scaling exponent was 0.3, whereas in high ferrofluid concentration of 1% vol. fraction, the scaling exponent was 0.9. Figure B shows the first results on the *in vitro* formation of chains of cells using inverse magnetophoresis, demonstrating its potential applications in tissue engineering.



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RAPID AND EFFECTIVE CELLULAR MANIPULATION AND SEPARATION USING FERROMICROFLUIDICS

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Manipulation of cells and micro-scale particles within microfluidics play a crucial role in cancer diagnosis, pathogen detection, and genomic testing. Optical tweezers, electric-based schemes – such as electrophoresis and dielectrophoresis – and magnetic tweezers are some of the common methods used for cellular manipulation and separation in this context. However, achieving high throughput and high resolution at the same time has been a challenge for most of these conventional techniques [1]. Among active methods, optical tweezers offer high resolution and sensitivity for manipulating single cells and molecules, though such manipulation is limited to a very small area due to the tight focusing requirements of the laser beam. Magnetic tweezers, on the other hand, are based on trapping and manipulating magnetically labeled particles within tailored magnetic field gradients [2]. The downside to this technique is the lengthy incubation times and wash cycles, and the difficulty of removing the label *post priori*. We have developed a microfluidic platform based on ferrohydrodynamics for the label-free manipulation and separation of cells, bacteria and microparticles within biocompatible ferrofluids. The principle of our technique depends on using the ferrofluid as a uniform magnetic medium within the microfluidic channel. External magnetic fields are applied via currents through integrated electrodes; the magnetic force on nonmagnetic particles which act as "magnetic holes" within the ferrofluid depends sensitively on their size and is typically an order of magnitude larger than what can be achieved by optical tweezers. Hundreds of microns particle separation is easily accomplished on a time scale of a few seconds under moderate currents within our microfluidic devices.

For our experiments involving microparticle and cell manipulation, we have developed a water-based, neutral pH ferrofluid comprised of cobalt-ferrite nanoparticles. The average diameter of individual nanoparticles as determined from transmission electron microscopy (TEM) images is 12 nm. Experiments carried out using different sizes of fluorescent latex microspheres have demonstrated controlled manipulation of different sizes of beads at different values of excitation frequencies and current values (fig.1a). By varying the excitation frequency or electrode spacing, we have achieved rapid separation of different size particles (fig.1b). We have performed a set of experiments to test our particle manipulation platform using *E. coli* bacteria. The experiments demonstrated that the bacteria immersed in the ferrofluid could be easily controlled and trapped by changing the direction and the frequency of the excitation. At moderate current values, bacteria achieved average velocities of 20 μm/sec (fig.1c). Overall, the high velocities for particles obtained as a result of our experiments, even under low and moderate current values, establish these ferromicrofluidic devices as highly effective platforms for cellular manipulation and sorting.

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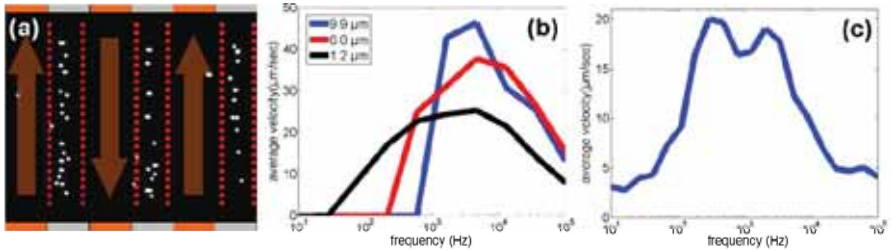

Figure 1: (a) Particle distribution within ferromicrofluidic channel after the excitation, at a frequency that results in particle trapping between electrodes. (b) Velocity versus frequency plot for 3 different sizes of fluorescent latex microspheres, depicting that the frequency to trap particles is specific to particle size. (c) Velocity versus frequency plot for *E. coli* bacteria. Bacteria can reach continuous velocities of 20 μm/sec and be easily manipulated at moderate current values.

On the encapsulation of superparamagnetic nanoparticles in icosahedral virus capsids

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Virus-like particles containing a superparamagnetic iron oxide core surrounded by a viral protein cage have been synthesized. The synthesis is based on spontaneous self-assembly. We will discuss here the surface properties that the magnetic core needs to possess in order to promote the self-assembly of a protein cage around it and the relation between the size of the cargo and the structure of the protein cage. These virus-like magnetic particles blend the innate biological properties of viruses with the physical properties of iron oxide nanoparticles and could be useful to probe the systemic movement of similar viruses by magnetic resonance imaging or for specific delivery of therapeutic purposes. First MRI results of magnetic virus-like nanoparticles in plants will be presented.

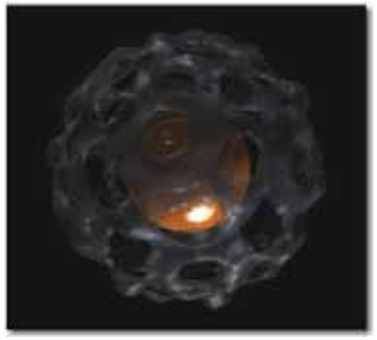

Figure: Single particle reconstruction image of a virus-like particle composed of an inorganic nanoparticle surrounded by a symmetric self-assembled protein cage. (Data was obtained by TEM and the 3D reconstruction was performed using the EMAN software suite.)

Applications of GoldMag® Particles in Molecular Biology

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The composite particles combining properties of gold colloid and magnetic nanoparticle have become a hot spot in recent years. The particles have advantages of large surface and superparamagnetic. Moreover, the nano-sized composite particles have characteristic optical spectra like nanogold. We have synthesized Fe_3O_4/Au particles with core/shell or assembled structure which have been proved a useful tool in assay or separation of biomolecules. The gold modified magnetic nano-particles were named GoldMag® and the related products have been commercialized by Lifegen in China(http://www.lifegen.com). Herein, we would like to give a brief introduction to the synthesis of GoldMag® particles and their applications in molecular biology.

GoldMag® particles have been successfully used in antibody/antigen immobilization, purification of nucleic acid and removal of high abundant protein from plasma. The capacity of protein coupling on 1 mg GoldMag® particles is 3~6 folds higher than those composed with polymer and magnetic particles. Avian IgY antibodies against high abundant protein was coupled on GoldMag® particle surface and used to remove some proteins in plasma such as human serum albumin (HSA) or IgG. The prefractionation technique play a very important role in the research of proteomics. Figure 1 shows the SDS-PAGE results of pre- and post-removal of HAS and IgG from human plasma.

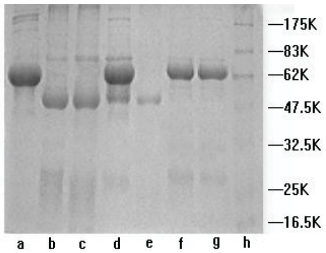

Figure 1 SDS-PAGE results of plasma treated by IgY-coupled GoldMag® particles
Lane a: HAS standard; Lane b and c: samples after HAS removal; Lane d: unprocessed plasma; Lane e: IgG standard; Lane f and g: samples after IgG removal; Lane h: Marker

The GoldMag® particles can also be used in purification of nucleic acid (including DNA and RNA). Compared with the traditional ways, this method has the advantages of the rapid performance, easy operation with high DNA/RNA yield and quality. In addition, it can be used in the automated purification system as well.

Vesicles encapsulating quantum dots and magnetic nanoparticles for multimodal imaging

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Semiconductor nanocrystals, known as QDs, are fluorescent nanoparticles of 1-10 nm in diameter that offer distinct spectrofluorometric advantages over traditional fluorescent organic molecules. QDs exhibit fluorescence characteristics that are 10-20-times brighter than conventional dyes, greater photostability, broad excitation wavelength range, size tuneable spectrum and narrow and symmetric emission spectrum. Owing to these photophysical characteristics they are being explored as potential imaging agents in fluorescence-based diagnostic applications. The properties of magnetic particles as T2 contrast agent are well known. The aim of this work is to combine these two properties in a unique carrier. The container is a liposome which allows to encapsulate hydrophilic and/or lipophilic nanoparticles. Moreover such magnetic containers may be guided in a magnetic field gradient in order to target a tumor for example. The properties of magnetic guidance of such liposomes have soon been established as well as their properties as contrast agent [1]. The process used to encapsulate the two types of nanoparticles is a reverse phase evaporation synthesis. The hybrid fluorescent and magnetic vesicles are characterized by optical, confocal and electronic microscopy [2]. They have been injected and followed in mice thank to a confocal fiber. The interesting point of this synthesis is their adaptability to a lot of nanoparticles, we will present a variety of hybrid vesicles encapsulating gold nanoparticles for examples.

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Giant vesicles containing magnetic nanoparticles and quantum dots: feasibility and tracking by fiber confocal fluorescence microscopy.

Magnetic Nanoparticles to Regulate Cardiac Metabolism

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In patients with diabetes, cardiovascular disease is the leading cause of death. Although coronary vessel disease and atherosclerosis have been identified to be the primary reasons for the increased incidence of cardiovascular dysfunction, the earliest change that occurs in the diabetic heart involves an alteration in energy metabolism. Diabetes impairs cardiac glucose utilization, leading to the exclusive use of **fatty acids (FA)** and eventually, lipotoxicity. Glucose utilization provides the heart with approximately 30% of its energy requirements, whereas FA contributes approximately 70% of ATP necessary for normal heart function (**Figure 1**). Following hypoinsulinemia or insulin resistance, impaired glucose transport and utilization switches energy production exclusively to oxidation of FA. Given the pivotal function of lipoprotein lipase LPL in FA delivery, and FA's contribution in mediating lipotoxicity, limiting FA delivery through this mechanism may be crucial for preventing diabetic heart disease.

We are investigating the use of magnetic nanoparticles for site-specific intracoronary heparin delivery to prevent the enhanced LPL activity in diabetic hearts and potentially modulate lipotoxicity. Heparin was covalently bound to functionalized magnetic polystyrene nanoparticles. Toluidine blue assay and activated partial thrombin time (APTT) confirmed and quantified the presence and quantity of heparin bound to the particles. *In vitro* studies of freshly prepared myocytes were undertaken to examine if the cardiac LPL could be targeted for prevention of altered metabolic processes that initiate and sustain heart failure during diabetes.

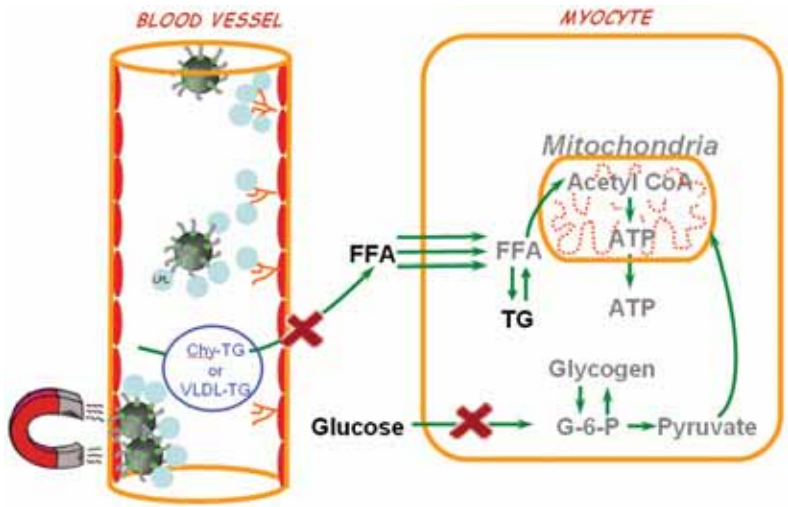

Fig. 1. Fatty acids are the main source of energy to the *diabetic* heart muscle cells (myocytes). With heparin-coated magnetic microspheres, we are attempting to reverse this pathway and prevent fatty acid toxicity.

Hydrogen Peroxide Removal with Magnetically Responsive *Saccharomyces cerevisiae* Cells

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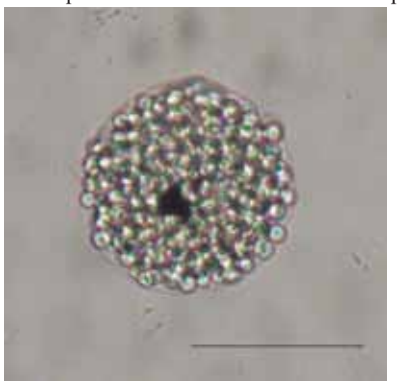
Hydrogen peroxide (HP) is a very effective microbicidal agent. A major reason for the growth in popularity of HP in the food, cosmetic, and medical fields is its low toxicity at use concentrations as well as its safe decomposition products (water and oxygen). HP is effective against a wide spectrum of microorganisms, including bacteria, yeast, molds, viruses and spore-forming organisms. The use of HP in food industry has been approved in several countries, for example in the production of Swiss cheese and for milk cold pasteurization.

The excess of hydrogen peroxide is usually destroyed by adding catalase (hydrogen-peroxide:hydrogen-peroxide oxidoreductase, EC 1.11.1.6). Commercial catalase preparations are rather expensive. In selected cases crude microbial extracts from approprite microbial producers can be used. Microbial cells can serve as inexpensive substitutes of purified enzymes. In the case of catalase, readily available *Saccharomyces cerevisiae* cells (bakers' yeast) are of special interest, due to their broad application in food industry; they are classified as "Generally recognized as safe (GRAS)". The cells can be used both in the native state and in the immobilized form.

In order to simplify manipulation with the biocatalyst, we have prepared magnetically responsive alginate beads containing entrapped *Saccaromyces cerevisiae* cells and magnetite microparticles. Larger beads (2-3 mm in diameter) were prepared by dropping the mixture into calcium chloride solution while microbeads (the diameter of majority of particles ranged between 50 and 100 μm) were prepared using the water in oil emulsification process. In general, microbeads enabled more efficient HP decomposition. The prepared microparticulate biocatalyst caused efficient decomposition of HP in water solutions up to the concentration of 2 %, leaving very low residual HP concentration after treatment (below 0.1 %). The biocatalyst was quite stable; the same catalytic activity was observed after one month storage at 4 ºC and the microbeads could be used at least five times. Due to the presence of variety of enzymes in the entrapped yeast cells, this inexpensive biocatalyst can also be used for other biotechnology processes.



Magnetically responsive alginate microbead containing entrapped *Saccharomyces cerevisiae* cells and magnetite microparticles. The scale bar corresponds to 50 μm.

Application of Magnetic Nano Particles to Full-automated Chemiluminescent Enzyme Immunoassay (CLEIA)

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Magnetic particles attract increasingly attention since their magnetic properties that enable magnetic particles to be used for biomagnetic separation and magnetic signal detection. The magnetic particles are typically on order of microns in size. A larger surface area is expected to show higher specificity for the reaction and shorter reaction time than those with conventional ones. However, when the size of the magnetic particles are less than several hundreds nanometers or lower they become difficult to magnetically collect. To resolve this dilemma, magnetic column was used to separate the nano-sized magnetic particles or the magnetic particles are coated with thermo-responsive polymers that reversibly aggregate and disperse through temperature change.

We have developed magnetic nanoparticles with diameter of less than 100 nm which can be magnetically collected by adding a small amount of flocculants at a constant temperature (Fig. 1). We used the magnetic particles as a carrier and developed an automated sandwich Chemiluminescent Enzyme Immunoassay (CLEIA) to detect Thyroid-stimulating hormone (TSH) successfully (Fig. 2). The TSH is sensitively and specifically detected by automation because unlike those micron sized magnetic particles, the completely dispersed magnetic nanoparticles in aqueous solution admit an accurate automatic handling. The convention micron sized magnetic particles are used to compare with our magnetic nanoparticles. Signals detected with magnetic nanoparticles are 8-fold higher than those with micron sized magnetic particles. A Within-reproducibility study shows coefficient of variation (CV, n = 10) less than 5%, suggesting a stable detect method.

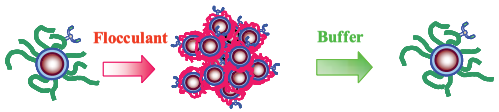

Fig. 1 Schematic diagram illustrating aggregation of magnetic nanoparticles

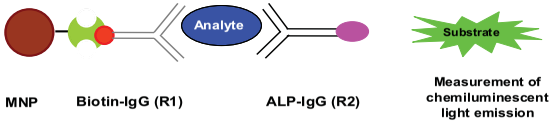

Fig. 2 Schematic diagram illustrating CLEIA by using magnetic nanoparticles

Monitor the kinetics of stem cells differentiation on nanosurfaces using optical waveguides

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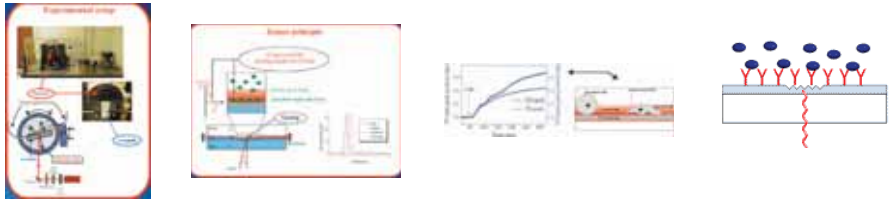
Abstract

Cell adhesion is an active process, carried out in vivo via receptor ligand-like interactions between cell surface adhesion molecules and the extracellular matrix. Initial cell surface reactions following contact may trigger multiple responses, which in turn result in either spreading or detachment of the cell. The set of adhesion and attachment molecules mediating the adhesive behaviour of stem cells and the kinetics of their interactions are as yet largely unknown. Substrate-stem cell investigations are important for therapeutic (such as 3D tissue growth) [1] and non therapeutic applications, e.g. cell development for therapy, disease modeling and protein production. Optical waveguide lightmode spectroscopy (OWLS) is a powerful technique for the precise quantifications of thin film material deposition on a solid waveguide surface [2] (within the sensing depth ~150 nm), due to the relevant shallow penetration depth cell focal adhesion events are mainly detected using OWLS whilst the majority of the cellular mass remains beyond the detection field. Using a novel waveguide design we are able to tune and predefine the probing depth and obtain more detailed information on the morphology of the cells and their behaviour at the surface [2], and has also been used to measure the kinetics of cell adhesion and spreading on predefined surfaces [3], and the attachment of bacteria [4]. We have therefore investigated the attachment and spreading of precursor cells on various surfaces ranging form glycoprotein to nanotextured metal oxides using OWLS. We suggest possible future non-therapeutic applications for these substrates.

Keywords: stem cells; optical waveguide; kinetics; attachment; spreading



Figures: Optical waveguide lightmode spectroscopy technique

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Red blood cells as carriers for magnetically targeted delivery of drugs

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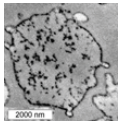
Although liposomal formulations of several drugs focus on the improvement of tolerability, certain disadvantages of liposomes, including their fast elimination from blood, failure to reach therapeutic concentrations at the target cells. Therefore red blood cells (RBC) were used and tested as natural carriers for nanoparticles.

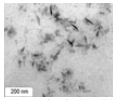
Erythrocytes were loaded with a nanosuspension of AmB and biocompatible, surface-tunable, luminescent, and magnetic nanoparticles. Additionally, the surfaces were modified with specific antibodies for targeting.

The loading procedure leads to a 10^4 times higher concentration of AmB per RBC compared to liposomes. It was proven that an amount of only 750 AmB-loaded erythrocytes per ml is sufficient to reach an antifungal effect, representing one millionth of the physiological erythrocyte concentration in human blood (4-6*10^9 per ml). Loading erythrocytes simultaneously with both, AmB and nanomagnets, did neither reduce the AmB concentration per erythrocyte nor its bioactivity. Additionally, nanomagnets loaded erythrocytes can be visualized by MRI. In-*vitro* phagocytosis assays using AmB loaded erythrocytes show a significant inhibition of free fungal activity. This effect can be observed in viability assays using flowcytometric analysis and direct cell culture assay.

The advantages of our carrier-system are not limited to AmB. Any other water insoluble drug has potential to be loaded onto erythrocytes. Additionally, coupling of specific antibodies can modify the surface of the carrier system, which is an effective tool for targeting. The carriers cannot just deliver a high dosage of different drugs but also protects them from inactivating effects and minimizes side reactions. Additionally they can be influenced by external fields. The diagnostic capacities as MRI contrast media and the possibility to focus on certain tissues are additional advantages of these drug carriers.



TEM image of Magnetite loaded RBC



TEM image of AmB and Magnetite loaded RBC

Nanocristals (AmB); dark spots (Magnetite)

CLSM image: Phagocytosis of FITC-labeled AmB-RBCs (green)

Magnetite loaded RBC can be moved by a magnet

Acknowledgements
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Lab On A Chip Detection of Biomolecules Using Magnetic Bead Labels

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We present our results on an integrated lab-on-a-chip (Fig. 1) for sensing biomolecules using inductive detection of magnetic beads at their ferromagnetic resonance (FMR) frequency. Detection of the beads indicates the presence of target molecules bound by a "sandwich" assay. This research consists of a system level design of four components: design and characterization of FMR-based bead detector, synthesis of custom nanobeads, biochemical functionalization of the bead and detector surfaces, and integration of on-chip electronics.

FMR detection of the magnetic beads, as shown in Fig. 2, occurs at a short circuited junction of two uniplanar microwave waveguides [1]. The junction is functionalized and serves as the active detector area. Commercially available magnetic labels are not optimized for FMR detection techniques. Consequently, our efforts on custom bead synthesis have focused on producing uniform beads with the desired properties - high magnetic moment, chemical stability and exhibiting superparamagnetism. The beads have a Co core with a SiO_2 shell and vary from 20 nm to 60 nm in diameter. Several reaction procedures were investigated to yield varying core configurations (Fig. 3). The SiO_2 shell protects the core from oxidation and facilitates biochemical functionalization of the beads. Fluorescence experiments have confirmed immobilization of these surface-modified nanobeads to a silica surface via biotin-streptavidin binding.

The sensitivity of an immunosensor, such as being discussed here, depends not only on the electrical sensitivity to the labels but also on the rate of the biochemical reactions. The reaction rate is determined by the functionalized area of the beads and the detector surface, and the rate of diffusion of the beads. The surface-to-volume ratio of our beads is designed to provide adequate surface area for functionalization and a detectable magnetic moment. The active sensor or junction area is large (3900 µm^2) to ensure a high interaction rate and high probability of immobilization.

Similar biosensor platforms have previously been designed using giant magnetoresistance (GMR) detection [2, 3]. Magnetoresistive sensors require the fabrication of specialty thin films. In contrast, FMR detection uses a single metal layer fabricated on top of a dielectric, and is easily realized in the top metal layer of an integrated circuit. We have designed the excitation and detection electronics for the detector in 0.18 µm CMOS technology. A lock-in frequency modulation method is used to robustly discriminate the FMR signal of the magnetic bead from stray coupling. Beads at the sensitive area are modulated in and out of FMR, resulting in an amplitude modulated output signal that represents a signature of the beads.

Fig 1. Integrated Lab-On-A-Chip

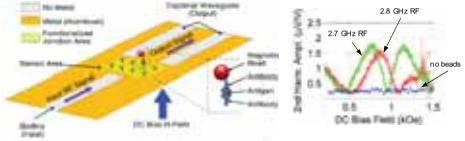
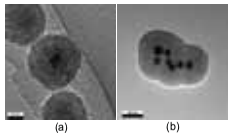


Fig 2. (left) Illustration of FMR detector. (right) Measured data for 4.5 µm M-450 Dynabeads placed at the junction.



(a) (b)

Fig 3. Transmission electron microscopy images showing (a) single Co core and (b) multiple Co core beads.

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[2] R. Edelstein, et. al, "The BARC biosensor applied to the detection of biological warfare agents", *Biosensors Bioelecton.*, vol. 14, no. 10, pp. 805-813, 2000.
[3] B. de Boer, et. al, "An integrated and sensitive detection platform for magneto-resistive biosensors", *Biosensors Bioelectron.*, vol. 33, no. 9-10, pp. 2366-2370, 2007.

Development of a magnetic lab-on-a-chip for point-of-care diagnosis

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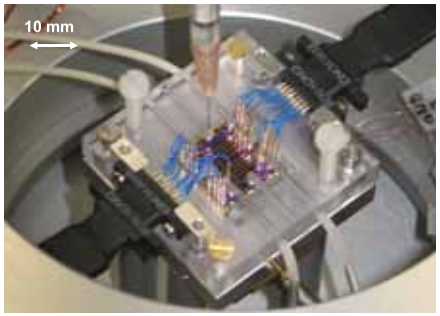
The term lab-on-a-chip defines microfluidic systems that automatically perform numerous tasks in the field of biomolecular diagnosis. The ultimate goal is to develop a system that is capable to sensitively and specifically detect multiple targets from natural sample material without any further input from the user, thus creating a compact instrument suitable for point-of-care diagnosis.

Very promising candidates for such labs-on-a-chip are magnetic biochips. In these systems, magnetic particles (markers) are employed both for sample pre-treatment and target molecule detection. We mix markers functionalized with primary antibodies against sepsis-indicative cytokines with the original sample solution. About 10 µl of the mixture is injected into a reservoir on our microfluidic chip, and the markers are magnetically stirred through the solution by gradient fields from small coils situated underneath the chip. In order to also generate repulsive forces, we superimpose a homogeneous magnetic field from Helmholtz coils that surround the entire chip. Due to the active motion of the markers, the diffusion limit normally encountered in molecular binding processes can be overcome. Afterwards, the markers are magnetically withheld during a replacement step of the sample solution with buffer, leaving only the markers and the molecules bound to their surfaces. Next, the markers are magnetically transported by a moving coil along a fluidic channel that contains detection zones of different sizes which are functionalized by secondary antibodies against the target cytokines, allowing sandwich-immunoassay type immobilization of the marker-bound cytokines. Underneath the detection zones, embedded magnetoresistive sensors (GMR-type $Ni_{80}Fe_{20}$/Cu-mulilayers) register the magnetic stray field of the markers by a resistance change that is proportional to the aerial density of bound markers. Thus, the markers function both as tools for sample preparation and molecular detection, and the entire process flow is ideally suited for an automated and compact point-of-care diagnosis instrument.

We will present our current progress on the ongoing development of our magnetic lab-on-a-chip demonstrator. At the time of abstract submission, this includes demonstration of the concept, verification of operation (i.e. the magnetic marker manipulation and detection) and preliminary results on the immunological functionality (i.e. antibody immobilization).



View of the microfluidic chip (center) with fluidic and electrical connections. The sample can be injected either by the fluidic system or directly with a syringe. Underneath the chip, millimeter-sized coils are situated for generating magnetic gradient fields, while Helmholtz coils enclose the entire setup and generate a homogeneous field.

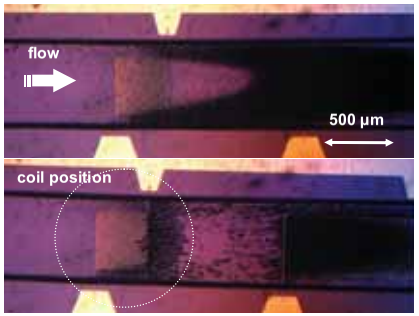


Image series demonstrating manipulation of magnetic markers (black). The markers flowing downstream along the channel (upper image) are recollected to the sensor site after turning on the gradient field of a coil that has been positioned underneath the respective sensor (lower image).

Magnetic beads on biosensor platforms for protein, DNA and cell detection

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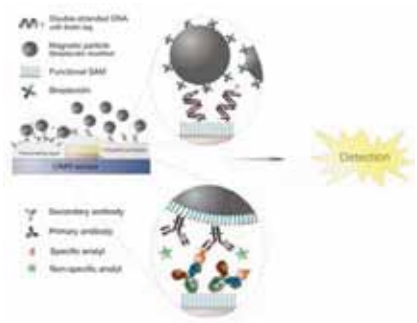
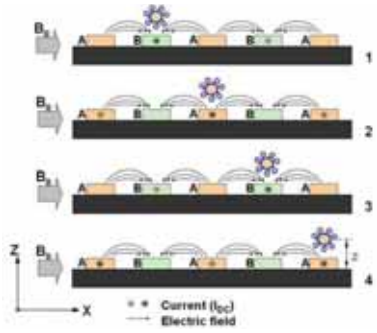
The presented work is supported by the IST program of the Commission of the European Communities (project 027652 MASCOT)



Despite great efforts in various application fields, most biosensors have not met the required sensitivity and specificity to allow for label-free detection. The use of superparamagnetic labels may form an interesting alternative to fluorescent labels. Not only can they be sensed at very low concentrations, their use for the isolation of cells, proteins, and nucleic acids makes it possible to combine sample (pre)purification steps and detection on a single lab-on-a-chip platform. For this reason, IMEC has examined both giant magnetoresistive (GMR) sensors for detection and current carrying conductors for the manipulation of magnetic beads. For magneto-resistive sensors, the sensor signal shows a clear dependence on the position of bound magnetic particles relative to the sensor location. Based on this phenomenon, we have explored the ability to enhance the sensitivity and specificity via active guiding of magnetic particles to the position that theoretically gives rise to the maximal signal. Exploiting this methodology, we have established the sensitive detection of both DNA and proteins (e.g. detection of the stroke marker S100ββ from ~1 ng/mL down to ~25 pg/mL). Aside from DNA and protein sensing, we present a modular disposable microsystem for the immunomagnetic isolation and enrichment of rare cancer cells. The actuation of labeled cells is achieved by sending an alternating current to an array of parallel GMR sensors. Separation is accomplished by the differences in the magnetic mobility between the cell complexes and unlabeled beads. Besides the magnetic force, a negative dielectrophoretic force is applied as well in order to control the distance between the device and the cell-bead complexes. Without the latter force, the complexes adhere to the surface as a result of weak electrostatic and attractive magnetic force components. Actuated by the traveling magnetic field, isolated cells can be counted while passing over a sensor.



In short, magnetic labels are of great value for biosensing or on-chip manipulations in general. This dual function makes them particularly suited to be used in point-of-care diagnostic devices. Such lab-on-a-chip systems are considered most promising as they allow for easy to perform, reliable, portable and cost-effective testing without major human intervention.

Affinity Analysis for Biomolecular Interactions Based on Magneto-Optical Relaxation Measurements

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Magneto-optical relaxation measurements of magnetically labelled biomolecules are a promising tool for immunometric analyses. Carcinoembryonic antigen (CEA) and its polyclonal and monoclonal antibodies (anti-CEA) were utilized as a model system for affinity analysis of the interaction between antibody and antigen. For this purpose antibodies were coupled with magnetic nanoparticles (MNP). Aggregation of these antibody sensors due to interactions with the antigen CEA was observed subsequently by measuring the relaxation time of the optical birefringence of a transmitted laser beam that occurs when a pulsed magnetic field is applied to the functionalized MNP. The added amount of antigen accounts for the aggregate sizes (Figure 1).

A kinetic model of chain-like aggregation of the particle complexes developed for these purposes allows the rapid and simple calculation of the equilibrium constant K_D and the concentration of antibodies c_{Ab} bound on MNP of the underlying protein interaction. From the known antigen concentration c_{Ag} and the increase in particle size during the interaction we are able to estimate the unknown parameters with standard methods for the statistical description of stepwise polymerization. We generated a special scaled diagram enabling the calculation of K_D and c_{Ab} in a very easy and rapid manner (Figure 2). An antigen amount dependent increase of the parameter y containing the conversion of the interaction and the aggregate size was observed. K_D and c_{Ab} can be directly determined from the regression parameters. This novel affinity analysis was successfully applied for the antigen antibody interaction described herein and can be applied to other biomolecular interactions. It offers the simple and rapid calculation of kinetic parameters in a magneto-optical immunoassay.

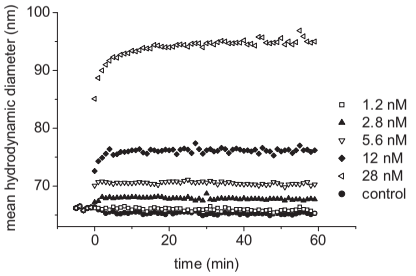

Figure 1. Mean hydrodynamic particle diameter from the incubation of monoclonal anti-CEA-MNP with different amounts of CEA

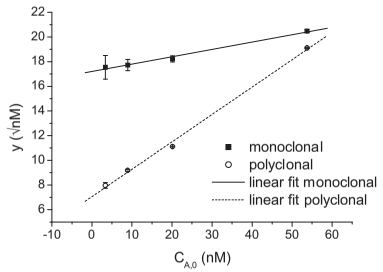

Figure 2. Scaled Plot of the interaction between CEA and the monoclonal and polyclonal antibody sensors. Mean y-values in steady state (30-60 min interaction time) ± SD.

Measurement of the specific binding of functionalised MNP in whole blood

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Specific molecular binding as a key principle in the metabolism in the living world is also the fundament of many techniques for the detection and quantification of biomolecules, where a specific detection molecule binds to an analyte. Often, the detection molecule is equipped with a marker, e.g. fluorescence molecule, radioactive label, producing a signal which can be measured. In this work, the markers are magnetic nanoparticles (MNP) which are detected by magnetorelaxometry (MRX). MRX measures the magnetisation decay of the sample after a magnetising field is removed. The signal is specific for the MNP and not influenced by the biological matrix matter and hence is detected virtually without any background perturbation . Furthermore, bound MNP-probes can be quantified in the presence of unbound ones because distinct relaxation signals are observed for both species. Therefore, utilizing MRX for the quantification of MNPs and their specific binding in whole blood, no other preparations than adding MNPs to the sample are required.

We quantified the total number of binding sites of thrombocytes using the specific binding between MNP-labelled monoclonal antibodies (mAb) anti-CD61 and the CD61 antigen expressed on the membrane of thrombocytes. Varying the concentration of thrombocytes by preparing a dilution series of the blood we observed a nearly linear relationship between thrombocyte concentration and the amount of bound MNP probes. The specific binding was validated by preincubation of the blood samples with an excess of unlabelled anti-CD61. For samples prepared in this way, no binding of magnetic probes could be observed. Our results demonstrate, that despite of the high variety of blood constituents which could unspecifically bind the MNP-probes and cause aggregation, the MNP probes bind specifically.

Using biotin bound to agarose beads as a test analyte and MNP-labelled streptavidin as the detection molecule, we investigated the influence of the medium on the specific binding. We incubated the biotin-agarose beads suspended in bovine serum albumin, human serum, tempered human serum and whole blood with the magnetic streptavidin probes. One of our findings was that after 30 minutes the fraction of bound probes in blood is only half as much as in BSA-buffer or in tempered human serum (figure). This indicates that the preparation of the whole blood sample has a significant impact on the binding.

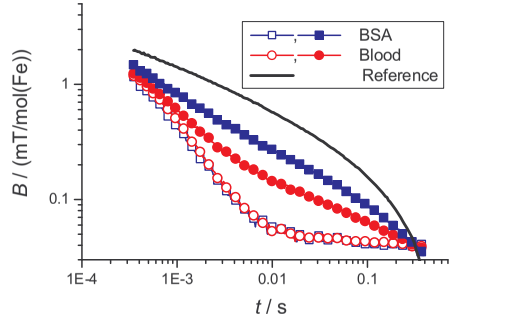

Figure: Relaxation signals of streptavidin-MNP probes added to biotin agarose which is suspended in BSA buffer or blood, respectively. Open symbols: probes are saturated with free biotin before incubation (controls). Reference: 100% immobilized MNPs.

Lateral Flow Immunoassay Using Magnetoresistive Sensors

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Magnetic particles have been adapted for use as biochemical labels in several assay formats. One format that, due to its low cost, has near-term potential for commercialization is the lateral flow strip test. Generally, a lateral flow strip test uses passive microcapillary action in a permeable material to draw a liquid sample through the detection system. Biochemically sensitive "capture sites" are created in the membrane such that the "analyte" that is to be detected will stick to the capture site while most of the other material in the sample flows by. Also sticking to the capture site is a "label" that is detectable in some way. Most commercially available strip tests (e.g. pregnancy test) are detected with the naked eye, and the label is a dye or cluster of colloidal gold. These visually read tests are inexpensive, but do not provide very quantitative information about the concentration of the analyte in the test sample. Rather, they give a YES/NO result.

More quantitative results can be obtained by using electronic detection systems. One magnetic detection system for lateral flow tests has recently been demonstrated for identification of e-coli strains using an immunoassay biochemical detection format [1]. The measurement apparatus was based on coil excitation and detection; if the magnetic labels were in the effective sample volume of the pickup coils, they produced a detectable change in magnetic susceptibility.

The present work is based on a similar magnetically labeled immunoassay format, but uses Giant Magnetoresistive (GMR) sensors instead of pickup coils. These small integrated sensor chips can detect the presence of magnetic labels in capture spots whose volume is approximately 150 μm x 150 μm x 150 μm. The range of linear detection is better than two orders of magnitude; the total range is up to 5 orders of magnitude. In this paper, we present results of a model immunoassay. The target analyte is mouse anti-rabbit IgG and the label is a 440 nm diameter magnetic bead (see Fig. 1 below). Initial experiments demonstrate detection levels at least 10 times lower than the manufacturers recommended concentration for chemiluminescence detection.

This demonstration involves bringing the sample to the magnetic detector using mechanical means. Ultimately the goal is for the detector to be fully integrated into the lateral flow strip backing to form a single consumable item [2].

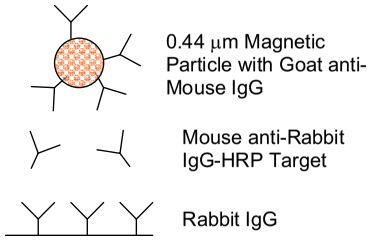

Figure 1. A cartoon schematic of model sandwich assays for magnetic detection on lateral flow strips.

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Multiparametric Magnetic Immunoassays

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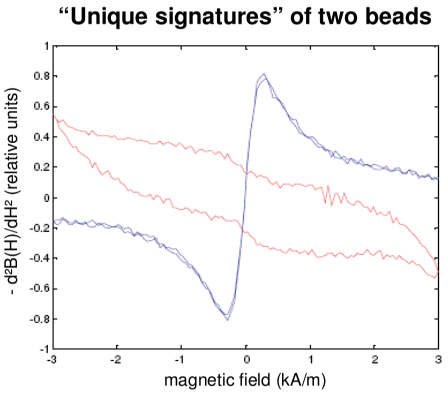
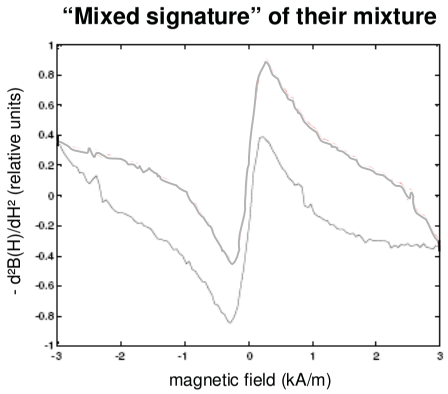
The use of magnetic beads as labels in bioassays has many advantages which are increasingly being recognized: intrinsic ability for quantification, time and temperature stability, easy transduction, commercial availability, high sensitivity. Utilizing the unique non-linear response of nanometric sized magnetic particles in the presence of a multi-frequency alternating field has enabled magnetic immunoassays with a lowered limit of detection and the flexibility to accommodate a variety of consumables applicable to many real-life applications.

Now, despite this increasing interest and intensive scientific efforts, magnetic beads were so far never reported as labels for multiparametric testing.

With the measurement of non-linear response as a starting point, we have shown that one can use a set of magnetic beads differing by their magnetic properties for the investigation of a single sample potentially containing a plurality of biological analytes. The method does not require aliquoting or any spatial resolution of the sample. It comprises the construction of a « unique signature » specific for the magnetic material included in each kind of magnetic beads. This signature depends on the nature of the magnetic material, i.e., composition, size, shape. A couple of magnetic beads differing enough by their magnetic signature is then selected, one specific for the analyte A (e.g., adenovirus), the other for the analyte B (e.g., rotavirus). The « mixed signature » of a mixture of both beads is then measured and compared to the unique signature for each. Proper data processing allows the determination of the presence/absence of each kind of bead, and also permits the quantification of each population, thus retrieving the amount of both analytes in the sample.

The MIAplex™ technology can be embodied in several formats including well known lateral flow membrane tests. Several families of magnetic materials differing by their signatures have already been identified.





Poster Abstracts

#	*First Author*		*Poster Title*
	Gene Delivery		
1	Jon	Dobson	Improved magnetic nanoparticle-based gene transfection using Halbach arrays
2	K.	Kamei	Direct Cell-entry of Gold/iron-oxide Magnetic Nanoparticles Enhanced Adenovirus Mediated Gene Expression in Adenovirus-resistant cells
3	Aparajita	Khatri	Adenoviral vectors and magnetic nanoparticles: Prospects in cancer gene therapy
4	Boris	Polyak	Superparamagnetic polymeric nanoparticles efficiently enhance non-viral nucleic acid delivery
5	Yolanda	Sanchez Antequera	Magselectofection – combined magnetic cell separation and magnetofection
6	Xiaoliang	Wang	Non-viral Gene Transfection Enhanced by Magnetic Nanoparticles
	Hyperthermia		
7	Daniele	Bonacchi	New nanodevice based on cobalt ferrite for magnetic fluid hyperthermia
8	Nikolai	Brusentsov	Combined MRI-adaptive Magnetohydrodynamic Thermochemotherapy for Improved Cancer Treatment
9	Tatiana	Brusentsova	RE –substituted Mn-Zn ferrite nanoparticles: size-dependent magnetic properties and AC-heating efficiency
10	Cindi	Dennis	The Influence of Temperature on the Magnetic Behavior of Colloidal Cobalt Nanoparticles
11	Atsuo	Hirukawa	Temperature rise of Fe3O4 nanoparticle measured under ac magnetic field and its evaluation by ac magnetization curve
12	Arkadiusz	Jozefczak	The effect of preparation of surface-active coating on the physical properties of biocompatible magnetic liquids
13	Timothy	Kline	Bio-Compatible High-Moment Magnetic Nanoparticles for Hyperthermia Treatment Optimization
14	Eva	Natividad	Specific Absorption Rate of Ferrofluids using a new adiabatic magnetothermal setup
15	Emil	Pollert	Search for new core materials for magnetic fluid hyperthermia
16	N. K.	Prasad	A novel approach to magnetic hyperthermia using biphasic gel of La1-xSrxMnO3 and maghemite
17	Amit	Sharma	Core shell iron-iron oxide magnetic nanoparticles for cancer treatment
18	Makoto	Suto	Heat dissipation mechanism of nanoparticle in magnetic fluid hyperthermia
19	Makoto	Suto	Heat diffusion characteristics of magnetite nanoparticles Dispersed Hydro-gel in AC magnetic field
20	Milan	Timko	Magnetic properties and heating effect in bacterial magnetic nanoparticles
21	Ainhoa	Urtizberea	Concentration effects in Maghemite-Based Ferrofluids
22	X.F.	Wang	Magnetic Hyperthermia in Tsinghua University
23	Liying	Zhang	Heating ability of monodisperse magnetite nanoparticles with various sizes by solvothermal method
	Magnetic Separation		
24	Entesar	Al-Hetlani	Generation and Manipulation of Magnetic Droplets
25	Jeff	Chalmers	Quantification of non-specific binding of magnetic nanoparticles: Implication for detection and magnetic cell separation
26	Haitao	Chen	3-D Modeling and in vitro investigation of portable high gradient magnetic separator device designs for blood detoxification
27	Ting-Hao	Chung	Application of Magnetic Poly(Styrene-Glycidyl methacrylate) Microspheres for Immunomagnetic Separation of Bone-Marrow Cells
28	D.	Huska	The effect of length of adenine chain on its isolation and detection using paramagnetic particles coupled with square wave voltammetry
29	Michail	Larin	Development of magnetic SiO2-coated, antibody-targeted particles
30	Miyaji	Masaaki	Development of low nonspecific bonding magnetic beads for bioseparation
31	Takashi	Nakabayashi	Magnetic particles with high magnetization for immunoassay
32	Joong Hee	Nam	Preparation and Characterization of Electrospinning NiZn-ferrite Nanofibers for DNA Separation
33	Joong Hee	Nam	Spray-dried Yttrium Iron Garnet Powders for Bio-purification
34	Pulak	Nath	A magnetophoresis chamber with 25 fractions output – design, fabrication and demonstration
35	Mohammad Reza	Nejadmoghaddam	Optimization of magnetic affinity separation method for Highly-efficient purification of polyhistidine-tagged Proteins
36	Sally	Peyman	On-chip deflection of magnetic microparticles in continuous flow. A comparative study.
37	Agmon	Porat	Combinatorial Magnetic Separation - a novel third generation concept
38	Thomas	Schneider	Scale-up and comparability study of T-cell depletion in single inlet – single outlet QMS
39	Adriana	Sprincova	Effects of ionizing radiation on induction of deletion mutants in yeast Saccharomyces cerevisiae
40	P. Stephen	Williams	Theory for Nanoparticle Retention Time in the Helical Channel of Quadrupole Magnetic Field-Flow Fractionation

#	*First Author*		*Poster Title*
41	Liping	Yu	Magnetic Focusing for Cell Enrichment and Analysis
42	Wen	Zeng	Research on relationship between surface structure and morphology of Fe3O4/Silica composite nanospheres and nucleic acid extraction
	Magnetic Drug Delivery		
43	Javed	Ally	Drag Force on Magnetic Microparticles at an Air-liquid Interface
44	Nathalie	Bock	Magnetic scaffolds for tissue engineering
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IMPROVED MAGNETIC NANOPARTICLE-BASED GENE TRANSFECTION USING HALBACH ARRAYS

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With the sequencing of the human genome and the advent of gene therapy has come the need to develop effective delivery and transfection agents. These agents must be able to target therapeutic and reporter genes to the relevant cells and organs both in vitro for basic investigations as well as in vivo for therapeutic applications. Recent safety concerns over the use of viral vectors has begun to shift the emphasis toward the development of non-viral delivery agents, primarily cationic lipids. At present, this is generally accomplished through lipid-mediated transfer or electroporation. However, these techniques suffer from significant drawbacks such as: (i) low levels of transfection in primary cells and some cell lines (ii) their inability to effectively transfect tissue explants (iii) detrimental effects on cell viability (primarily with electroporation) and (iv) difficulty in translating to in vivo (clinical) applications[1].

We have developed a novel gene transfection system based on attaching DNA to magnetic nanoparticles. High-gradient Halbach-type[2] magnet arrays direct the particle/gene complex to cells in vitro resulting in significantly faster transfection times and higher transfection efficiencies (up to 100x at short exposure times) in comparison to the best cationic lipid-based agents available (Figure 1). It also improves speed and efficiency in comparison to commercially available magnetofection systems.

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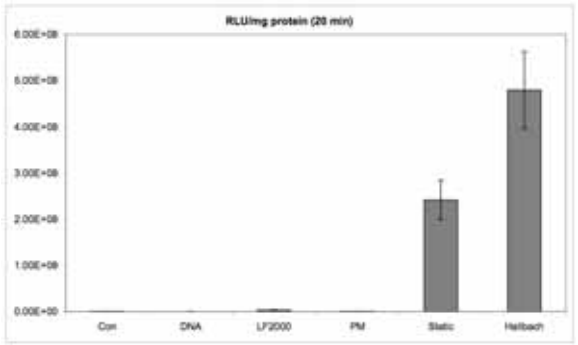

FIGURE 1: Luciferase activity in H292 human lung epithelial cells transfected with OzBiosciences Polymag particles coated with pciLux luciferase reporter construct in response to standard magnetofection plates and Halbach magnet array. All transfections were performed in serum free RPMI 1640 media as follows; Polymag 0.1ug DNA/0.1ul PolyMag per well and LF2000 0.1ug DNA/0.3ul LF2000). The plates were transferred to an incubator and exposed to standard magnetofection plates or a Halbach array for 20 minutes (N=12). At 2hr post transfection, the magnets were removed and at 48hr post transfection, the media was removed from each well and the cells lysed by the addition of 30µl of cell reporter lysis buffer (Promega CCLR). Samples were assayed for Luciferase activity using a Luciferase assay reagent (Promega) and the total protein concentration determined using a BCA assay reagent (Pierce, USA).

Direct Cell-entry of Gold/iron-oxide Magnetic Nanoparticles Enhanced Adenovirus Mediated Gene Expression in Adenovirus-resistant cells.

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Gold/iron-oxide Magnetic Nanoparticles (GoldMAN) comprise a magnetic iron-oxide core with smaller gold nanoparticles immobilized on its surface (Fig. 1). The immobilized gold nanoparticles allow the simple conjugation of functional biomolecules containing cysteine and methionine via an Au-S bond without requiring a linker molecule.

On the other hand, among the various methods of gene transduction, adenovirus gene delivery vector (Ad) can provide high-level transduction efficacy to a variety of cell types. However, some tumor cells exhibit a resistance against Ad-mediated gene transduction due to a decline in the expression of a coxsackie-adenovirus receptor (CAR), primary Ad-receptor, on their surface. In this study, we have developed a novel intracellular transduction method of Ad against CAR (-) cells using GoldMAN. GoldMAN can conjugate with Ad which is containing cysteine and methionine. We demonstrated that Ad/GoldMAN complex could more efficiently transduce marker genes (EGFP) into CAR (-) cells in a magnetic field compared with conventional Ad alone (Fig. 2).

This technology makes possible the effective use of various biomolecules within the cell because, in contrast to previous carriers, the cell entry mechanism is not dependent on surface receptors and endocytosis. In addition, because transduced cells that are functionally modified by gene expression, gene knockdown, and activation have GoldMAN-derived magnetic properties, this technology can be extended to image-analysis of artificially differentiated stem cells or immune cells. This novel technology will accelerate the development of cellular science, including functional analysis of the cell and cell-based therapies.

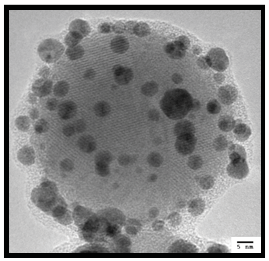

Fig. 1 TEM image of GoldMAN

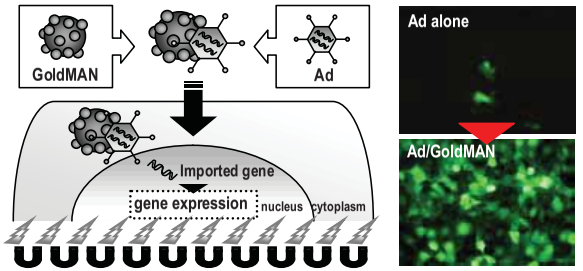

Fig. 2 Schematic illustration of gene delivery with GoldMAN

ADENOVIRAL VECTORS AND MAGNETIC NANOPARTICLES: PROSPECTS IN CANCER GENE THERAPY

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Cancer is a complex disease and still poses a formidable challenge. Given the inadequacy of traditional therapies, novel therapies such as gene therapy have generated major interest. A major obstacle to cancer gene therapy is the challenge of developing reliable and efficient gene transfer techniques. Adenoviral (Ad) vectors are the most efficient for gene delivery and hence are the most explored for cancer therapy, however, the efficiency is not adequate. Nanoparticles (NP) provide a novel and promising option; by virtue of their size (<100-200nm), these preferentially accumulate in cancer cells. Especially, inorganic iron oxide NPs have attracted interest for cancer imaging and targeted drug delivery; some formulations are now FDA approved for these purposes.

We have developed magnetic nanoparticles (MNP) (magnetite and maghemite) of different shapes/surface areas and investigated them for delivery of Adenoviral vectors into cancer cells. The particles were coated with cationic polyethylenimine (PEI, 25 KDA) to facilitate MNP linkage to Ad. The steric effects of PEI also conferred stability to NPs: dynamic light scattering measurements showed that the average size of the MNP aggregates was reduced to less than 200 nm and remained stable for over 14 days. Ad expressing Green Fluorescent Protein (AdGFP) or Firefly Luciferase (AdLuc) were used. Nanoparticles significantly improved AdLuc/AdGFP gene transduction to non-permissive ovarian cancer cells (SKOV3) by up to 1400-fold. (Figure1). While all morphologies tested showed significant enhancement in comparison to virus alone, some were clearly better than others. Intracellular iron was visualised using Prussian Blue staining and generally correlated with the extent of gene transduction. A two way effect was seen; when cells infected with virus/MNP were stained for iron uptake, it was clear that MNP uptake was also facilitated by Adenoviruses thus resulting in cumulative effects (Figure 2). Overall, when conjugated to MNPs the dose of Ad vector required to achieve the desired gene expression was dramatically reduced. This potential reduction in Ad dose would have significant clinical implications as a major hurdle to adenoviral delivery is the vector immunogenicity. This system also dramatically enhanced Ad gene delivery to non-Ad permissive mesenchymal stem cells (by>100,000fold), thus broadening the scope and options even further in cancer imaging and therapy. On the flip side, however, when AdGFP specifically modified to enter cancer cells (and not normal cells) were conjugated with MNPs, this specificity was lost. Hence, alternate ways of targeting these complexes requires exploration such as transcriptional targeting or use of cell carriers like mesenchymal stem cells. In conclusion, the Ad-MNP system constitutes a viable and potentially more effective alternative for gene delivery to clinical cancers; these may have greater penetration within the tumour mass and hence the potential to greatly improve *in situ* gene delivery.

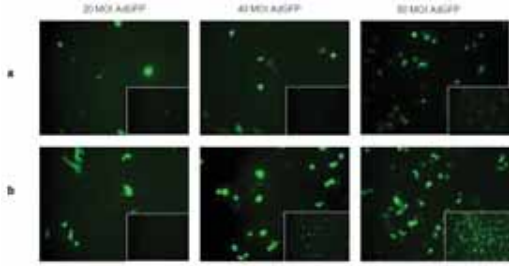

Figure 1: GFP expression in SKOV-3 cells following AdGFP-spMNP magneto-transduction: UV fluorescence micrographs of *SKOV-3 cells magneto-transduced with AdGFP-spMNP at spMNP concentrations of (a) 5 μg/mL and (b) 10 μg/mL are shown (20x magnification; inset 5x magnification)*

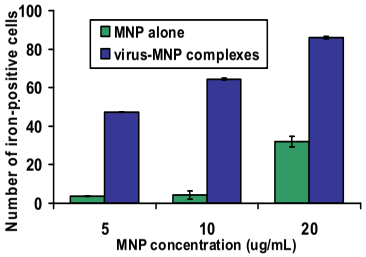

Figure 2: *Iron was detected by Prussian Blue staining after cells were incubated with MNP alone or virus-MNP complexes. Cellular iron content was greater in the latter.*

Superparamagnetic polymeric nanoparticles efficiently enhance non-viral nucleic acid delivery

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Non-viral delivery of nucleic acids for therapeutic purposes remains a challenge mainly due to a comparatively low efficacy. We addressed this problem by using non-viral gene carriers possessing magnetic targeting properties. The achievable via magnetic force targeted delivery of nucleic acids may provide a clinically viable solution for effective and non-toxic gene transfer. In our previous work we developed formulations of polylactide (PLA)-based biodegradable nanoparticles (NP) surface modified with branched polyethylenimine (PEI 25K). Recent scientific literature showed that deacylation of commercial preparations of linear PEI dramatically boosted its gene delivery efficiency due to increase in the number of protonatable nitrogens, which presumably results in a tighter condensation of nucleic acid and a better endosomal escape of the PEI/nucleic acid complexes. The present studies investigated the hypothesis that non-viral gene transfer can be enhanced via magnetically driven delivery of superparamagnetic NP formulated with deacylated linear PEI. The linear PEI synthesized by acid-catalyzed hydrolysis of 200-kDa poly(2-ethyl-2-oxazoline) and adjusted to pH7 was used to formulate iron oxide laden NP by means of modified emulsification-solvent evaporation methodology. NP containing 35% iron oxide by weight had an average size of 360±25 nm, zeta-potential of 43±3 mV and exhibited superparamagnetic properties (magnetic remnance less than 0.5% of their magnetic saturation value). The ability of linear PEI-NP formulation to deliver nucleic acids was examined *in vitro* in cultured A10, rat aortic smooth muscle cells (SMC) and bovine aortic endothelial cells (BAEC), using green fluorescent protein (GFP) as a reporter gene. NP formulated with branched PEI were used for comparison. NP complexed with nucleic acids were applied to cells for 15 min under magnetic field (500G) in serum-containing cell culture medium. In one set of experiments, GFP encoding plasmid DNA was delivered to the cells and the transfection efficiency was measured fluorimetrically 2, 4 and 8 days post treatment. Intracellular NP levels were directly dose dependent in examined NP concentration range for both PEI formulations. The GFP expression reached its maximal level for both PEI formulations at day 4 resulting in 2.5-3 times higher GFP levels for both cell types transfected with linear PEI-NP formulation. In another set of experiments, enhanced GFP (eGFP) short interfering RNA (siRNA) was delivered to the cells and the suppression of eGFP expression in lentivirus transduced smooth muscle and endothelial cells as well as cell viability (by AlamarBlue) were measured fluorimetrically 5 days post treatment. In GFP silencing experiments, efficient eGFP suppression was achieved using magnetic NP formulated with either branched or linear PEI. The eGFP suppression in A10 cells depended directly on the NP dose for both formulation types. The suppression of eGFP was directly siRNA dose dependent in the case of linear PEI-NP and inversely dependent for branched PEI-NP resulting in a maximal suppression of 40% for both NP types. In BAEC, the eGFP suppression depended directly on the NP dose for the branched PEI-NP only. The eGFP suppression was not dependent on the siRNA doses for both NP types resulting in a maximal suppression of 50% for both NP formulations. Studied NP/siRNA complexes did not significantly compromise cell survival showing more than 90% of viable cells 5 days post treatment at maximal NP dosages for both NP and cell types. It is concluded that magnetically responsive linear PEI-NP demonstrated increased delivery efficiency of plasmid DNA versus branched PEI-NP while in the gene silencing experiments no difference in capacity to suppress genes for both types of NP was observed.

Magselectofection – combined magnetic cell separation and magnetofection

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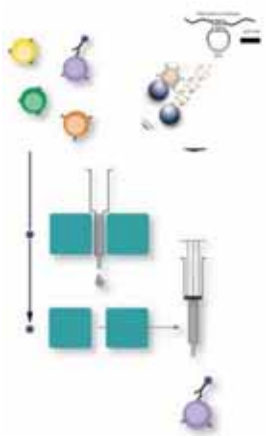
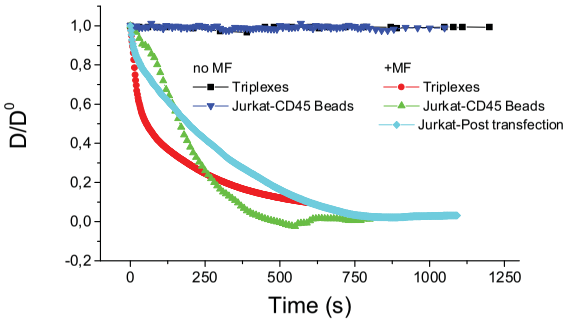
Magnetic cell separation is the method of choice for isolating specific cell populations from tissue samples. Magnetofection is a highly efficient transfection method exploiting the influence of magnetic field acting on nucleic acid vectors associated with magnetic nanoparticles. Here we examined whether Miltenyi MACS® magnetic cell separation technology and magnetofection can be combined to one integrated procedure of cell isolation and transfection. **Methods.** Standard



magnetofection procedure was optimized on a 96-well plate to be used in suspension type Jurkat cells using magnetofection vectors formulated with novel magnetic nanoparticles in combination with the Dreamfect-Gold transfection reagent (OZ Biosciences). In a second step, loading of Miltenyi MACS® columns exploiting magnetic retention of magnetofection vectors was established. In a third step, on column magnetofection of Jurkat cells was established. In a forth step, mixtures of K562 and Jurkat cells were treated with Jurkat-specific CD2 Microbeads (Miltenyi), applied to magnetofection vector loaded MACS® columns placed on MidiMACS™ magnets and stepwise eluted first in the presence of the magnetic field followed by pressure enforced elution in the absence of the magnetic field. Cell separation specificity, vector association with and internalization into target cells, cell recovery, cell viability and the efficiency and specificity of reporter gene expression were analyzed. Magnetic responsiveness of the magnetically labelled cells (Jurkat-CD45 Beads), transfection triplexes and cells post-transfection on column was evaluated using simple turbidity measurements (D/D_0) upon application of the magnetic field (MF). **Results.** A protocol for magselectofection was established. Procedure for vector loading on columns was developed enabling 100% retention of the magnetic complexes. Vector association with target cells after 30 min incubation of the column on the magnet was higher than 90%. The vector internalisation 6 h and 48 h post-transfection was > 40% and > 50%, respectively. Cell recovery from vector-loaded columns was quantitative. Purity was 95 ± 0.4% for the K562 population and 96 ± 0.4% for the Jurkat fraction retained on the column. Overall reporter gene expression was high and specific for Jurkat cells, and cell viability was >80%. **Conclusions.**



Proof of principle for combined magnetic cell separation and magnetofection on column (magselectofection) was provided. This technology may become a useful tool in nucleic acid therapy approaches involving ex-vivo genetically modified cells such as hematopoietic stem cells in the case of SCID-X1 gene therapy. Funded by EU LSHB-CT-2006-019038.

Non-viral Gene Transfection Enhanced by Magnetic Nanoparticles

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Many viral and non-viral gene vectors have been developed to delivery nucleic acid into cells. Safe, reliable and effective gene delivery, however, is yet to achieve, especially in vivo applications[1] . Coupling magnetic nanoparticles (MNPs) to gene vectors to enhance transfection efficiency of reporter genes by applying a magnetic field (magnetofection) has proved to be an excellent approach for gene delivery [2] . I n this paper, we describe an approach for functionalizing magnetic nanoparticles with molecules containing amino or carboxyl group such as dopamine and citric acid . The prepared particles and their aqueous colloids were characterized by Zeta Sizer, Thermal analysis (TGA), Transmission electron microscopy (TEM) and Dynamic Light Scattering (DLS). Then these particles were used to enhance PEI transfection of plasmid DNA to NIH 3T3 (Mouse embryonic fibroblast cell line) in vitro. We find that both two kinds of particles can form stable complexes with the polyplex, although the complex size and colloidal behavior vary dramatically with different experiment parameters such as weight ratio of MNPs to DNA and so on . By exploiting magnetic force, MNPs greatly enhance the transfection of PEI, which is more obvious at higher N/P ratios. Interestingly, if no magnetic force is applied, MNPs hindered the transfection of PEI , which is absolutely different from many other kinds of nanoparticles such as silica beads.

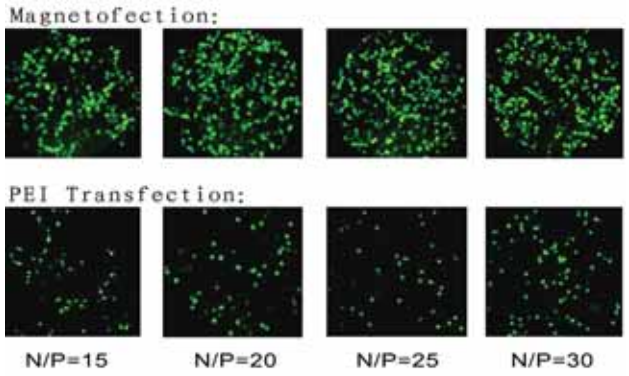

Fig. 1 Fluorescence photos of the NIH-3T3 cells 24 hours after conventional transfection with PEI for 4 h or magnetofection for 15 minutes.

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NEW NANODEVICE BASED ON COBALT FERRITE FOR MAGNETIC FLUID HYPERTHERMIA

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Keywords: Magnetic nanoparticles, hyperthermia, MFH, iron oxide, cobalt ferrite

Magnetic Fluid Hyperthermia by biocompatible nanodevices is one of the most promising technological application forecasted for nanomedicine as the heating effect could act as a therapeutic agent by itself or be combined with pharmacologically active molecules.

Early result made by Jordan [Jordan et al. 2001] show that this approach is feasible and could lead to innovative therapies of cancer. Despite these interesting preliminary results many aspects are still to be investigated. Following our magnetic study on cobalt ferrite that foresees MFH applications for cobalt ferrites [Baldi et al 2007], in this contribution we would like to present our recent investigation on cobalt ferrite as MFH material and especially on a new cobalt ferrite based nanodevice. The presentation will be introduced by a comparative study with magnetite of the same dimension but will focus on MFH efficiencies of cobalt ferrite nanoparticles of different sizes at different frequencies.

In the last part of the presentation we would like to present an innovative system [patent pending] consisting of magnetic cobalt ferrite nanoparticles embedded in poly-DL-Lactic-Glycolic acid with the outer surface functionalized with Bovine Serum Albumin (Figure 1) presenting its hyperthermic and MRI properties, early in vitro toxicity and efficiency investigations.

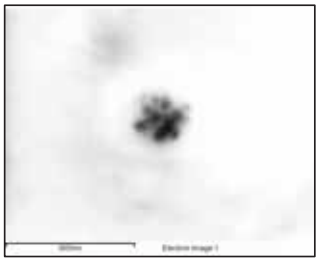

Figure 1 FEG-SEM image of biocompatible hyperthermic device

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Giovanni Baldi, Daniele Bonacchi, Claudia Innocenti, Giada Lorenzi and Claudio Sangregorio
Journal of Magnetism and Magnetic Materials, 311, Issue 1, April 2007, Pages 10-16

Combined MRI-adaptive Magnetohydrodynamic Thermochemotherapy for Improved Cancer Treatment

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Early detection of oncogenesis and metastases by MRI-monitoring is an important problem in detection of invasive tumors. We have synthesized and tested ascorbinate-ferrite sol (AFS) for the early detection of oncogenesis and metastases at combined MRI-adaptive magnetohydrodynamic thermochemotherapy with slime aspiration (AMT) to improve cancer treatment. During investigation of early stage oncogenesis and metastases by BIOSPEC BC 70/30 (Bruker) we have found that weak proton signals from small sites of pathogenic cells are neutralized by strong signals from normal tissues. To solve the problem, ferrite nanoparticles can be used as MR-negative contrast agents [1]. The enhanced MR-images of oncogenesis are represented in Figs. 1-10. In our experiments, hypodermic and skin tumors were treated with magnetically controllable drugs. Increase of drug concentration in tumor tissues due to magnetic field was achieved by use of $SmCo_5$ bandages (0.2-0.5 T induction) according to recommendations given in Ref. [2] and by a superconducting coil (7.0 T). Quantification of magnetic nanocarriers of such antitumor drugs *in-vitro* and *in-vivo* in mice bodies were carried out by "BioMag" device based on non-linear magnetization of nanoparticles [3]. Initially, 60 female mice with mammary adenocarcinoma Ca 755, 60 female mice with nick of uterus carcinoma and 60 male mice with melanoma B16 underwent non-enhanced MR-imaging with T_1-weighted {500/15 [repetition time msec/echo time msec]} and T_2-weighted {1900/80} spin-echo and T_2-weighted gradient-echo (GRE) {500/15} sequences. Then 1.0 ml AFS (particle diameters 6–18 nm, dose 10.0 mg Fe/kg) were injected in mice caudal vein. Within 2-24 hours the second MRI and AFS-enhanced T_2-weighted sequences were performed. Decrease of the signal intensity was recorded, and visual analysis was performed. At the early stages of oncogenesis thermochemotherapy at +46 C for 30 min using dextran-ferrite sol (DFS) with the dose of 60 mg Fe/kg containing of Cyclophosphamide (CPh) and Melphalan (MPh) were performed. The DFS (Fe_3O_4 weight -83 mg; CPh -4 mg; MPh -4 μg) was injected into tumor sites and was concentrated in the tumor with magnetic bandages. Treatment of mammary adenocarcinoma Ca 755 tumor ~60 mm^3 (stage shown in Fig. 2,3) by AC magnetic field (0.88 MHz, 7.3 kA/m, 0.15 kW) led to tumor regression in female mice before metastases by 30%, and 240% increase of life span has been achieved. The slime aspiration and CPh metastases treatment (at the stage shown in Fig. 6,7) led to 180% increase of life span.

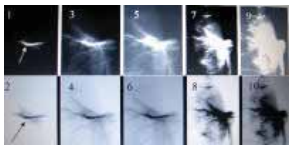

Fig. 1-10: MR-monitoring of oncogenesis of mammary adenocarcinoma Ca 755, which was implanted subcutaneously under the first right dug mammary of the female mouse C57Bl/6j, with subsequent ascorbinate-ferrite intravenous injections: (1,2)- implantation canals (arrows) 4-24 hours after 200 μl injection of 10^6 cells Ca 755 suspension and ascorbinate-ferrite injection; (3,4)- after 24-48 hours: (4-8)-fold increase of implantation canal and network branching lateral canals are observed; (5,6)- after 48-96 hours: basic and lateral canals became10 times larger, body height large canals and foggy boundaries of invasion of tumor cells in normal tissues are shown; (7,8)- after 96-192 hours: volume increase of canal network and its merging in nodules which results in large units node formation are observed; (9,10)- after 192-384 hours: merging of large nodules in dense tuberous formations as well as separate tuberous formations merging in a solid tumor are observed.

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RE –substituted Mn-Zn ferrite nanoparticles: size-dependent magnetic properties and AC-heating efficiency

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The nanocrystalline particles of spinel ferrites $Mn_{1-x}Zn_xFe_{2-y}RE_yO_4$ (RE – La^{3+}, Ce^{3+}, Gd^{3+}, Dy^{3+}, Er^{3+},Yb^{3+}; x=0.4, 0.5, 0.6; y=0, 0.1, 0.2, 0.3) have been synthesized by chemical co-precipitation method, according to the procedure reported before [1]. Additionally, a cyclic growth method (2 and 3 cycles of precipitation), similar to the one described in [2], was applied to obtain the particles of same compositions but with bigger diameters. X-ray diffraction analysis, TEM, SAED, SEM, mid- and far-IR absorption spectroscopy, and elemental analysis were performed in order to confirm the formation of pure spinel phase, to study the mean size, morphology and crystallinity of the particles, and to define the cation distribution in the spinel ferrites obtained. Neutron diffraction study was used to determine the magnetic structure within the RE-substituted spinel system. Magnetic properties (Fig. 1) were studied in a temperature range from 4.2 K up to 120 °C.

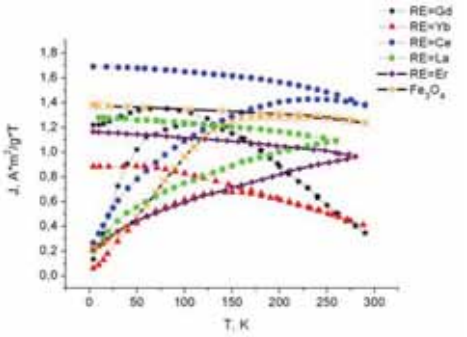

Fig. 1: ZFC and FC temperature dependencies of the reduced magnetization (specific magnetization divided by sample mass and applied field value) for particles of $Mn_{0.6}Zn_{0.4}Fe_{1.9}RE_{0.1}O_4$ (d_{mean} -10 nm).

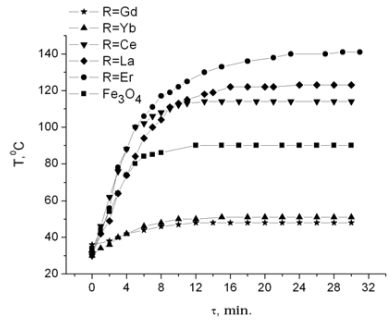

Fig. 2: Time-dependent temperature curves of powder-like nanoparticle samples in an external AC-field.

The main magnetic parameters of the particles (blocking temperature, specific magnetization values, T_C) demonstrate an unambiguous correlation with RE-type and quantity, as well as with a mean particle size. The experiments on AC-heating (880 kHz, 9 kA/m, at environmental temperature of 37 °C) for the samples with different RE-substitutions and particle sizes, in powder-like state (Fig. 2) and as particles dispersed in agarose gel (in concentration 0.1 g of ferrite per 1 ml gel) were conducted. The goal was to evaluate the heating efficiency of currently investigated RE-ferrite particles in comparison to the one for Fe_3O_4 : γ-Fe_2O_3 nanoparticles, currently applied as a heating agent in magnetic fluid hyperthermia. The results demonstrate different temperatures of thermo-equilibrium and the heating rates for the substituted RE-ferrite particles comparable to that of Fe_3O_4 : γ-Fe_2O_3 particles obtained by similar method.

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The Influence of Temperature on the Magnetic Behavior of Colloidal Cobalt Nanoparticles

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The applications of magnetic nanoparticles, including hyperthermia for cancer treatments, require knowledge of how the colloidal environment affects the magnetic properties of the nanoparticles. Here, 10 nm diameter cobalt nanoparticles synthesized via thermo-decomposition in 1,2-dicholorbenenze (DCB) are used to study the effects of the colloidal environment on the magnetic behavior of such materials. The magnetic properties are investigated via hysteresis loops and magnetization (M) vs. temperature (T) measurements performed on the samples under both zero-field-cooled (ZFC) and field-cooled (FC) conditions. Of particular interest in the ZFC/FC data is a continuous rise in the magnetization observed around the DCB melting point in the ZFC curve and a discontinuous drop around the DCB freezing point in the FC curve. When the DCB is exchanged with other solvents (ethanol, hexane, and toluene), only the continuous rise in the magnetization in the ZFC loop around the solvent melting point appears. (Both the rise and drop appear when the DCB is exchanged for more DCB.) The rise near DCB melting point in ZFC curve is believed to be predominantly associated with a transition between two different spin rotation mechanisms: (1) Néel rotation at lower temperatures when the solvent is frozen and (2) Brownian rotation at higher temperatures when the solvent is liquid permitting increased magnetic alignment with the field due to both physical movement and rearrangement of the induced dipolar chains. First Order Reversal Curve (FORC) measurements demonstrate a startling change in the distribution of coercivities on warming, from a very broad (>2kOe) distribution below the melting point of DCB, compare with a significantly narrower (<0.2kOe) distribution above the melting point. This corresponds to further chain formation as the nanoparticles align with the field, increasing the interparticle dipolar coupling. On cooling, torque magnetometer measurements highlight the portion of the sample that does not respond to the applied field. This frozen-in component doubles with decreasing temperature as the sample cools through the freezing point in DCB. There is also a slight increase in the effective uniaxial anisotropy inherent to the system as the liquid to solid transition is traversed.

Temperature rise of Fe_3O_4 nanoparticle measured under ac magnetic field and its evaluation by ac magnetization curve

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Hyperthermia is a therapeutic procedure of raising the body temperature for cancer. It has an advantage that risks of scar and harmful side effects are reduced, which is not achieved by various established treatments of surgical operation, radiotherapy and chemotherapy. Magnetic nanoparticle exhibits self-heating under an ac applied magnetic field due to hysteresis loss and magnetic relaxation loss. The self-heating property of nanoparticles dispersed in medium is significant for the hyperthermia application, but the nanoparticles packed in tiny container is also attractive because of its higher temperature rise. In this paper, the self-heating property and the corresponding magnetization curve of Fe_3O_4 nanoparticles are discussed.

The sample was commercially distributed Fe_3O_4 nanoparticle of 20-30 nm. The temperature rise of the Fe_3O_4 nanoparticle of 4 mg packed in the glass tube was measured by applying the alternating magnetic field at up to 20 kHz. The magnetization curve of the sample was also measured under dc magnetic field and ac magnetic field.

Figure 1 shows the magnetic field dependence of the temperature rise of the sample heated by the applied field at 20 kHz. The self-heating under the magnetic field of 200 Oe seems to be effective in the figure. This field dependence was agreed with the area of the BH loops. The area of the BH loop measured at dc field corresponds to dc hysteresis loss, whereas measured at ac field includes magnetic relaxation loss as well as hysteresis loss. Comparison of the self-heating temperature and ac magnetization curve is significant to evaluate the heating mechanism of the nanoparticles. Also the tiny tube of 1mm diameter and 4 mm length packed in Fe_3O_4 nanoparticle is proposed as heating implant for hyperthermia, and its advantages are discussed.

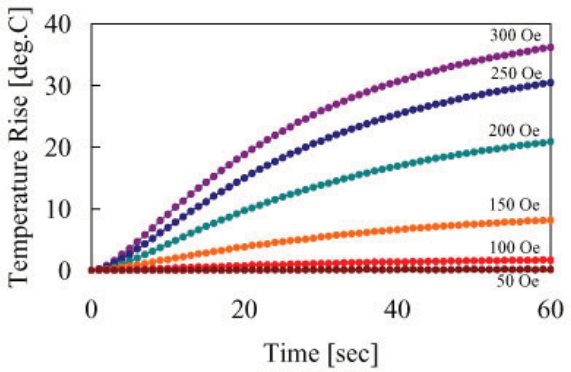

Figure 1 Magnetic field dependence of the temperature rise of Fe_3O_4 nanoparticles measured at 20 kHz.

The effect of preparation of surface-active coating on the physical properties of biocompatible magnetic liquids

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Biocompatible magnetic liquids are stable colloidal suspensions of nano-sized magnetic particles in a carrier liquid. Their biocompatibility and stability are achieved by coating them with one or more surfactant layers that produces entropic repulsion. The aim of this study was to investigate the influence of preparation of second layer of the surface-active coating on the magnetic, thermal and mechanical properties of biocompatible magnetic liquids. The surfactant used for preparation of the second layer of coating was PEG (polyethylene glycol). In sample 1 (denoted as Merck) the PEG from Merck (5000-11250 g/mol) and in sample 2 (denoted as Sigma) PEG from Sigma (1000 g/mol) were used, respectively. Samples denoted as Sigma1, Sigma2 and Sigma3 contained different ratios of magnetite (Fe_3O_4) to PEG, i.e. 4 mg/1 mg in case of Sigma1, 0.4 mg/1mg in case of Sigma2 and 40 mg/1 mg in case of Sigma3. In all samples the first layer of coating consisted of sodium oleate.

Magnetic measurements of the samples were carried out with the aid of a vibrating sample magnetometer (VSM) at the temperature of 300 K, within the range of variation of external magnetic field strength, $\Delta B = \pm 1$ T. On the basis of the initial magnetization curve the saturation magnetization (from the numerical extrapolation of quasisaturation part of the entire curve), Ms, was obtained, which allowed us to determine the volume fraction, ϕ_V, of magnetite phase (Fe_3O_4). From the hysteresis loop data coercive force, H_c, and remanence magnetization, M_r, as well as the energy loss during a single cycle were determined. Moreover, mean magnetic diameter and the standard deviation of particles were determined from magnetization curves.

Magnetic nanoparticles offer some attractive possibilities in biomedicine. One of such important applications is magnetic fluid hyperthermia offering a possibility of increasing the tumour temperature to 41–46°C and killing tumour cells. Magnetohyperthermia is realized upon the application of external, low amplitude, ac magnetic field to the target tissue, which is previously labeled with magnetic nanoparticles. The heating ability and biocompatibility of magnetic liquids which is measured by specific absorption rate (SAR) depends on physical (particle size, shape and distribution) as well as magnetic properties of particles. Hyperthermic measurements were performed at frequency $f = 750$ kHz vs. the AC-field amplitude in the range of 0–2 kAm^{-1}. The observed H^n–law-type dependence of the temperature increase rate, $(\Delta T/\Delta t)_{t=0}$, on the amplitude of the magnetic field, where $n \cong 2$, indicated the presence of superparamagnetic particles in the magnetic fluid (see fig.1). The SAR values as a function of external magnetic field show suitability of the studied magnetic liquids in the biomedical applications.

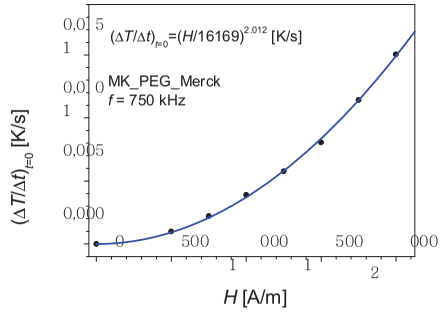

Fig. 1. The dependence of $(\Delta T/\Delta t)_{t=0}$ on the magnetic field amplitude H for the magnetic fluid at the selected frequency 750 kHz. The solid line represents the fit of the function $(\Delta T/\Delta t)_{t=0} = (H/16169)^{2.012}$ to the experimental data.

The mechanical properties were studied using broadband ultrasonic spectroscopy. In this approach a broadband ultrasonic pulse is used, *i.e.* a single pulse which contains a wide range of different frequencies. After the pulse has traveled through the sample, it is analyzed using a Fourier transform algorithm to determine the values of velocity and attenuation as a function of frequency. It was found that in the absence of external magnetic field the coefficient of absorption expressed as α/f^2 decreases monotonously and the attenuation can be attributed to the friction and heat exchange between the particles and the surrounding medium. On the basis of speed of sound measurements the values of adiabatic compressibility were determined.

The studies were supported by Polish Ministry of Science and Higher Education grants No. 4T 07B 04130 and N202 097 32/2406.

Bio-Compatible High-Moment Magnetic Nanoparticles for Hyperthermia Treatment Optimization

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Hyperthermia is a method already in use in the medical area of oncology for the treatment of cancer and is based on cancer cell death upon cell temperature elevation to the range of 42-45°C.

A great deal of attention has been paid to the use of magnetite nanoparticles as heating elements. The main reasons appear to be magnetites known bio-compatibility and also their commercial availability. However, these particles have a relatively low magnetization and as a result, have low heating efficiency as well as difficulties in detection applications.

To maximize heating efficiency we propose and show the use of high moment magnetic nanoparticles, particularly the material with the highest naturally occurring magnetic moment, CoFe. The use of high-moment magnetic nanoparticles increases the specific loss power. This is due to the increase of heating efficiency since relaxational losses are proportional to the square of the magnetic moment. High moment particles offer the optimal physical characteristics to increase heating efficiency.

Using a physical vapor nanoparticle-deposition technique the high-moment nanoparticles were synthesized from the gas phase. The particles, immersed in deionized water, were placed in an AC magnetic field (1kW Hotshot, Ameritherm Inc., NY), of variable magnetic field amplitudes and frequencies, centered in a 3-turn water-cooled conducting coil. The temperature rise was measured with a fluoroptic thermometry system (Luxtron 3100 thermometer, Luxtron Inc., CA), collected at one second increments.

Fig. 1 displays the temperature rise of the Co(Fe)-Au nanoparticles under exposure to three different magnetic field frequencies. The amplitude of the magnetic field was 6kA/m, and the particle concentration was 11mg/ml. Further testing will be into the applied magnetic field amplitude dependence, as well as particle size variation. Further analysis will go into theoretical comparisons of the power loss dependencies. This further research will allow for a better physical understanding of the optimization of these particles for hyperthermia applications.

High-moment, water-soluble, bio-compatible magnetic nanoparticles offer the highest degree of promise as a heating agent for the application of magnetic hyperthermia. It is seen that Co(Fe)-Au nanoparticles can successfully be heated when exposed to an AC magnetic field.

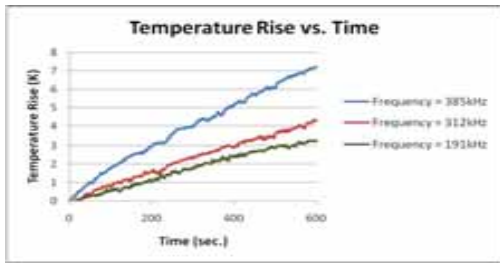

Fig. 1 Temperature rise vs. time of Co(Fe)-Au nanoparticles exposed to a magnetic field of 6kA/m and of varying frequency

Specific Absorption Rate of Ferrofluids Using a New Adiabatic Magnetothermal Setup

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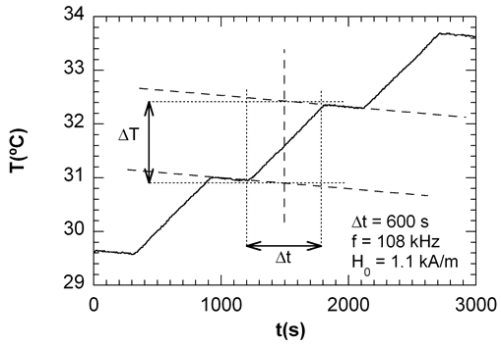
In magnetic fluid hyperthermia for cancer treatment, the heat generated by the magnetic fluid under an alternating magnetic field is used to weaken or destroy cancerous cells. The heating efficiency is quantified by the specific absorption rate, $SAR = (1/m)\cdot C\cdot(\Delta T/\Delta t)$, where m is the mass of dissipating material, C, the heat capacity of the sample, and ΔT, the temperature increase during the ac-field application interval, Δt. Current SAR installations cannot use this expression since heat losses (conduction, radiation, convection) are not minimized. Then, SAR is calculated as SAR=C β/m, where β is the initial slope of the temperature-time curve during Δt. This procedure leads to incorrect SAR values if initial thermal losses or non-homogenous temperature distributions across the sample are present.

To overcome these limitations, we have developed the first adiabatic magnetothermal setup in which the generated heat is entirely invested in the sample temperature raise, allowing direct measurement of ΔT. Setup validation has been performed with a copper sample that provided comparison with theoretical values: adiabatic conditions gave SAR values only 3% higher than theoretical ones, while the typical non-adiabatic method, performed with the same setup, disabling the adiabatic control and the vacuum, underestimated SAR by 21%.

We report the results obtained with several commercial ferrofluids. As an example, the figure shows three adiabatic heating steps performed on the fluidMAG-UC/A, from Chemicell GmbH. The typical step-like behavior of the adiabatic processes is observed, in which temperature linearly increases during ac-field application intervals. In such conditions, a SAR value of 0.217 W/g was obtained with good reproducibility (4%).

Moreover, the setup adiabaticity and sensitive thermometry allow measuring small heating powers. Attempts to measure the SAR of the contrast agent Endorem (Guerbet) are reported in literature, and an upper limit of 0.1 W/g for 6.5 kA/m and 400 kHz is given. With our setup, Endorem SAR was quantified as 0.18 W/g only using 2.1 kA/m and 108 kHz.

In summary, the reported setup provides accurate SAR measurements, of great interest for the studies on correlation between SAR and material properties, simulations of temperature distributions in tissues or phantoms and optimization of hyperthermia therapies.



Three consecutive heating steps of the FluidMAG-UC/A.

SEARCH FOR NEW CORE MATERIALS FOR MAGNETIC FLUID HYPERTHERMIA

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Today the use of nanoparticles for magnetic fluid hyperthermia application is preferentially oriented on magnetite Fe_3O_4 and maghemite γ-Fe_2O_3. The control of their magnetic properties in a desirable way is, however, rather difficult. A possibility to solve this task is either the use of complex magnetic oxides or multiphase materials allowing to achieve the fundamental requirements put on the cores, i.e. high specific power losses and adjustment of the self-controlled heating mechanisms ruling out the risk of local overheating of the healthy tissue.
The possibilities studied systematically in our laboratory are as follows:
- Modification of the intrinsic properties depending on the composition and structure. $La_{1-x}Sr_xMnO_3$ perovskites are an example where magnetic properties are tuned by the controlled-valency mechanism, variation of the Mn^{3+}/ Mn^{4+} ratio [1].
- Modification of the extrinsic properties. $CoFe_2O_4$ spinel is an example where a suitable coercivity may be adjusted by the crystallite size [2].
- Use of Sr-hexaferrite/maghemite composites, where combined contribution of different phases allows to adjust suitably the resulting properties [3].
In reality these approaches are usually combined.
The saturated magnetization and Curie temperature of $La_{1-x}Sr_xMnO_3$ perovskites strongly depend on the size of crystallites and are decisive for the resulting behaviour of a given composition. The coercivity values, depending on the crystallite size for cobalt spinel and on the phase ratio for the Sr-hexaferrite/maghemite composites control the power losses in both these materials.
A particular attention is paid to the heating efficiency measured calorimetrically on fluid suspensions and to dc hysteresis losses, measured on dry nanopowders.

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A novel approach to magnetic hyperthermia using biphasic gel of $La_{1-x}Sr_xMnO_3$ and maghemite

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The temperature rise and hence the damage to the normal tissue during magnetic hyperthermia (MHT) treatment using magnetic nanoparticles (MNPs) of Fe_3O_4, γ-Fe_2O_3 or their derivatives may not be controlled due to their high Curie temperature (T_C). On the other hand T_C of $La_{1-x}Sr_xMnO_3$ (LSMO) could be tuned near therapeutic temperature (45 °C). But, LSMO is biocompatible for a comparatively smaller dose. Thus a biphasic gel using agarose and the mixture of LSMO and an iron oxide derivative (Al-substituted maghemite, AlFe) in different proportions were prepared to study their heating abilities. It was observed that AlFe component provides sufficient heating ability and biocompatibility whereas the LSMO provides controlled temperature rise.

The temperature vs. time curves for only LSMOs based gels suggest that the temperature get stabilized below 60 ºC due to their low T_C whereas for AlFe based gel temperature rise continued even after. The SAR values for the LSMO based gels varied between 11 and 18 W/g of MNPs. For the gel prepared for the mixture of $La_{0.75}Sr_{0.25}MnO_3$ (L75C) and AlFe, the maximal temperature increases (Fig. 1) whereas the time to reach highest temperature decreases with the increased ratio of the later. The domination of magnetic properties of AlFe in the mixture was also observed in the SAR values which have increased with the increased percentage of it. With the gel based on $La_{0.73}Sr_{0.27}MnO_3$ (L73E) the highest temperature achieved was 40 °C which rose to 45 °C with a mixture of 10 % AlFe in it (Fig. 1). Further, the temperature of gel rose to 50 and 53 °C with 20 and 25 % of AlFe respectively in the mixture gel. As observed earlier, the SAR value of mixture get increased with increased percentage of AlFe and it was 2, 3 and 4 W/g of MNP for 10, 20 and 25 % of it respectively. The current study suggests a new way for *in vivo* control of temperature and avoids excessive heat generation during MHT.

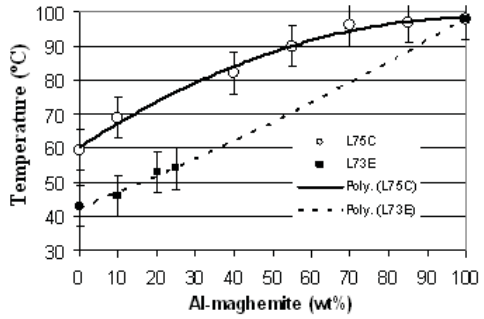

Figure 1: Tmax evolution of magnetic gel of mixture of LSMO and AlFe with increased percentage of the later.

Core shell iron-iron oxide magnetic nanoparticles for cancer treatment

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Magnetic nanoparticles (MNPs) offer excellent treatment option in medical world. We can compile magnetic nanoparticles with size range from 2 to 200 nm to various cells and tissues for target drug delivery, magnetic resonance imagining (MRI) and hypothermia to name few. One of important factors for the medical applications of MNPs is high magnetic moment. We have produced monodispersive core shell Iron-iron oxide magnetic nanoparticles with very high magnetic moment ~180 emu/g in our novel cluster deposition system. Core iron enhances the heating effect for hypthermia applications. We tested stability of MNPs by observing the rate of oxidation of core shell iron/iron oxide nanoparticles using X-ray diffraction after 0, 24, 48, 96, 208 hours. High magnetic moment substantially reduces the dosage of the nanoparticles for hypothermia applications. We tested the non toxicity and uptake of magnetic nanoparticles on LX-1 small cell lung cancer cells found in rats (Figure 1). Both dextrin coated and non coated nanoparticles were uptaken uniformly by the cancer cells without using any transfection. The test of trypan blue stain shows nontoxicity for the cells. Due to high magnetic moment offered by our MNPs we predict that even in low applied external alternating field the desired temperature can be reached. Magnetic nanoparticles produced in our lab also do not require any transfection agent, proving a cost effective means of treatment, while commercial iron oxide MNP products FERIDEX must add a protamine transfection agent for uptaking process with large MNP aggregations inside the cancer cells. Those aggregations must be overheated during hypothermia.

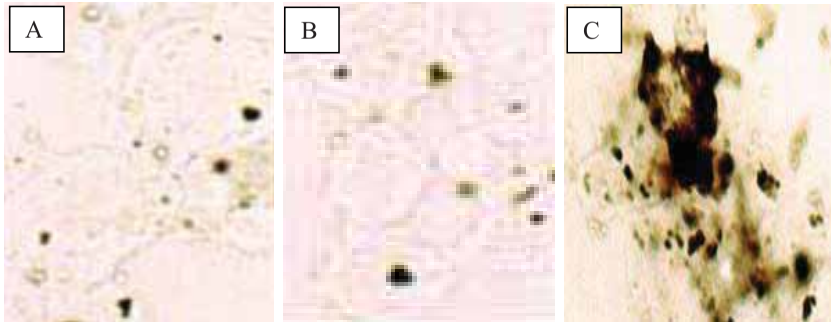

Figure 1: A) 250µl pure core-shell MNPs incubated by the LX-1 SCLC cancer cells without protamine; B) 250µl dextrin coated Core-shell MNPs incubated by the LX-1 SCLC cancer cells without protamine: C) 250µl commercial iron oxide Feridex incubated by the LX-1 SCLC cancer cells with 10 microgram protamine.

Heat dissipation mechanism of nanoparticle in magnetic fluid hyperthermia

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[2] Ferrotec Corporation Chuo-ku, Tokyo, Japan, [3]National Institute for Materials Science, Nanomaterials Laboratory, Tsukuba, Japan

The thermal seeds used for Magnetic Fluid Hyperthermia (MFH) are known to dissipate heat through either (a) the relaxation of magnetic vector within the particle (Neel relaxation) or (b) the rotation of the particle itself (Brownian relaxation). Though considerable studies are being carried out to determine the optimum size of thermal seeds suitable for MFH, no detailed studies have been carried out to investigate respective contributions from the above two mechanisms. In this paper, we report the study undertaken to determine the optimum particle diameter that dissipates heat manly through Neel relaxation.

Magnetic particle suspensions dispersing magnetite particles of varying sizes and concentrations in water and non-polar solvents with different viscosities are prepared and their heat dissipation characteristics were measured in an ac magnetic field strength and frequency of 3.2 kA/m and 600 kHz, respectively. The TEM micrograph of particles from the representative samples are given in Fig. 1. The heating characteristics of corresponding samples are also given in Fig. 2. The results of specific heat absorption of magnetite samples under various experimental conditions have also been carried out. The details will be reported and discussed.

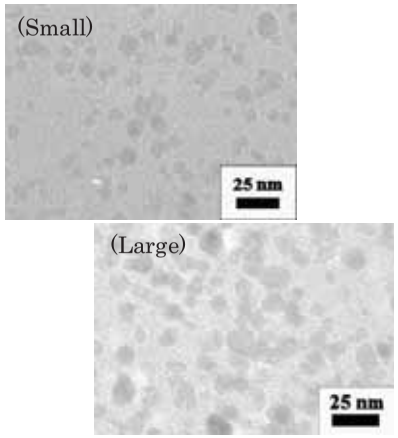

Fig. 1 TEM micrograph of magnetite nanoparticle sample (small and large sample)

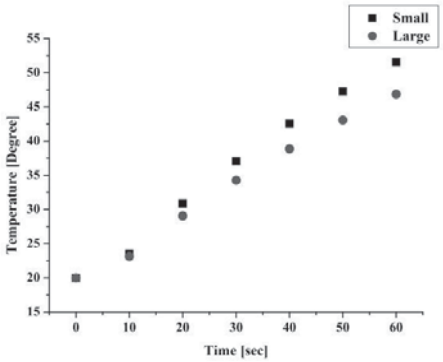

Fig. 2 Mean temperature rise of small and large magnetite nanoparticle dispersions in AC magnetic filed as a function of time.

Heat diffusion characteristics of magnetite nanoparticles Dispersed Hydro-gel in AC magnetic field

M. Suto[1], H. Kosukegawa[1], K. Maruta[1], M. Ohta[1], K. Tohji[1] and B. Jeyadevan[1]

[1]Tohoku University, Sendai, Japan,

Magnetic fluid hyperthermia is a potential method to treat cancer, and demands detail studies on the development of efficient thermal seeds with heat dissipation and diffusion characteristics suitable for *in vivo* applications where cancer cells are killed selectively. Here, we report the results of numerical and experimental investigations on the heat diffusion characteristics of a heat source dispersing magnetite in hydro-gel and exposed to an AC magnetic field strength and frequency of 3.2 kA/m and 600 kHz respectively. The numerical estimation assuming one dimensional spherical model (Fig. 1) and constant heat evolution from the heat source suggested that a large temperature difference existed between the values at the center and the surface of the heat source. This reflected the local heating characteristics of magnetic fluid hyperthermia. On the other hand, similar behavior was also observed experimentally, except for a large temperature gradient at the magnetite-dispersed and magnetite-free hydro-gel interface for a rectangular model of PVA hydro-gel shown in Fig. 2.

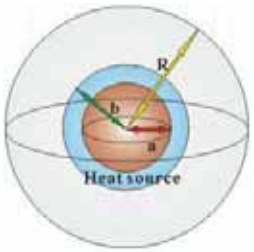

Fig. 1 Numerical model to investigate heat diffusion characteristics, a: domain of heat source MNPs are dispersed, b-a: domain of the magnetite free hydro-gel surrounding heat source, R-b: domain of air

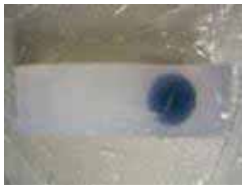

Fig. 2 Experimental specimen composed of spherical magnetite- dispersed hydro-gel.

Though a qualitative agreement between numerical estimation and experimental observation was recorded for magnetite concentrations 1, 2, and 4 wt. %, a quantitative difference existed in the temperature distribution (Fig. 3). The estimated value was always lower than the observed and the difference was greater for higher magnetite concentration. The ac magnetic measurements confirmed that the deviations between numerical and experimental values were mainly due to the difference in particle size distribution considered for numerical estimation rather than magnetic interaction. Thus, it was concluded that the algorithm used for the case which has no fluid flow is valid and studies are in progress to consider fluid flow conditions.

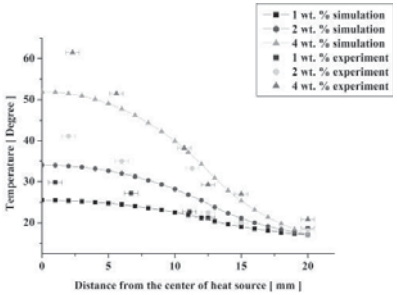

Fig.3 Temperature distribution along the radius of the heat source.

Magnetic Properties and Heating Effect in Bacterial Magnetic Nanoparticles

M. Timko[1*], A. Džarová[1], V. Závišová[1], J. Kováč[1], A. Šprincová[1], M. Koneracká[1], P. Kopčanský[1], N. Tomašovičová[1], A. Skumiel[2], A. Jozefczak[2], I. Vavra[3]

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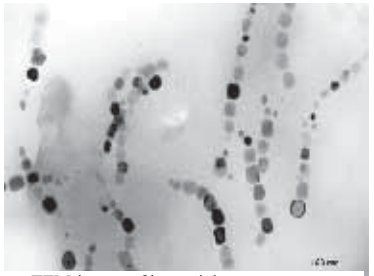
Magnetic bacterial nanoparticles have an huge amount of potential value for various technological applications not only scientific interests. Using of magnetosome was exerted for the immobilization of relatively large quantities of bioactive substances, for immobilizing the enzymes glucose oxidase and uricase as components of medically important biosensors, for incorporated bacterial magnetite particles into eukaryotic cells, which could be manipulated by a magnetic field, for the introduction of DNA into cells, for the detection of mRNA, as a contrast agent for magnetic resonance imaging and tumor-specific drug carriers based on intratumoral enrichment, for biomedical applications. One of the special potential application areas of magnetic bacterial particles is hyperthermia. As it was pointed out recently an enhancement of specific heating power is of importance for reducing the useful dosage applied to the tumour. By this way a gain in reliability of magnetosomes as tumour therapy is expected.

In our experiments bacterial magnetic nanoparticles (magnetosomes) were prepared by biomineralization from magnetotactic bacteria Magnetospirillum sp. strain AMB-1. The magnetic separation was used for the isolation and purification of magnetosome particles without of breaking of surrounding membrane. Electron micrograph (TEM) of the magnetosomes revealed that magnetosomes dispersed very well are arranged in bent chains in suspension so as to minimize their magnetic stray field energy. The mean particle size estimated from TEM is 34 nm. The XRD powder diffraction peaks of magnetosomes fit very well with standard Fe_3O_4 reflections. The average particle size, calculated by the Debye-Scherrer formula from XRD line width of the (311) peak is 37 nm, what corresponds to TEM value. Magnetic properties were examined by SQUID magnetometer. The saturation magnetization of the magnetosomes was estimated to be 62 emu/g. The curve of field dependence of magnetization at 293 K exhibited the remanence of 18 emu/g and coercivity of 145 Oe what is connected with fact that the mean particle size is larger than critical size for transition from superparamagnetic to ferromagnetic behavior. The heating of magnetosome solution which was measured by specific absorption rate (SAR) depends on physical (particle size, shape and distribution) as well as magnetic properties of particles. Hyperthermic measurements were performed at frequency f = 750 kHz vs. the AC-field amplitude in the range of 0–5 kAm^{-1}. The found value for SAR 270 W/g at 5 kA/m and 750 kHz means that magnetosomes may be considered as good material for the biomedical applications in hyperthermia.



TEM image of bacterial magnetosomes

Acknowledgement This work was supported by the Slovak Academy of Sciences (grant No. 6166) and by the Slovak Research and Development Agency under the contracts No. APVV-26-026505 and APVV-0173-06.

Concentration Effects in Maghemite-Based Ferrofluids

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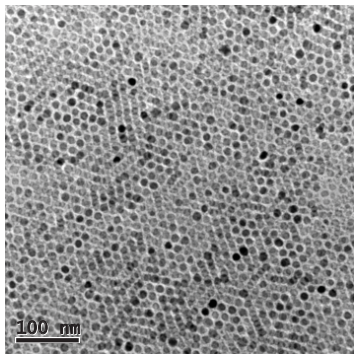
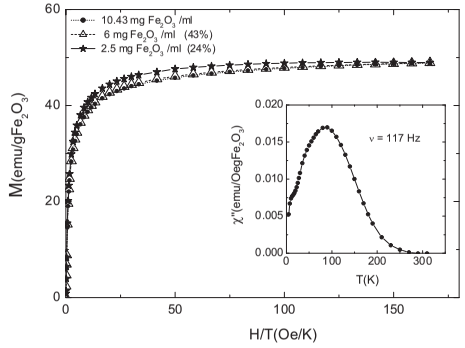
The use of ferrofluids with narrow size distribution allows optimizing specific absorption rate (SAR) values in magnetic fluid hyperthermia. Relaxation time is similar for most of the superparamagnetic particles in the ferrofluid, and dissipation is maximized when the ac magnetic field frequency is tuned with such time. However, factors like cluster formation or particle interactions may deviate the real SAR values from theoretical estimations.

The synthesis of highly crystalline and monodisperse maghemite nanoparticles was carried out in organic medium by the Hyeon method. This procedure, which allows varying particle size by controlling the experimental parameters, is based on the thermal decomposition of iron pentacarbonyl in the presence of oleic acid. The resulting iron nanoparticles were transformed to monodisperse maghemite by controlled oxidation using trimethylamine oxide as a mild oxidant. The final concentration of the ferrofluid was 10.4 mg (γ-Fe_2O_3)/ml. The TEM image reveals the narrow size distribution of the sample under study, with diameters of about 13 nm. DLS distributions (not shown) reveal the absence of aggregates.

Ac susceptibility measurements were performed by quenching the ferrofluid at 5K, so that each nanoparticle becomes immobilized and the magnetic interactions between dipoles can be considered to be the same as that in room temperature, when the solvent is liquid. The temperature-dependence of the out-of-phase component (χ'') showed a broad peak representative of interactions between nanoparticles. So that several dilutions from the original sample were studied. The magnetization curves at room temperature display different behaviors: for the original concentration and for 6.0 mg/ml, sample reaches saturation more slowly than for 2.5 mg/ml, indicating that those samples are more stable against magnetic orientation. These results suggest that dipolar interactions influence the magnetic response and that it is possible to enhance susceptibility diluting the ferrofluid.

The SAR of both of the original and the diluted samples was determined using an adiabatic magnetothermal setup, which provides accurate and reproducible values due to the possibility of measuring direct temperature increments with no heat losses. The obtained results are discussed on the basis of the microstructure and magnetic properties.





Left: TEM image. **Right:** Magnetization curves at r.t.. **Inset:** χ'' of the original sample.

Magnetic Hyperthermia in Tsinghua University

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Heating magnetic material implanted in site of tumor by their magnetization loss in alternating magnetic field is a resurged hyperthermia treatment for targeted cancer therapy. The Institute of Medical Physics and Engineering in the Department of Engineering Physics (DEP) in Tsinghua University, Beijing, has been addressed on magnetic hyperthermia research since 2000, including: 1) hyperthermia facility development as the research platform, 2) material research for targeted hyperthermia and drug delivery and 3) bio-medical research and application of hyperthermia.

A series of hyperthermia facilities has been constructed in the laboratory, including a prototyping hyperthermia system for clinical trial, an animal experiment facility and desktop instruments for small animal and material experiments.

Investigation on the magnetic materials concerning the physical mechanisms of magnetization loss, bio-heat transfer and thermal dosimetry is ongoing in both theory and experiments (Fig1). A kind of magnetic thermo seeds (Ni-Cu alloy, mm size) has been developed with suitable heating performance; while magnetic micro sphere and nano particles as the carrier for targeted hyperthermia and drug delivery, are also in research and development with related laboratories.

So far we have finished the bio-medical experiments using thermo seeds on levels of cell, tissue and in vivo heating therapy of animal tumors (Fig.2) [1, 2]. Further laboratory studies are ongoing for targeted hyperthermia and drug delivery using micro/nano particles**.** In near future, the first-stage clinical trial initially using magnetic thermo seeds will be started in collaboration with related hospitals.

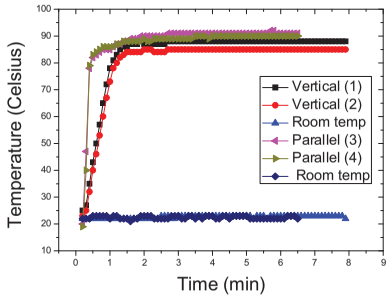

Fig.1. Heating performance of Ni-Cu alloy thermo seeds, with the Tc around 85°C

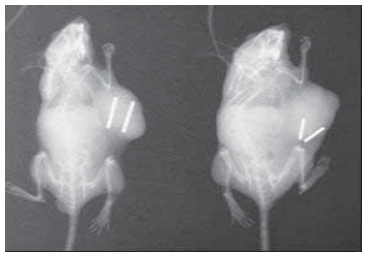

Fig.2 Radiographs of mice after thermo seeds implanted

Keyword: magnetic hyperthermia, thermo seed, magnetic nano particle

Reference:

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[2]. R. Hu, X. liu, et. al, Chn J Min Inv Surg, 2007, Vol.7.No.11, 1043-1045

Heating ability of monodisperse magnetite nanoparticles with various sizes by solvothermal method

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Abstract: Different sizes of monodisperse hydrophilic iron oxide nanoparticles were prepared by solvothermal method to investigate the size dependence of heating ability for the purpose of magnetic hyperthermia application. The particles can be modified by amino group and also can be coated by silica. The highest heating efficiency with specific loss power of 97 W/g.Fe_3O_4 was found on 35 nm nanoparticles under *ac* magnetic filed of 11 kA/m at frequency of 55 kHz. The transmission electron microscopy, x-ray diffractometer and vibrating sample magnetometer were used to characterize the morphology, crystal structure and magnetic properties. The heating mechanism was discussed.

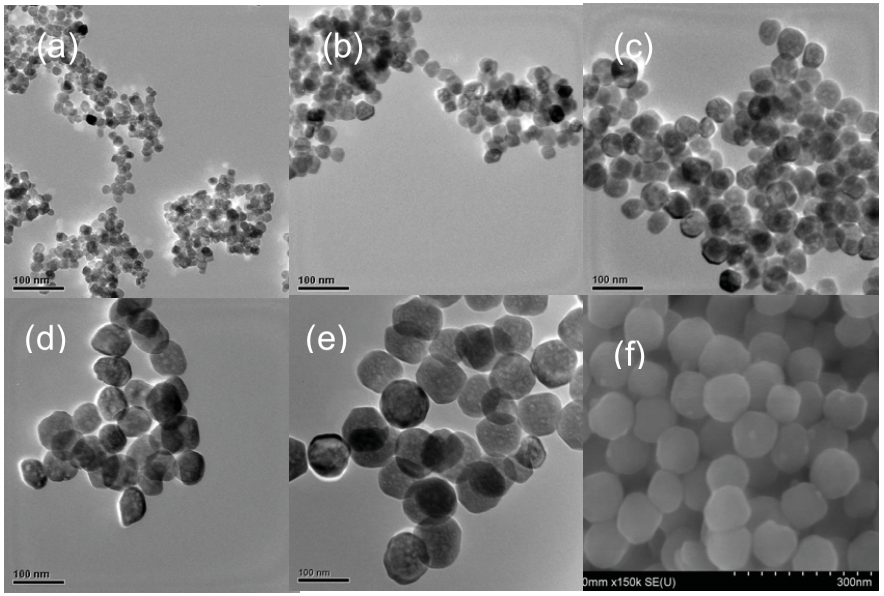

Fig. TEM images of Fe_3O_4 nanoparticles with various sizes: a) 20 nm; b) 35 nm; c) 50 nm; d) 70 nm; e) 100 nm. f) SEM image of 100 nm nanoparticles.

Generation and Manipulation of Magnetic Droplets

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The on-chip generation of microdroplets in an immiscible phase is currently of great interest in the microfluidics community. Such droplets represent picolitre reaction vessels and have been used for kinetic studies, bioanalysis, protein crystallisation studies as well as the synthesis of nanomaterials.[1] Manipulation of droplets such as the controlled movement, merging or splitting has so far mainly been achieved by smart channel design or by electric forces such as dielectrophoresis.[2] Magnetic droplets have so far received relatively little attention, despite the fact that they could be manipulated conveniently with external magnetic fields.

Here were present a microfluidic platform for the generation and downstream manipulation of magnetic droplets in continuous flow. A microfluidic device as shown in figure 1 was fabricated from glass. The internal surfaces were silanised with C18 groups to render them hydrophobic. At a T-junction, aqueous ferrofluid droplets were generated in an immiscible phase of oleic acid. Downstream, the droplets entered a wider chamber. When a magnetic field was applied by means of a small NdFeB magnet, the droplets could be deflected from the direction of laminar flow (figure 2). For example, magnetic droplets of 210 µm diameter were deflected towards the magnet at a speed of 1.6 mm s^{-1}, indicating a magnetic force in the range of nanoNewtons was acting on the droplets.

The extent of magnetic deflection depends on the magnetic loading and size of the droplets as well as on the applied magnetic field. Continuous flow droplet manipulation via magnetic forces represents a viable alternative to electrical manipulation. Further studies will be aimed to use the droplets as reaction vessels.

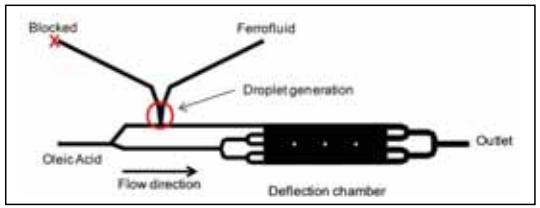

Figure 1: Chip design featuring one inlet for the continuous phase (ferrofluid) and another inlet for the dispersed phase (oleic acid).

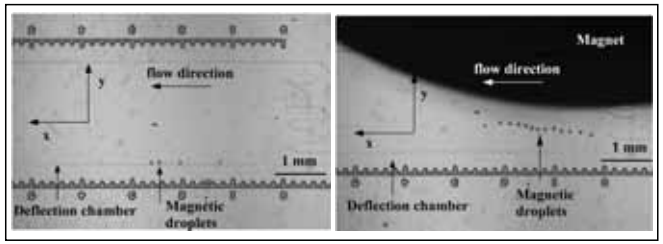

Figure 2: (left) In the absence of a magnetic field, ferrofluid droplets were flowing near the channel wall. (right) With a magnetic field applied, the droplets were deflected from the direction of laminar flow.

References

1. Song, H., D.L. Chen, and R.F. Ismagilov, *Reactions in Droplets in Microfluidic Channels.* Angewandte Chemie International Edition, 2006. **45**: p. 7336 – 7356.
2. Teh, S.-Y., et al., *Droplet microfluidics.* Lab on a Chip, 2008. **8**: p. 198–220.

Quantification of non-Specific Binding of Magnetic Nanoparticles: Implication for detection and magnetic cell separation

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The maturity of magnetic cell separation technology, as indicated by both the commercial success of a number of companies, and high number of citations indicating its use, also places increasing demands on magnetic cell separation performance. While a number of factors can cause sub-optimal performance, one of the major challenges can be non-specific binding of magnetic nano or micro particles to non-targeted cells. Depending on the type of separation, this non-specific binding can have a negative effect on the final purity, the recovery of the targeted cell, or both. In this work, we quantitatively demonstrate that non-specific binding of magnetic nanoparticles can impart a magnetization in cells such that they thee cells can be retained in a MACS separation column in such a way to significantly impact the purity of the final product. We also demonstrate that the nonspecific binding can negatively impact the recovery of desired cells. While highly variable, cells without specifically or non-specifically bound MACS beads can be retained in a MACS column, compromising the performance of the separation. How, this phenomenon is not easily reproducible. We also demonstrate experimentally, and through theoretical arguments that the number of MACS magnetic particles needed to impart a magnetization that can cause non-targeted cells to be retained in the column to be on the order of 500 to 1000 nanoparticles. This number was demonstrated experimentally with an instrument, cell tracking velocity, CTV, and it is demonstrated that the sensitivity of the CTV instruments for Fe atoms is several order of magnitude more sensitive than ICP-MS.

3-D MODELING AND IN VITRO INVESTIGATIUON OF PORTABLE HIGH GRADIENT MAGNETIC SEPARATOR DEVICE DESIGNS FOR BLOOD DETOXIFICATION

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A portable magnetic separator for the selective, on-demand removal of magnetic drug carriers from the flowing blood stream after drug targeting or blood detoxification is theoretically evaluated for optimal carrier removal performance. In the separator design, an array of biocompatible capillary tubing and magnetizable wires is immersed in an external magnetic field. The wires are magnetized and the high magnetic field gradient from the magnetized wires helps to collect blood-borne magnetic carriers from blood flow. In this study, a 3-D numerical model was created and the effect of tubing-wire configurations on the capture efficiency, defined as the percentage of magnetic carriers retained in the device for one pass-through, of the system was analyzed using COMSOL *Multiphysics* 3.3®. The results showed that the configuration characterized by bi-directionally alternating wires and tubes was the best design with respect to the four starting configurations. A more detailed parametric study was carried out to investigate this selected design configuration. The test parameters included mean blood velocity (1 to 20 cm/s), magnetic field strength (0.1 to 2.0 T), carrier size (500 nm to 1000 nm in radii), carrier-incorporated magnetic materials (iron, two types of magnetite) and magnetic content (0.05 to 0.8 by weight), wire material (nickel, stainless steel 430, wairauite), length (2.0 to 20 cm), and size (0.125 to 1.0 mm in radii) and tubing size at a fixed tubing-to-wire diameter ratio. The results of our mathematical simulations defined capture efficiencies well over 90% are achievable under reasonable physiological and engineering boundary conditions (i.e., mean blood velocities within the tubing <5.0 cm/s). Further, the magnetic separator performance was improved by maximizing an applied magnetic field strength of up to 1.0 T, reducing the size of the separator unit, and employing tubing and wires of equal radii. I*n vitro* experiments verified the numerical predictions. In summary, the results support the feasibility of a magnetic separator technology for the efficient and selective removal of magnetizable drug carriers from the blood stream.

Application of Magnetic Poly(Styrene-Glycidyl methacrylate) Microspheres for Immunomagnetic Separation of Bone-Marrow Cells

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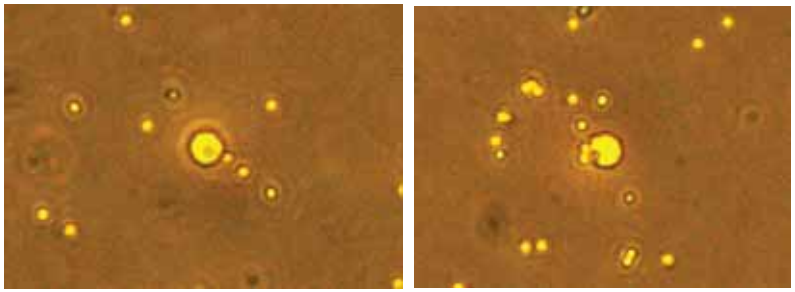
Magnetic poly(styrene-glycidyl methacrylate) (PS-GMA) microspheres containing either amino or carboxyl groups on the surface were prepared and then coupled with antibodies that could specifically recognize the surface proteins of stem cells in bone marrow. Prior to antibody coupling, carboxyl group-containing magnetic PS-GMA microsphres were chemically bound with avidin. Immunomagnetic separation of cells from the suspension of mononuclear cells (MNCs) from mice femurs based on the positive selection of Sca-1 surface marker was carried out by either direct or indirect approach. In the direct method, biotinylated Sca-1 antibody was coupled directly to the avidin-bound magnetic microspheres; while in the indirect method, a goat anti-mouse antibody (secondary antibody) was oxidized and immobilized onto the amino group-containing magnetic microspheres by the site-directed manner, and the resultant conjugate was then coupled with non-modified Sca-1 antibody (primary antibody) for cell selection. By the indirect selection the purity of isolated Sca-1^+ cells increased with bead-to-cell ratio up to 10. When the bead-to-cell was 10, a purity of 85 % Sca-1^+ cells and an enrich fold of 17 were achieved by the indirect selection. The purity was 62 % and the enrich fold was 11 by the direct selection using the same bead-to-cell ratio.



Isolated Sca-1 positive cells on magnetic PS-GMA microspheres.

The effect of length of adenine chain on its isolation and detection using paramagnetic particles coupled with square wave voltammetry

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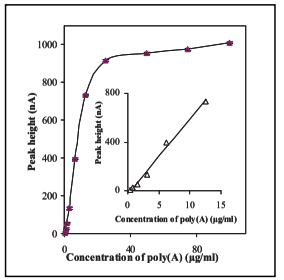
Isolation of nucleic acids with high yield and efficiency is needed for molecularly biological studied on physiological processes in living organisms. Recently the attention was devoted to the identification and characterization of the small RNAs. For these purposes new processes and techniques have been developing. One of these technologies paramagnetic particles with differently modified surface represent. Various magnetic particles with surfaces modified by nucleic acids, proteins and/or antibodies are available commercially. The main aim of our work was to utilise paramagnetic particles with the surface modified by oligonucleotides containing 25 thymine bases for isolation of homooligonucleotides containing various number of adenines. It is well known that different physico-chemical factors influence quantitative isolation of molecules of interest. The influence of the size of the isolated molecule can be very important factor, because of this our experiments were aimed on the investigation of the isolated nucleic acid molecules size (length). For this purpose the oligonucleotides of the length A5, A10, A15, A20, A25, A30 and Poly(A) were chosen. Isolation was carried out in the buffer of total volume 10 - 30 μl. The hybridisation process was optimised. The solution contained of 0.1 M Na_2HPO_4 + NaH_2PO_4; 0.5 M NaCl; 0.6 M guanidium thiocyanate and 0.15 M Tris-HCl (pH = 7) was found as the optimal for the highest yields.. Moreover we determined that the most suitable time of hybridisation of poly(A) varied from 35 min to 40 min when after 40 min the change in detectable amount was negligible. In the case of the shorter molecules (A5, A10 and A15) the most suitable time of hybridisation was from 20 min to 25 min. This interval varied from 25 min to 35 min in the case of A20, A25 and A35. The washing and hybridisation steps were automated using apparatus (MSC 3000 multi spin) that enables short centrifugation and shaking. The temperature of hybridisation was also studied. Based on our results the temperature doesn't play essential role and the procedure can be carried out at laboratory temperature without loss of the nucleic acid recovery. Quantifications of captured nucleic acids were carried out by square wave voltammetry. Typical calibration dependence is shown in the figure (y = 61.717x - 27.378, R^2 = 0.9934). The limits of detection for nucleic acids were estimated as tens of ng/ml.



Acknowledgement: The financial support from the grant KAN 208130801 is highly acknowledged.

Development of magnetic SiO_2-coated, antibody-targeted particles

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Magnetic separation of cell suspension mixtures using magnetic antibody-targeted particles (MAPs) is widely used in current scientific and medical practice. MAP development involves an important and laborious step of antibody immobilization on solid magnet-sensitive carriers, and there is a need for an immobilization method, which would not require carrier modification and can be performed quickly at room temperature and neutral pH.

A method of metal-chelate protein immobilization on solid surfaces is known from the literature. We suggested applying this method to produce magnetic particles carrying monoclocal antibodies on their surface.

To this aim, magnetic carrier particles 0.4 mcm large were produced from magnetite $FeO{\cdot}Fe_2O_3$ with SiO_2-coating formed by polycondensation of silica-organic olygomer AquaSil™. The carrier was activated by $TiCl_4$ for 4 hours at room temperature. 1 mg of activated particles were shown to covalently bind 80 mcg of immunoglobulin and retain this binding for 6 months.

Study of method feasibility was performed using anti-CD3-, CD4-, CD5-, CD20- and CD34 antibodies (RPC Medbiospectr Ltd., Moscow, Russia), which were immobilized at a level of 15 mcg per 1 mg of carrier. To prevent non-specific MAP-cell interaction, the carrier surface was treated by bovine serum albumin, gelatin, Tween-20 and dextran 10 kDa or 70 kDa.

Validation of antibody immobilization was performed by assessment of cell separation efficacy using a mixture of Jurkat, Raji and KG-1 cells. Separation was performed by means of a constant magnet, which allows concentration of magnet-sensitive MAP-cell complexes on the side wall of the vial.

MAPs blocked by 70 kDa dextran ensure reliable separation of the model cell mixture. At MAP/cell ratio = 4-32/cell the separated target cell fraction exceeded 60 % with purity >95 % and more than 90 % viable cells. Non-specific MAP-cell binding did not exceed 4 %.

The proposed method of MAP production can be applied to development of magnetic antibody-targeted particles, which are used for separation of peripheral blood and bone marrow cells during management of cancer diseases and immune dysfunction.

Development of low nonspecific bonding magnetic beads for bioseparation

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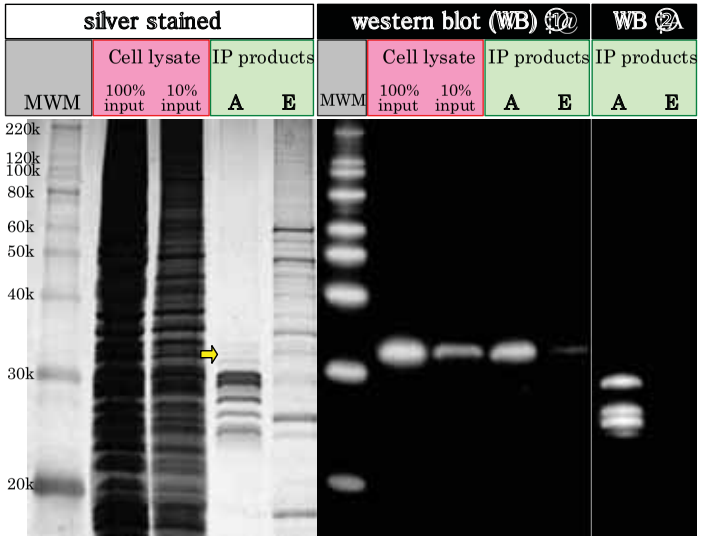
Magnetic beads have been popular in the field of clinical diagnostics as a solid support for enzyme-linked immunoassay (ELISA) because of their advantage on automation suitability, easy handling and rapid separation. In the bioseparation process, it is important to reduce contaminations caused by nonspecific bonding (NSB) of proteins to the solid surface. Magnetic beads have not been widely used as solid support for bioseparation as much as agarose beads because of disadvantage of NSB.

In this study, we discuss influence of functional group and hydrophilicity of the surface on NSB. Condition of antibody conjugation on NBS were studied and optimized through immunoprecipitation (IP) of 20S proteasome complex from jurkut cell lysate.

We also show the performance of optimized low NSB magnetic beads in bioseparation of cells, tagged protein in cell lysate and low abundance proteins such as alpha -fetoprotein in serum. Furthermore, immunoassay using our low-NSB magnetic beads has high signal to noise ratio.



Identification of the immunoprecipitated 20S proteasome. The point of the arrow shows the band of α6 subunit of 20S proteasome.
WB1 : α6 subunit was detected
WB2 : α5/ α7, β1, β5, β5i, β7 subunits were detected

Magnetic particles with high magnetization for immunoassay

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Magnetic particles have been widely used in bio-medical fields. Conventional particles are usually made of iron oxides because of their chemical stability. The response to a magnetic field depends on magnetization of magnetic materials. Therefore, particles composed of metallic Fe exhibit higher magnetization than iron oxides and are expected to show a stronger response to a magnetic field. However, chemical instability of Fe, oxidation, has prevented their practical use. A preferable method of preventing oxidation is encapsulation of Fe particles. We have developed metal Fe fine particles encapsulated by titanium oxide (Ti-O/Fe), and applied them to cell separation (presented at the last 6th meeting).

The average diameter of Ti-O/Fe particles was controlled in the range from 0.7 μm to 8.0 μm. The particles' magnetization of 120-140 Am^2/kg resulted in significantly faster response to a magnetic field compared to conventional magnetite particles. Furthermore, the Ti-O/Fe particles were non-toxic to tumour cells when tested in an MTT cell viability assay (Fig. 1). This suggests that they are biocompatible.

Highly magnetic particles of 0.8 μm in diameter were prepared by coating Ti-O/Fe particles with silica and functionalization with streptavidin (HMMI particles), followed by conjugation to antibodies. These particles were then used in an immunoassay to detect adiponectin antigen and to compare them to conventional magnetic particles (Fig. 2). The results suggest that HMMI particles are more sensitive than conventional particles and that they might be candidates for next generation magnetic particles.

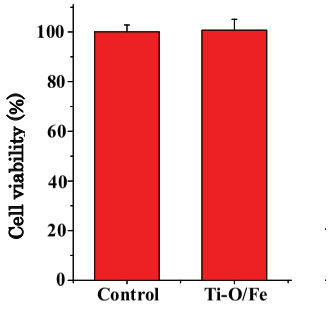

Fig. 1. Cell toxicity test of Ti-O/Fe particles in mesothelioma H226 cells.

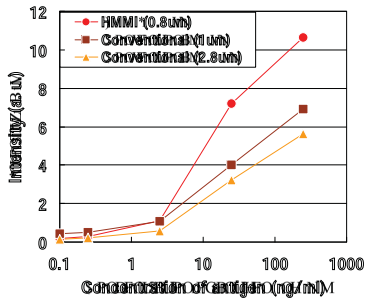

Fig. 2. Immunoassay sensitivity for the antigen adiponectin.

Preparation and Characterization of Electrospinning NiZn-ferrite Nanofibers for DNA Separation

J. H. Nam[1*], Y. H. Joo[1], J. H. Lee[2], J. H. Chang[2], J. H. Cho[1], M. P. Chun[1], and B. I. Kim[1]

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Magnetic separation is an emerging technology that uses magnetism for the efficient separation of micrometer-sized magnetic carrier from chemical or biological suspensions. It also has many advantages compared to other techniques used for bio-separation or purification. Recently, many previous works have been investigated to use as magnetic carriers of iron oxide nanoparticles. The ceramic nanofiber exhibit both large surface area and specific nanostructures, which tend to be mutually exclusive. A new challenge to use for magnetic carrier of ferrite fiber is to engineer materials surfaces that allow for a two-way communication between adsorption and desorption of DNA.

The spinel ferrite MFe_2O_4(M=Ni, Zn) nanofibers were synthesized by a electrospinnng process in this study. The raw materials as chemical reagents were iron(Ⅲ) chloride, nickel(Ⅱ) acetate tetrahydrate and zinc acetate dihydrate. The aqueous metal salts/polymer solution was prepared with polyvinyl pyrrolidone in N,N-dimethylformamide and metal salts under stirring at room temperature. The applied electric field and spurting rate for spinning conditions were 10kV, 2㎖/h, respectively. The obtained fibers were calcined at 600℃ for 3h and annealed at 900℃ in air. By tuning the viscosity of batch solution before electro-spinning, we were able to control the microstructure of NiZn ferrite fiber in the range of 70~200nm at 770 cp. The primary particle size in a ferrite fiber was about 10~15nm. The properties of those NiZn ferrite fibers as magnetic carrier were determined from x-ray diffraction, electron microscopy, thermal analysis, and magnetic measurement. The DNA adsorption efficiency yields compared to magnetite nanoparticle showed about 50% for UV light wavelength, which can be modified and utilized for DNA separation with magnetic nanofiber in clinical applications.

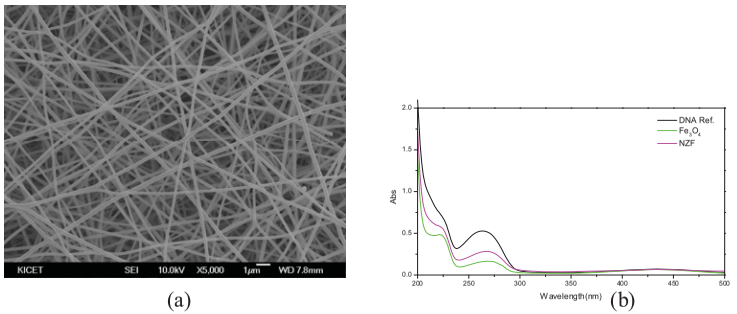

Fig. Characterizations of NiZn ferrite nanofiber ; (a) FE-SEM images of those nanofiber(NSF), (b) DNA adsorption efficiency compared with magnetite spherical nanoparticle, respectively.

Spray-dried Yttrium Iron Garnet Powders for Bio-purification

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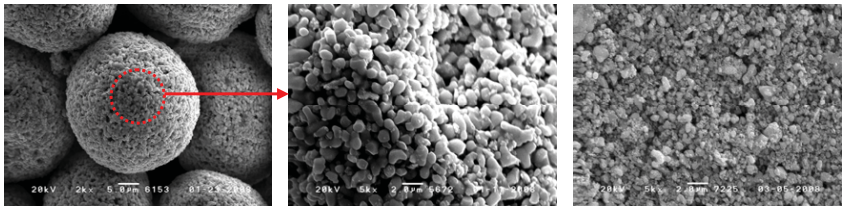
Some of magnetic oxide particles as Fe_3O_4 and γ-Fe_2O_3 in medicine and biology has been attracting significant attention in vitro applications as enzyme linked immuno-sorbent assay test, cell separation, bio-application. Recently, it has been studied that other magnetic oxide materials as yttrium iron oxide were prepared to use for biomedical and biochemical applications. In the present paper we describe a new challenge of submicron yttrium iron garnet in the area of bio-chemical application.

YIG ferrite was prepared by conventional ceramic process and ball-milling for dispersion of particle aggregation. After ball-milling, spray drying process was applied to form spherical YIG particles with typical surface microstructure. Additional mechanical milling was also applied to reduce the particle size. The properties of YIG ferrite as magnetic carrier were determined from x-ray diffraction, electron microscopy, thermal analysis, and magnetic measurement. The DNA adsorption efficiency produced as absorbance for UV wavelength has increased and showed the enhanced property for milled YIG ferrite with particle size reduction, which can be modified and utilized for DNA separation. The presence of particle size effect of YIG ferrite resulted in improvement of DNA separation therefore we can control the spherical particle size by synthesis processing and expect to produce the better efficiency for clinical application.



(a) (b) (c)

Fig. SEM micrographs of YIG ferrite material ; (a) as spray-dried, (b) magnified surface morphology of spray-dried YIG particle, (c) mechanically milled particles.

Table Characterization in DNA separation of spray-dried YIG ferrite powder.

Adsorption time	UV-Light Absorbance (%)		
	DNA	Spray-dried YIG	**Milled YIG**
2 h	0.53	0.53	**0.33**
4 h	0.53	0.50	**0.32**
6 h	0.59	0.58	**0.31**

A magnetophoresis chamber with 25 fractions output – design, fabrication and demonstration

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ABSTRACT

Our goal is to develop an instrument that will aid biomedical capabilities by offering a technique for high throughput, high sensitivity biomolecular analysis. We are developing a magnetophoresis instrument that incorporates a microfluidic flow chamber located inside a magnetic field gradient. This system is used to sort magnetic microparticles according to their magnetic moment for eventual use as biological labels.

Here we will discuss the chamber fabrication process, concentrating on the development of our techniques that have allowed us to increase our sorting capability from 8 channels to 25 channels. Key to the process is the use of double-sided tape to join the 3 layers in the chamber. We will present data for the sorting of magnetic microparticles and the subsequent resorting of a selected bin to give an indication of the reproducibility.

Optimization of magnetic affinity separation method for Highly-efficient purification of polyhistidine-tagged Proteins.

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Abstract:

Although purification of histidine-tagged proteins using Immobilized Ni^{2+}-Affinity Chromatography by magnetic bead supports can be achieved in a single capture step, the inability of a procedure to achieve uniform quality purification results for each recombinant His-tagged protein presents a major limitation. In this study we are present our practical experiments for greatly improved purification of three types of recombinant His-tagged proteins by commercial Ni-magnetic beads. According to results introducing unique procedure for Immobilized Ni^{2+}-Affinity Chromatography by magnetic bead supports dependents on different conditions such as particles concentration, the ratio of particles and targeted protein, particles surface, targeted protein molecular weight, degradability of targeted protein and host cell lysis method.

On-chip deflection of magnetic microparticles in continuous flow. A comparative study.

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The magnetic deflection of seven commercially available microparticles from three different manufactures (Dynal, Bangslabs and Micromod), with diameters between 1 to 10 µm was compared in a microfluidic device using free-flow magnetophoresis.

Magnetic microparticles are being used with increasing popularity as solid supports for analytical systems due to the large surface to volume ratio and their ease of manipulation using external magnets.(1) Recently, a method for the separation and isolation of magnetic particles (2.0 and 4.5 µm) in continuous flow has been developed, termed on-chip free-flow magnetophoresis (figure 1).(2) Here, we investigate the deflection behaviour of a wide range of commercially available particles with different flow velocities and magnetic field settings.

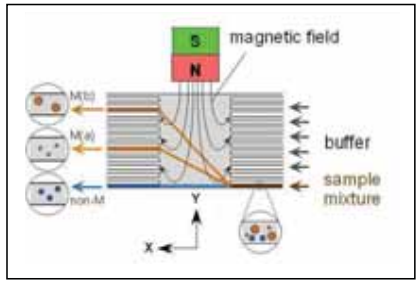

Figure 1. The principle of free-flow magnetophoresis. Magnetic particles flowing in the x-direction experience a force from a magnetic field in the y-direction resulting in the particle being deflected.

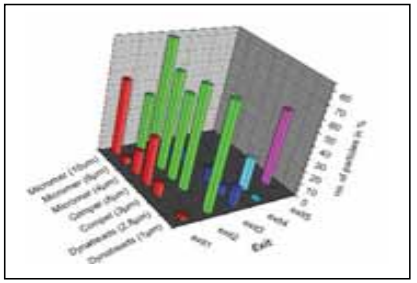

Figure 2. The deflection behaviour of micro-particles from three different suppliers measured for a given flow rate and magnetic field was found to differ considerably.

The deflection behaviour of magnetic particles in continuous flow depends on their size and magnetic properties. The degree of deflection is proportional to the ratio of magnetisation of the particle, M_s, over its radius, r.(3) A typical example for the observed deflection behaviour at a flow rate of 0.2 mm s^{-1} is given in figure 2. Considerable differences between manufacturers were noted. When the observed deflection was compared to the magnetic properties provided by the manufacturers, it was found that some did not behave as expected. Also, there was a wide distribution in the deflection of a particular particle type suggesting differences in magnetite content within a single batch. To further characterise the microparticles, independent magnetisation measurements using a Vibrating Sample Magnetometer (VSM) were performed. These results were consistent with the particle deflection behaviour observed on-chip.

In addition, the effect of temperature and liquid viscosity on the deflection behaviour of one type of particle was investigated. Particles experienced a greater degree of deflection from laminar flow as fluid temperature increased due to lower fluid viscosity. This could greatly affect applications that incorporate magnetic particles as labels in magnetophoretic measurements.

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Combinatorial Magnetic Separation- a Novel third generation separation

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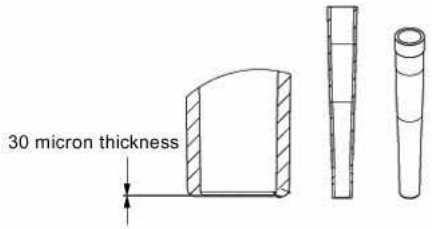
Magnetic separation technology has become a quick, easy and cost efficient method for reliably capturing targeted cells and bio-molecules from biological and other samples.
Many robotic manufactures have developed automated magnetic separation machines; while most of these manufacturers utilize a simple first generation machine that applies a magnet outside of the well plate and handles the liquids in the well plate, some have made a second generation machine which moves the magnetic particles instead of the liquids. Bio-Magnetics has developed a combinatorial magnetic separator (CMS) that is a third generation concept. With CMS the magnets can move with much more freedom in that the user can select and move any one or combination of desired magnetic pins into a standard 96 and 384 well plate. It is particularly important when conducting continuation tests, and for confirming positive detections. The CMS controls the magnetic particle and allows these particles to be moved between the wells providing a unique, efficient method of washing particles, increasing significantly the interacting surface area and saving time in the process. The liquid does not move but rather, the particles. We have developed a tip whose thickness is under 30 microns to be used in a second generation separation that achieves a 99.98% of the particles and saving 60 % of protocol time compared to first generation. We have developed a separation tools that give a purer, faster and costs are significantly saved.



Scale-up and comparability study of T-cell depletion in single inlet – single outlet QMS

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Pre-clinical scale T-cell depletion (2×10^8 processed cells) using a single inlet – single outlet, pump controlled quadrupole magnetic flow cell sorter (QMS) was under investigation to show reproducibility of published results in a process scale-up. Buffy coats from haemochromatosis patients were spiked with cultured Kg-1a cells to mimic peripheral progenitor cells. The target T-cell fraction was immunomagnetically labeled in a one or two step labeling protocol without washing and depleted by negative selection at sorting speeds of up to 3.3×10^6 cells per second. A mean $\log_{10}$ T-cell depletion of 2.9 (range 2.13 to 3.74, n=4) for a one-step labeling strategy and a mean $\log_{10}$ T-cell depletion of 3.1 (range 2.58 to 3.59, n=2) for a two-step labeling strategy showed a reasonable agreement with published data describing $^1/_{10}$ as many cells processed compared to this study. The depletion experiments show high total cell recovery (92.7 ± 7.7%) and high viability (95.0 ± 3.8%) and recovery of the spiked cell fraction (81.7 ± 14.7%). The study also revealed challenges and limitations for a scale-up into clinical scale T-cell depletions ($> 1 \times 10^9$ - 1×10^{10}). The current goal is a 4 $\log_{10}$ T-cell depletion. The composition of different cell fractions for an ideal depletion is shown in the Figure.

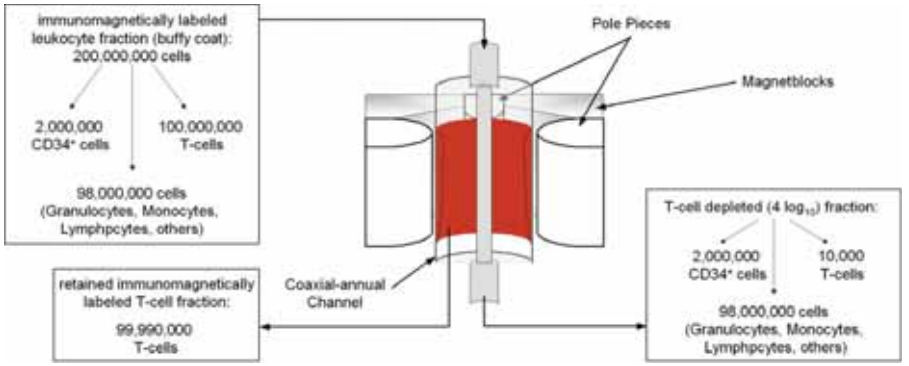

Figure. Idealized 4 log_{10} T-cell depletion by QMS.

Effects of ionizing radiation on induction of deletion mutants in yeast *Saccharomyces cerevisiae*

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Molecular studies in yeast *Saccharomyces cerevisiae* often require the isolation of both plasmid and chromosomal yeast DNA. Highly purified DNA is necessary for analytical procedures such as *in vitro* mutagenesis, molecular cloning and sequencing. But most methods utilize PEG precipitation and phenol/chloroform extraction procedures for the purification of DNA. These preparations are often contaminated with impurities that interfere with restriction enzyme digestion, cloning, sequencing, etc. Isolation of DNA using magnetic nanoparticles is quick, cheap and simple [1].

The objective of this study is to determine the effects of ionizing radiation (gamma-rays with the flux 0.7 Gy/min and energy 1.3 MeV and heavy ion ^{11}B with energy 45 keV) on induction of deletion mutants. We used a plasmid system developed in Tokyo University by Hideo [2] for quantitative analysis of deletion formation. The plasmids rescued from the Can^R Cyh^R (resistant to canavanine and cycloheximide) mutant cells (eight clones *Saccharomyces cerevisiae* strain CRY1-2c with genotype *MAT*α *RAD*53 *ade*2-1 *ura*3-1 *trp*1-1 *his*3-11,15 *leu*2-3,112 *can*1-100 *cyh*2[YCpL2]) induced by radiations were introduced into *E. coli* strain TG1 and analyzed by agarose gel electrophoresis. Plasmid DNA isolation from *E. coli* was carried out according to Birnboim and Dolly [3] with modification using magnetic nanoparticles. Restriction mapping of the plasmid DNA allows localizing deletion on the plasmid. Restriction fragments of plasmid DNA prepared from mutant YB100-2-2 is shown in Figure1.

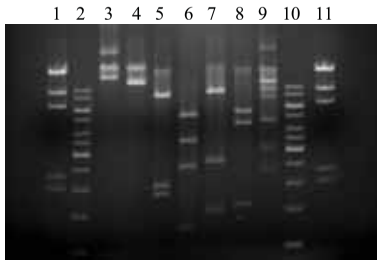

Fig 1. Restriction analysis of plasmid DNA isolated from mutant YB100-2-2 using magnetic nanoparticles. Lanes 1 and 11: λ phage DNA/*Hind*III digest, lanes 2 and 10: DNA molecular weight marker (1 kb ladder), lane 3: plasmid DNA from mutant YB100-2-2, lane 4: *Xba*I digested mutant YB100-2-2, lane 5: *Kpn*I digested mutant YB100-2-2, lane 6: *Eco*RV digested mutant YB100-2-2, lane 7: *Eco*RI digested mutant YB100-2-2, lane 8: *Hind*III digested mutant YB100-2-2, lane 9: *Dra*I digested mutant YB100-2-2.

Genetic analysis shows that the rescued plasmids from all the analyzed mutants were smaller than the parental plasmid YCpL2. Furthermore, the resistance to canavanine and cycloheximide is more likely due to a deletion in the *CAN1-CYH2* region of the plasmid YCpL2.

Additionally, using Schmitt et al. procedure [4] as a base we have developed a modified procedure for chromosomal DNA isolation from yeast using magnetic nanoparticles as a solid phase support.

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Theory for Nanoparticle Retention Time in the Helical Channel of Quadrupole Magnetic Field-Flow Fractionation

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Quadrupole magnetic field-flow fractionation (QMgFFF) is an analytical and characterization technique for particulate magnetic materials [1-4]. The magnetic nanoparticles used for cell labeling and for targeted drug therapy tend to be composite materials consisting of a magnetic core with biocompatible coatings carrying antibodies or therapeutic drugs. The coatings stabilize the materials in suspension and also reduce the magnetic dipole-dipole interactions between the particles in a magnetic field.

The FFF techniques are similar to chromatography in that different components of a small sample elute from a separation channel at different times. Chromatography exploits differences in partition between the mobile and stationary phases to separate sample components as they are carried along a column, but FFF separation is achieved within the flowing mobile phase and does not utilize a stationary phase. Quadrupole magnetic field-flow fractionation uses a thin helical channel mounted in a radially symmetric field gradient. Magnetization of the particulate sample is induced by the applied magnetic field and the particles are driven toward the outer channel wall by their interaction with the field gradient. Due to viscous drag, the mobile phase velocity profile across the channel thickness is close to parabolic, with highest fluid velocity near the channel center and zero velocity at the walls. For particles smaller than about a micron in diameter, an exponential concentration profile results from the opposing influence of the field-driven and diffusive transport mechanisms. Particles that interact strongly with the field gradient form thin zones adjacent to the wall, and are confined to the very slow moving fluid close to the wall. Particles that interact less strongly with the field gradient form more diffuse zones, and they sample faster fluid streamlines in addition to those close to the wall. A separation between the particles is thereby induced as they are carried along the channel.

The fluid velocity profile in the thin helical channel deviates from the parabolic profile found in a parallel plate channel. Approximate equations describing the fluid velocity profile in the helical channel will be presented together with derived equations for the so-called retention ratio. This is the ratio of the mean fluid residence time in the channel to the elution time of a retained material. The quantitative theoretical foundation of FFF allows the determination of the strength of interaction of particles with the field gradient as a function of their elution time.

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Magnetic Focusing for Cell Enrichment and Analysis

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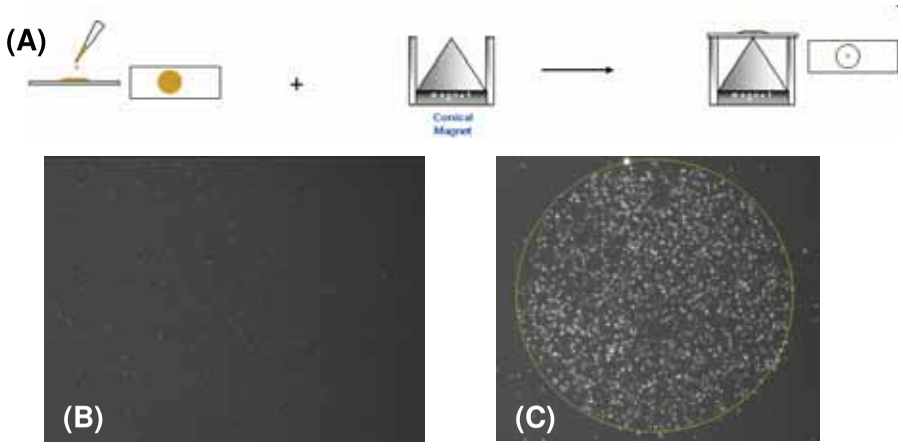
Conventional optical analysis of biological cells in a fluid sample typically requires separation of target cells from other biological components. Cells can be labeled with magnetic particles and separated using a magnetic field. Typically, such methods require repeated washing after separation and an optical scanning/imaging of the separated samples for analysis, which are both time consuming and usually require expensive instrumentation.

In this study, we present a magnetic separation method that is suitable for concentrating specific type of cells or other particles in samples of micro-liter volume. The method uses a conical magnet, constructed with a soft magnetic pole piece attached to a rare-earth permanent magnet, capable of concentrating magnetically labeled cells into an area that is small enough to be imaged within a single microscopic objective field of view, eliminating the need for optical scanning. This magnetic focusing and optical imaging system can be used to detect and enumerate subtype blood cells and rare cells in a biological sample in a non-wash single-solution format and yields quantitative results that correlate well with results obtained by commercial flow cytometry systems.

This magnetic focusing method provides a fast, economical and controllable way to separate and enrich cells or other particles in a biological sample, and potentially can be combined with a variety of detection and quantization methods.



(A) Schematic of the mechanism of magnetic focusing separation. Images of fluorescently labeled cells in the same sample before(B) and after(C) magnetic focusing

Research on relationship between surface structure and morphology of Fe_3O_4/Silica composite nanospheres and nucleic acid extraction

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Fe_3O_4/Silica composite nanospheres are widely used in nucleic acid extraction due to the adsorbing effect between silica and nucleic acid in specific solvent. Currently, the synthesis of Fe_3O_4/Silica composite nanospheres with different surface structure and morphology and the adsorbing effect between silica and nucleic acid are respectively widely investigated. To enhance nucleic acid extraction efficiency, it's necessary to investigate the effect of surface structure and morphology of Fe_3O_4/Silica composite nanospheres on nucleic acid extraction.

In this paper, we focused on the synthesis of Fe_3O_4/Silica composite nanospheres with different surface structure and morphology via modified Stöber methods, and compared the effect of different surface structure and morphology of Fe_3O_4/Silica composite nanospheres on nucleic acid extraction. We firstly prepared sphere-like magnetite nanoparticle aggregations with a typical hydrodynamic size ~150nm [1], these aggregations were used as core of Fe_3O_4/Silica composite nanospheres. Secondly, the core-shell Fe_3O_4/Silica composite nanospheres were synthesized via modified Stöber methods [2]. The surface structure and morphology of Fe_3O_4/Silica composite nanospheres such as size, thickness of silica shell, surface area, pore volume, pore diameter distribution and the silanol group density on the surface could be controlled by adjusting the reaction conditions such as catalyzing condition, tetraethyl orthosilicate (TEOS) concentration, reaction temperature, methyltrimethoxysilane (MTMS)/TEOS molar ratio and aging solvent. Finally, the synthesized Fe_3O_4/Silica composite nanospheres with different surface structure and morphology were used to extract herring sperm DNA, salmon sperm DNA and λ DNA, the related effects of surface structure and morphology on DNA extraction were investigated.

The Fe_3O_4/silica composite nanospheres were characterized by HPPS, TEM, N_2 Adsorption and TGA. The extracted DNA was characterized by Nanodrop. It was learned that when the density of the silanol group is in a certain number, the sample with a surface area of 60.37m^2/g extracts DNA most effectively. When the surface area was in a certain number, the more silanol groups, the more DNA was extracted, the results were shown in Figure 1 and Figure

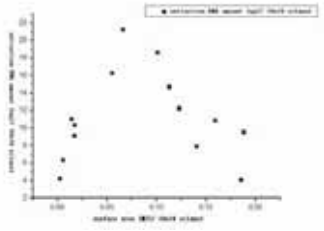

Figure 1 Effect of surface area on DNA extraction

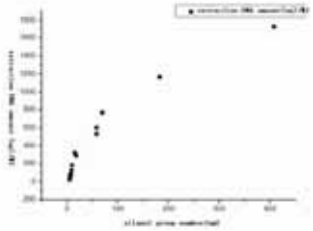

Figure 2 Effect of Si-OH density on DNA extraction

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Drag Force on Magnetic Microparticles at an Air-liquid Interface

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Magnetically targeted inhaled aerosol drug delivery to the human airways has the potential to improve chemotherapy for lung cancer treatment. To retain inhaled particles at the target site, the body's natural defenses against inhaled particles, i.e. mucus clearance, must be overcome. A magnetic field can be used to hold particles at the target site in the airways against the mucus clearance mechanism. This poses unique challenges, as the applied magnetic field must overcome drag on partially immersed particles at the air-liquid interface of a viscoelastic liquid.

Unlike retention of fully immersed magnetic particles, e.g. in the bloodstream, retention of magnetic particles at the airway surface has not been extensively considered in the literature. Previous studies have shown that the drag force on particles and particle aggregation are key factors for particle retention in the airways. To determine the drag force and particle mobility, the drag coefficient of the particles and aggregates along the air-liquid interface of the mucus layer must be known. Although individual particles will be pulled into the mucus layer by surface forces, larger particles and particle aggregates will remain at the interface. Experimentally, the motion of particles at the air-liquid interface of various liquids in a magnetic field is observed in order to determine the drag coefficients by trajectory analysis. A simplified system consisting of magnetic particles at an air-liquid interface is considered in order to perform a parametric study.

Various liquids were used (water, silicone oil and polyethylene oxide-water mixtures) to test a range of viscous and viscoelastic liquid properties. Rheological characterization of the liquids was done using a rheometer. The particles used were 4 μm diameter polystyrene magnetic particles. In addition to particle size, the drag coefficient also depends on the extent of particle immersion, i.e. the particle contact angle, which was measured using the film trapping technique.

Particles were placed at the interface of the liquid and a magnetic field applied. The resulting particle motion was observed under a microscope, and particle trajectories determined from captured image data. The drag coefficients were then determined from a force balance between the magnetic and drag forces for the observed trajectories, as illustrated in Figure 1. This is a first set of data of its kind as it relates to potential application of targeted drug delivery to the airway surfaces.

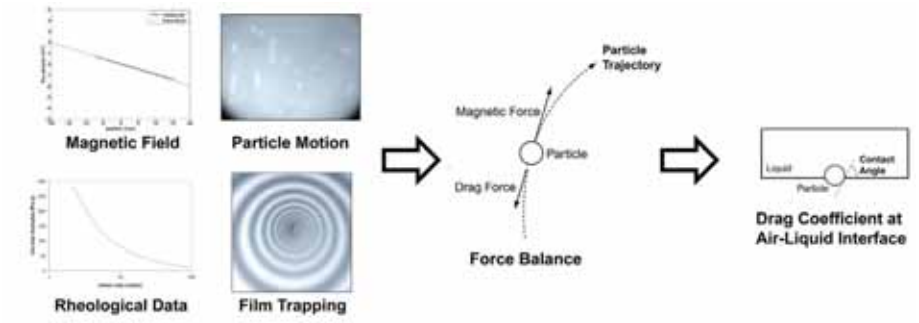

Figure 1: Flowchart for determining drag coefficients at an air-liquid interface.

Magnetic scaffolds for tissue engineering

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The use of scaffolds for tissue engineering is increasing due to their efficacy in helping the body rebuild damaged tissue. One of the current issues concerning this technique is how to improve the re-growth of damaged tissue by supplying growth factors; these have to be readily available to the live tissue that is growing in the scaffold.
In the present work we address this problem by magnetizing the scaffolds. We show an example of our magnetizing technique on a bone graft substitute, which is used to replace damaged or diseased bone tissue. The susceptibility of the scaffold is sufficiently high that, if placed in a magnetic field, it acts as magnet. In our vision this will allow growth factors, which will be attached to magnetic nanoparticles, to be attracted by the scaffold and hence be available to the growing live tissue.

ADSORPTION OF DOXORRUBICIN ONTO MAGNETIC NANOPARTICLES FOR DRUG TARGETING

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Magnetic targeting has being investigated as a means of local delivery of drugs, combining precision, minimal surgical intervention, and satisfactory concentration of the drug in the target region. In view of these advantages, it is a promising strategy for improving the pharmacological response of drugs with low therapeutic index as anticancer drugs.

Magnetic nanoparticles have been largely used in biotechnology. These particles are attracted by a magnetic field gradient, and drugs bound to them can be driven to their site of action by means of the selective application of magnetic field on the desired area. Doxorubicin (Dox) is a promising anti-cancer drug and previous studies with iron complexation have been done aiming a reduction of its cardiotoxicity. We have explored the binding of Fe to Dox (Fig.1) to develop a magnetic delivery system. The goal of this work was to evaluate the adsorption of doxorubicin in magnetite nanoparticles, with dimensions around 230nm.

The magnetic particles were prepared by a coprecipitation method in the presence of the PVA. An aqueous solution of Doxorubicin was added to the magnetite suspension. The suspensions were stirring for three minutes and than, the magnetite particles were allowed to settle down in the presence of a 100 mT magnetic field. The analysis of the DOX adsorption on magnetite was made UV-visible spectrophotometric studies of the supernatant.

We have found that 21% of the doxorubicin in the suspension was adsorbed onto the magnetic nanoparticles. In addition, our results suggest the possibility of direct binding of other antracyclines to surfaces of metallic particles. We notice that for the purpose of vectorization, the direct adsorption of Dox to magnetite has the advantage of larger magnetic response and smaller particle size as compared to polymeric formulations.

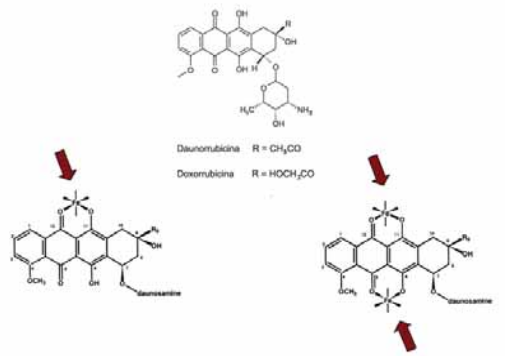

Fig.1. Chemical structure of doxorubicin and places of iron complexation.

Calculation of Dipole Interactions in Magnetic Drug Targeting

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The magnetic targeted drug delivery system of Aviles, Ebner and Ritter [1], which used SS 409 as the seed ferromagnetic material and iron for the magnetic drug carrier particles, is considered. Agglomeration of the particles is known to occur is such systems and here the effect of magnetic (dipole) interactions between the particles is included. The dipole interactions were calculated previously by Mikkelson et al. [2] under low magnetic fields.

Here, for higher magnetic fields, the effect of the magnetic interactions on the two particles is calculated using a reference particle and particle tracking. The calculations were performed with the open source software OpenFOAM. The system performance is assessed in terms capture cross section [1]. In the simulations agglomeration is seen to occur leading to larger capture cross section.

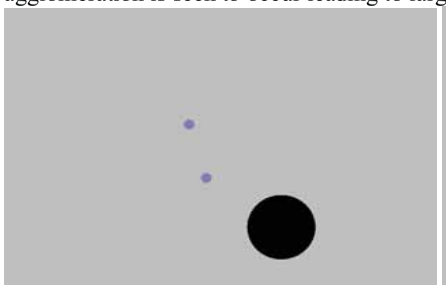

Figure 1a

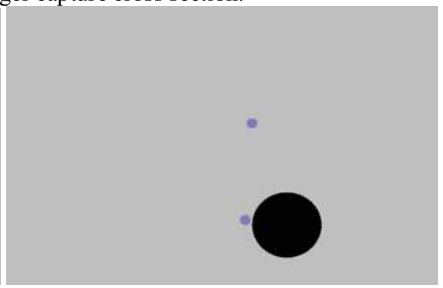

Figure 1b

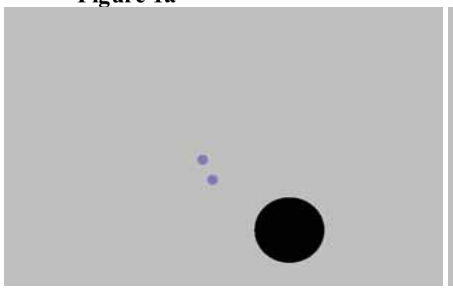

Figure 2a

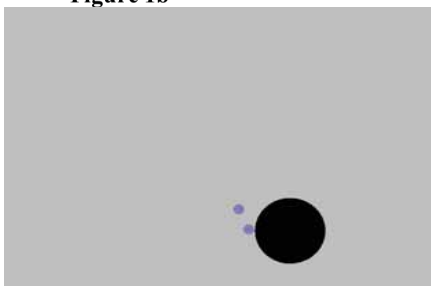

Figure 2b

Figures 1a and 1b are the output of the OpenFOAM program and show the particles behaviors without dipole interactions. Figures 2a and 2b show the effect of the dipole interaction. Iron (50 nm particle radius) for the magnetic carrier particles and SS 409 (1000 nm seed radius) as the seed ferromagnetic materials is considered under high magnetic field.

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Magnetic Drug Targeting to the Lung: Preliminary In vitro Investigations

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Purpose: To improve the pulmonary deposition of active substances by means of superparamagnetic aerosols and high-gradient magnetic fields.

Introduction: The major concern of drug targeting is to enhance the concentration of an active substance at its site of action while minimizing the systemic exposure and thus adverse reactions. Tumor diseases of the lung, especially non-small cell lung cancer (NSCLC), are difficult to treat, with poor prognosis for patient survival. The major drawback of systemic chemotherapy is the low availability of *in vitro* effective drugs. This is partly due to low perfusion of the tumor and over-expression of efflux-transporters like p-gp and MRP. Inhalation of chemotherapeutic agents as a simple way of drug targeting to the lung is limited due to intensive deposition into the larynx caused by impingement of large droplets associated with therapy-limiting adverse reactions. When inhaling smaller particles, huge amounts of drug are exhaled. In this case, we suggest that magnetic retention forces to the aerosol particles may increase the deposition probability. In addition, due to the presence of appropriate nanoparticles the uptake mechanisms may switch to endocytosis, thus avoiding the elimination by efflux-transporters.

Materials and Methods: Preliminary investigation was performed using a commercially available water-based ferrofluid composed of maghemite, proven to be biocompatible. The ferrofluid was atomized by a pneumatic nebulizer (Pari Boy SX, Pari GmbH, Germany) that generates an aerosol with a median mass diameter (MMD) of 2.9 µm. Different magnetic gradient fields were generated by several permanent magnets in varying arrangement. Magnetic induction was measured in order to calculate the magnetic field gradient around the magnetic poles. Approximately 2 mL of ferrofluid (~100 mg Fe) were atomized and deflected onto the flappers aligned parallel to the air stream by the magnetic field. These flappers were placed in varying distances from the magnetic poles. The intercepted particles were decomposed and analyzed using flame atomic absorption spectrometry.

Results: The gradients directly upon the magnetic poles were calculated to be approximately 40 $T \cdot m^{-1}$. With optimal arrangement of the magnets, 10 $T \cdot m^{-1}$ were generated in a distance of 2 cm from the poles. Due to the dark-brown color of the ferrofluid the intercept was easily visualized. Upon atomization of a concentrated ferrofluid up to 2% of iron intercepted onto the flappers. This amount decreased considerably with increasing distance between flappers and poles and with dilution of the ferrofluid.

Conclusion: The extent of droplet deposition under the tested conditions is still far below therapeutically meaningful desposition rates. Until now, the effective droplet size and size distribution during the atomizing process is unknown. Future effort will be directed towards the generation of smaller droplets with diameters between 0.5 µm and 1 µm and narrower size distributions and the generation of stronger field gradients within a larger operation range using electromagnets.

Magnetically Responsive Core-shell Microspheres for Smart Drug Delivery

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Abstract

We report the fabrication methodology of magnetically-functionalized core-shell microspheres by microfluidic flow-focusing (MFF) approaches. The MFF chip, fabricated with PDMS by soft lithographic technique, has a main channel flowing with three laminar streams including the carrying fluid, drug solution and magnetic colloid, respectively. The outmost layers of the spherical droplets formed in the main channel were solidified by an additional chemical reagent injected through a side channel and the core-shell microspheres were thus obtained. Since the shell of microsphere is embedded with magnetic nanoparticles, it can experience the change in shapes like ellipsoidal with different aspect ratios under an external magnetic field. Therefore, the pumping-like behavior resulted from the compress-expansion of shell can be observed under an AC magnetic field and the drug stored in core-shell microsphere would actively release by controlling the magnetic intensity, frequency as well as excitation signals. UV absorption measurement of cumulative aspirin release was preformed to decide the influence of such factors. It is found that obvious difference of drug release behaviors can be detected when sine and step signals are applied, respectively.

Amphotericin B magnetic carriers for lung diseases treatment

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Nowadays, drug delivery is certainly one of the most important challenges in the biomedical area. The desirable characteristics of drug carriers may be achieved by systems containing magnetic nanoparticle (MNP). This system could minimize the collateral effects of very toxic drugs, such as Amphotericin B (AmB). AmB is a useful drug for the treatment of severe lung diseases, as Paracoccidioidomycosis (PCM). AmB needs a very long time treatment and induces nefrotoxicity, leading the PCM patient to interrupt the treatment. A new magnetic fluid sample based on maghemite nanoparticles associated to AmB (MF-AmB) and presenting an AmB release control was developed. This work had the aim of evaluating the MF-AmB sample toxicity and biodistribution in comparison with the free AmB effects. Experiments were performed from 12 hours until 80 days after treatment with MF-AmB (injections at each 3 days; free AmB equivalent concentration), AmB (diary injections) and PBS, as control. Cytometry analysis showed that both samples induce few and temporary alterations in both blood and peritoneum leukocyte populations. However, MF-AmB leukocyte alterations were fewer than the AmB changes. Samples did not cause any alterations in the blood enzyme levels that could indicate hepatotoxicity or nefrotoxicity. However, after longer treatment, AmB induced changes in the serum levels of alanine amino transferase enzyme, suggesting induction of hepatic damage. The Nigrosin exclusion test showed that peritoneal cells viability was slightly affected by the treatments. The only value less than 90% was seen after 80 days MF-AmB treatment. Micronucleus test showed that AmB caused genotoxicity in both polychromatic and normochromatic erythrocytes. However, MF-AmB did not present this effect. Histology analysis showed MNP clusters in the liver, spleen, and lungs; no damage in the spleen; more severe inflammatory process in the liver and kidney of AmB treated animals, but more enlargement of the alveolar wall after MF-AmB treatment, probably due to the MNP clusters. Interestingly, the MNP preferential target was the lung. Samples did not induce alterations in the body weight. MF-AmB caused less stress, but induced death after 70 days treatment. This result was particularly interesting because evidenced that is possible to administrate until 23 injections of MNP without severe effects, thus opening other opportunities to MNP biomedical applications. In conclusion, the data suggest MF-AmB sample is biocompatible, organ-specific, and may be used as an Amphotericin B carrier. Further it can perform the PCM therapy decreasing the observed collateral effects commonly observed after the free Amphotericin B treatment, thus evidencing the potentials of nanostructured drugs.

Work supported by CAPES, CNPq/MCT, FINATEC, FAP-DF, and CNANO

Kohler N, Xu C, Xie J, Ho D, Walsh E, Kalluri R, Sun S.

Title

Tumstatin Conjugated Iron-oxide Nanoparticles for MRI Contrast Enhancement and Anti-Angiogenic Drug Therapy.

Purpose

To develop a multifunctional nanoparticulate system as MRI contrast agents for angiogenic tumor imaging and anti-angiogenic therapy.

Methods

10 nm Fe_3O_4 nanoparticles were prepared by reductive decomposition of iron acetylacetonate in the presence of oleic acid and oleylamine. The as synthesized nanoparticles were further functionalized with dopamine and a 3000 MW poly(ethylene glycol) linker which was coupled to tumstatin, a low molecular weight peptide specific for the $\alpha_V\beta_{III}$ integrin expressed on the surface of endothelial cells. The hydrodynamic size of the tumstatin nanoparticles were measured by dynamic light scattering analysis. The stability of the nanoparticles was tested in phosphate buffered saline + 10% fetal bovine serum. Reduction in CPAE (endothelial cell) viability was tested in vitro through WST-1 assay analysis. Tumstatin nanoparticle uptake was quantified through Inductively Coupled Plasmon-Atomic Emission Spectroscopy (ICP-AES). Visualization of nanoparticle uptake was demonstrated through scanning confocal microscopy. T2 MRI contrast enhancement was tested using a Siemens Trio 3T scanner.

Results

DLS analysis demonstrated nanoparticle stability of 24 hours in simulated human plasma. In vitro cell culture with tumstatin conjugated nanoparticles demonstrated an 80% mean reduction in cellular viability of CPAE cells compared to the control without tumstatin, and a 29% reduction in cell viability compared to free tumstatin control. Nanoparticle uptake showed up to 3.03E-3 ng/cell iron through ICP-AES analysis. Scanning confocal microscopy of

Targeted delivery of SPIO-labelled progenitor cells to a site of vascular injury using an external magnetic actuator

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Introduction: Endothelial progenitor cells (EPCs) are proliferative cells that can adopt an endothelial-like phenotype and are known to be involved in pathological and physiological processes such as vascular re-endothelialisation and post-ischaemic neovascularisation (1,2,3). Labelling of cells with superparamagnetic iron oxide MRI contrast agents is becoming an established method of tracking cells *in vivo* in animals and has recently entered the clinical setting (4). It may also be possible to exploit the magnetic properties of these particles by imposing an external magnetic force on the labelled cells both *in vitro* and *in vivo* (5,6). The long-term aim of our study is to target EPCs to a site of vascular injury using an externally applied magnetic force, thus enhancing re-endothelialisation, and to monitor their homing and retention non-invasively using MRI.

Our group has studied CD133+ derived progenitor cells acquired from peripheral blood in vascular repair. Cultivation of CD133+ cells for 10 days in pro-angiogenic conditions ("d10-CD133") results in maturation to committed cells that become adherent, express endothelial markers (VE-Cadherin, VEGF receptors) and are able to engraft into the de-endothelialised carotid artery after balloon angioplasty in an animal model. Here we present results of the viability assessment of these cells, MRI visibility, preliminary results from our magnetic targeting *in vivo* study and finally data from an *in vitro* system for the magnetic capture of cells in flow.

Methods: EPCs were obtained from culture of fresh human CD133+ cells. For differential labelling with SPIO (Endorem, Guerbet, 500ugFe/ml), the suspension d9-CD133 fraction was separated and labelled for 23h followed by additional labelling for 1h with the adherent fraction (24:1 labelling). For standard labelling the whole cell population was labelled for 24 h (24:24 labelling). Iron uptake was quantified with a superconducting quantum interference device. Cellular function was tested with MTS and Annexin-V assays and flow cytometry. Magnetic actuation devices were designed using FEM software and constructed using NdFeB magnets. For *in vitro* MRI, agarose suspensions of labelled cells were scanned in a 9.4T MRI system. For *in vivo* studies, balloon angioplasty was performed on rat common carotid arteries, followed by administration of iron-labelled CD133s with and without magnetic actuation. Arteries were excised at 24 hours and confocal microscopy was performed.

Results: Figure **1a** and **1b** shows that standard labelling (24:24) of d10-CD133s results in decreased viability leading to cell lysis after application of a magnetic force (BgradB=8 T^2/m). Fig. **1c** demonstrates that 24:1 labelled cells are not compromised by the same magnetic force. These differentially labelled cells contain, on average, 3.9 pg iron oxide per cell, which is sufficient for visualisation in an agarose phantom at concentrations fourfold lower than those observed in our *in vivo* study (**Fig. 2**). Specific magnet arrays have been modelled and constructed to maximise and homogenise the applied force for *in-vivo* magnetic targeting (not shown). Most importantly, preliminary *in vivo* and *in vitro* data indicate a 2.3-fold increase in EPC adhesion to the injured artery (**Fig. 3a**) and to the tubing in a 1mL/min flow phantom (**Fig. 3b**) following magnetic targeting.

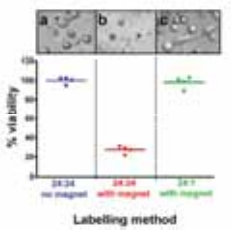

Figure 1: Viability assessment (MTS assay).

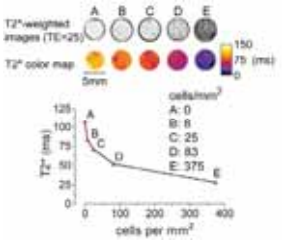

Figure 2: MRI visibility.

Figure 3a: *In vivo* targeting. Sample en face confocal microscopy images of arterial lumen from i) control and ii) magnetic targeting group.

There are increased numbers of adhering cells (green) in the targeted group, max 2000 per artery (130/mm²).

Figure 3b: Cells capture in flow phantom. Side view of 1mm tube with cells captured in flow (yellow arrows) using the magnet array (red arrows).

Discussion
A method of labelling the suspension and adhesive cell fractions differentially has been developed to maintain cell viability following magnetic actuation. Here we have provided *in vitro* evidence for cell viability following labelling and magnetic actuation, as well as MR visualisation at low cell concentrations. Computer modelling has improved magnet design for *in vivo* experiments, and our preliminary *in vivo* results indicate that *in vivo* targeting using an externally applied magnetic field is a promising approach to increase engraftment of stem cells to a site of vascular injury.

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In Vitro Study of Magnetite Nanoparticles as the Implant for Implant Assisted-Magnetic Drug Targeting

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Ritter and co-workers have been exploiting nanostructured magnetic materials as a scaffold for implant assisted-magnetic drug targeting (IA-MDT). In MDT, an external magnetic field is used to attract and retain magnetic drug carrier particles (MDCPs) at a specific site in the body. However, the use of an external field alone is limited by the small magnetic forces exerted on the MDCPs, which leads to poor retention. IA-MDT represents a possible solution to this dilemma.

One IA-MDT approach Ritter and co-workers have been testing uses magnetic nanoparticles, such as magnetite, to enhance the capture of the MDCPs at the target site. These magnetite nanoparticles, which are far more magnetic than the MDCPs, are easily collected at the target site when placed in the magnetic field. So they are collected first, followed by the MDCPs. This approach works because the magnetic nanoparticles become magnetized in the magnetic field and thus impart a force on the MDCPs that is larger than that generated by the magnetic field alone. In effect, the magnetic nanoparticles act as a scaffold for the MDCPs to be attracted to, with both being collected and retained magnetically at the target site.

This IA-MDT system was studied *in vitro* using a porous polyethylene cylinder to simulate capillary tissue. The magnetite nanoparticles were first captured within the porous polymer using an external magnet. They ranged in diameter from 10–100 nm. The MDCPs were then captured using the magnetite nanoparticles as a magnetic scaffold or implant, as explained above. The MDCPs were 0.87 μm in diameter and embedded with 25 wt% magnetite.

This presentation will disclose the effects of several variables on the performance of this unique IA-MDT approach in terms of the MDCP capture efficiency (CE). The fluid velocity, the distance to the magnet, the applied magnetic field, the magnetite nanoparticle size and concentration, and the MDCP concentration were studied. The results showed a significant increase in the capture of the MDCPs surrogates when the magnetite nanoparticles were present. The results also revealed considerable insight to the design of such an IA-MDT system.

Magnetic Polymer Brushes for Bioseparation and Magnetoresponsive Drug Delivery Systems

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Magnetic polymer brushes[1] receive growing interest as functional magnetic nanocarriers for biomedical uses like hyperthermia, bioseparation or magnetic drug targeting. The surface-grafted polymer shell allows on the one hand the steric stabilization of single magnetic cores with excellent dilution stability, and on the other hand permits a great diversity of chemical and physical properties by varying the chemical structure of the polymers. By using functional comonomers a high degree of functional groups can be achieved in the polymer shell.

The concept of our work is based on the combination of magnetic heating, thermoreversible dispersion behavior and high functionality for release and separation applications. Therefore we combine magnetic FeO_x nanoparticles (d_{core} = 12 nm) via surface initiated ATRP[2] with copolymer shells showing a LCST in water. The obtained particles are characterized with respect to their functionalization, composition, thermal and magnetic properties and their dispersibility in water or physiological buffer.

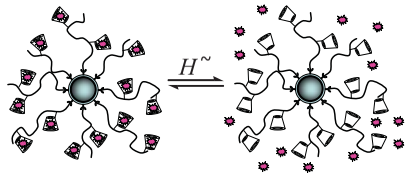

Fig. 1: Drug release from nanoparticle via intrinsic heating

β-cyclodextrine (β-CD) is well known for its ability to form host/guest complexes with a large number of substances. To achieve magnetoresponsive drug delivery systems (Fig. 1) we copolymerize oligo(ethyleneglycol)methylether methacrylate (OEGMA) with a β-CD bearing comonomer in order to allow a reversible complexation with active guest compounds. The decomplexation can be obtained by extrinsic or intrinsic heating in an oscillating magnetic field. We use phenolphthalein as a model substance to investigate the thermally induced release of loaded model copolymers and loaded magnetic nanoparticles.

Our second approach is based on FeO_x brush particles with a carboxy-functional polymer shell by using methacryloxysuccinimide (MASI) as a functional comonomer. Via carboxydiimide activation amines or proteins can be bond covalently (Fig. 2). By increasing the temperature above the LCST of the copolymers, the particles precipitate in the carrier medium due to the collapse of the shell, allowing the separation of the species by low magnetic field gradients. On the poster we present results of the selective separation of trypsine from bovine pancreas solution.

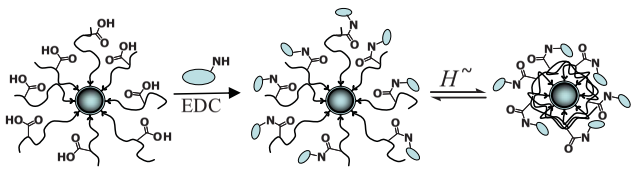

Fig. 2: Binding of amines and sedimentation of the particles for separation

The selected results show the high potential of functional magnetic copolymer brushes for separation and release applications because of their high functionality and magnetoresponsivity.

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Magnetic Drug-Targeting Carrier Encapsulated with Thermo-sensitive Smart Polymer

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Abstract

The primary challenge in the delivery of a drug to a tumor site is to target the anticancer drug specifically into and around tumors at concentrations that will decrease their growth and/or viability. Excellent vehicles for targeted drug delivery are magnetic nanocarriers. We have fabricated a novel temperature and pH-responsive magnetic nanocarrier that combines tumor targeting, carrier monitoring, and controlled drug release. The carrier is characterized by a functionalized magnetic Fe_3O_4 core conjugated with the therapeutic agent doxorubicin, which is crosslinked with dextran-g-poly(N-isopropylacrylamide)-co-N,N-dimethylacrylamide [dextran-g-poly(NIAAm-co-DMAAm)] biodegradable thermosensitive polymer shell. The dextran-g-poly(NIAAm-co-DMAAm) smart polymer shell exhibits a lower critical solution temperature (LCST) of ~ 38°C, which is representative of a phase transition behavior. This behavior allows for triggering on-off mechanism. At an experimental temperature lower than LCST, the rate of drug release was low. However, at a temperature greater than LCST, there was initially a higher rate of drug release followed by a controlled released in the second stage; a behavior that is attributed to the collapse of the outer shell of the thermosensitive biodegradable polymer. The proposed carrier is appropriately suitable for magnetic targeting drug delivery system with longer circulation time, lower side effects, and controlled drug release in response to change in external temperature.

Biocompatible Magnetic Fluid Application in Drug Targeting Methods

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The utilization of ferrofluids as modern tool in biomedical applications contnues to represent a challenging reasearch direction for both physicians and physicists. The possibility of magnetically delivery of drug molecules results from the ability of the paramagnetic nanoparticles attached to these molecules as carriers to accumulate in the target tissue, selectively and quantitatively, under the action of external magnetic field, independent of the site and methods of its administration. The leading of active molecules toward the maligne tissue under magnetic control makes chemotherapy more effective by increasing the drug concentration at the tumor site, while limiting the systemic drug concentration.

The authors of this experimental study aimed to investigate the behaviour of magnetic nanoparticle - drug complex (based on two aqueous ferrofluids and two molecule types, i.e. a non-steroidian anti-inflammatory drug – the sodium diclofenac and, respectively an antibiotic - the rifampin) in animal blood. The investigation focused on the capacity of the ferrofluid to form reversible complexes with sodium diclofenac respectively rifampin molecules. The expected reversible and controllable interactions between the magnetic nanoparticles (magnetite and maghemite) and drug molecules under the influence of adequate magnetic field induction were evidenced for two of the four tested combination: nanoparticles coated with sodium oleate – sodium diclofenac and, respectively, nanoparticles coated with citric acid – rifampin. In each case the optimal concentration ranges were revealed. The spectrophotometric assay (Shimadzu-UV1700 device) of sodium diclofenac release in blood serum was based on the ultraviolet range absorption at 275 nm while two absorption bands of rifampin (in visible and ultraviolet range) were used: at 474 nm and 334 nm.

The magnetic fluids diluted to various concentrations were let to interact with blood serum during 24 hours of incubation at physiological temperature, the controlled releasing of the drug molecules in the living tissues being monitored for several time durations in the presence of the magnetic field: 0.5 h; 1.0 h; 1.5 h; 2.0; 2.5 h and 24 hours following the incubation was accomplished (24 hours). The generalization to the case of *in vivo* situation needs to be further investigated – which is planned in the frame of future project.

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Multifunctional Magnetic Nanoparticles: Drug Delivery and Imaging of Malignant Glioma

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Malignant glioma is the most frequent primary brain tumor. Current post surgical therapy involves simultaneous TMZ (Temozolomide) and ionizing radiation treatment while patient survival has only modestly improved over the past 30 years. Novel targeted treatment modalities have failed to make a considerable clinical impact, while combinatorial therapeutic methodology such as TMZ and cytotoxic cytokines (e.g., TNFα) are essential to improve treatment efficacy. Magnetic nanocarriers are ideal for blood brain barrier applications because of their size (25 – 300nm), their ability to encapsulate a variety of therapeutic agents, and the potential to functionalize their surface for cell targeting and improved imaging capabilities. Cellular uptake of nanoparticles with TMZ as payload of two narrow size distributions, 100 nm and 280 nm, were confirmed with clonogenic assays. Magnetic nanocarriers enter cells by endocytosis and can effectively deliver protein, radiation induced genes, and other therapeutic agents to tumor tissues. Results indicate that the TMZ, TNF protein, and gene-loaded magnetic nanocarriers can penetrate cells and distribute directly within brain tissue by convection enhanced delivery. The TMZ dose used on mice was 5mg/kg and measured using UV-VIS spectroscopy. The release of the magnetic nanocarrier payload was optimal based on release rates between 24 and 72 hours. The magnetic nanocarriers were characterized with X-ray fluorescence microprobe and XANES to determine their distribution to be a majority of magnetite and a smaller fraction of hematite. Rat brains were injected 5 mm in depth with nanocarriers by the Convection Enhanced Delivery technique (Figure 1) and cross sections were characterized using Fluorescence Correlation Spectroscopy combined with Confocal Microscopy the diffusion coefficients obtained were between 0.52 and 34 $\mu m^2/s$. The Stokes-Einstein relationship was used to calculate particle diameter and the values were compare with experimentally obtained Dynamic Light Scattering effective diameter measurements. Magnetic nanocarriers of 40 and 80 nm in size were also imaged in vivo by using Magnetic Resonance Spin Echo technique and the magnetite contrast agent were visible in the images at the treatment dose rate. Other physical characterization will be presented to describe the development of unique nanocarrier systems for selective cancer treatment and imaging both in vitro and in vivo.

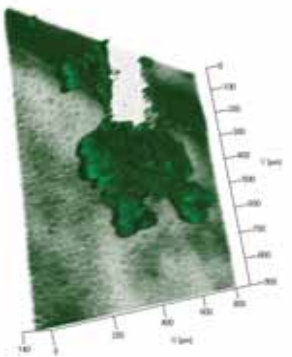

Figure 1. Confocal microscope image of plume of injected fluorescein-labeled magnetic nanocarriers within the cross section of rat brain tissue. The fluorescence allows the study of the distribution and dispersion of the nanocarriers injected by Convection Enhanced Delivery (*Courtesy of Argonne's Center for Nanoscale Materials*).

Physical and Biochemical Targeting of Cisplatin loaded Particles in Squamous Cell Carcinomas of the Head and Neck (SCCHN)

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Squamous cell carcinomas of the head and neck (SCCHN) have a worldwide incidence of 900,000 cases annually making it the sixth most common cancer. In the United States, approximately 40,000 cases will be diagnosed annually. The majority of patients with SCCHN are diagnosed with locally or regionally advanced disease. Therapy has therefore concentrated on locoregional control with radiotherapy and surgery as the traditional mainstays. Almost all patients with recurrence will succumb to their disease. Thus, SCCHN is a worldwide health problem with devastating consequences but provides an ideal model to implement and improve local treatment delivery and modalities. Past attempts with intratumoral gene therapy have shown promise but are limited due to technical difficulties associated with intratumoral injection and concerns regarding safety of viral gene delivery. Newer technologies such as magnetic nanocarriers are becoming available that can address these concerns and simultaneously allow physical and chemical targeting of specific chemotherapy agents, proteins, and genes. PLA (poly-lactic acid) or PLGA (poly-lactic-co-glycolic acid) polymers were used as encapsulation materials combined with nanoparticles of Fe_3O_4 in the 2-10 nm range to provide the magnetic characteristics of the nanoencapsulation system.

Cellular uptake studies of an SQ20B cell line with a Confocal Microscope for nanocarriers with cisplatin as payload and effective size distribution of 200 nm shows successful endocytosis through the cell membrane (Figure 1). Magnetic nanocarriers with and without an epidermal growth factor (EGF) monoclonal antibody on the nanocarrier surface enter cells by endocytosis and can effectively deliver protein, cytotoxic genes, and other therapeutic agents to tumor tissues. The cisplatin doses used on mice were between 5 mg/kg and 0.05 mg/kg and cisplatin was measured using ICP-MS analysis of Pt. The release of the magnetic nanocarrier payload was optimal between 24 and 72 hours. The magnetic nanocarriers were characterized with magnetic susceptibility and ICP-MS measurements and showed magnetization of 0.4 emu/g. Other physical characterization and in vivo measurements will be presented to describe the preliminary efficacy of physical and biochemical targeting for SCCHN cancer treatment.

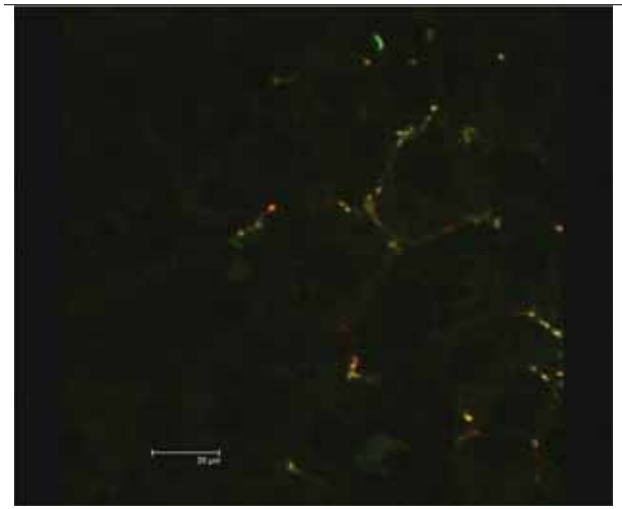

Figure 1. Confocal Microscope image of SQ20B cells shows successful endocytosis of the nanocarriers. The magnetic nanocarriers contained fluorescein-labeled polymer with an Alexa red-labeled BSA payload. The payload appears as red color once released intracellularly from the nanocarriers. The unreleased payload appears as yellow, while the empty fluorescein-labeled magnetic nanocarriers appear as green. The figure shows an elapsed time of 72 hrs.

Synthesis and Characterization of Magnetite HP-β-CD Composite and Its Application as a Carrier of Doxorubicin

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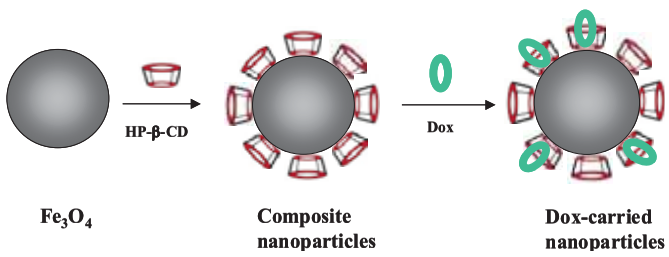
Keeping the cavity of β-cyclodextrin with higher aqueous solubility and lower toxicity, hydroxylpropyl-β-cyclodextrin (HP-β-CD) has been shown to be suitable for parenteral excipient approved by FDA. It plays a role as "reservoir" of drugs without interfering with their activities in aqueous solution. Herein HP-β-CD was introduced to the surface of magnetic nanoparticles (MNps) in the presence of $NH_3 \cdot H_2O$ to synthesize magnetite HP-β-CD composite that can be used as a carrier in magnetic targeted drug delivery. The composite nanoparticles was characterized by FTIR, ICP-AES, TEM and VSM. The results showed that the composite nanoparticles contain 23.6% HP-β-CD in weight and have saturated magnetization of 59.9 emu/g with a size range of 10-20 nm. The capacity of composite nanoparticles for doxorubicin adsorption is 87.8 μg/mg. The cumulative percentage of released doxorubicin in PBS buffer (pH=7.0, 0.01M) in 1d, 4d, 10d were 35.5%, 49.3%, 76.5%, respectively. Thus, the magnetite HP-β-CD composites could be a potential carrier in the magnetic targeted drug delivery.



Schemetic structure of magnetite HP-β-CD composite

Internalization of Metal Oxide Nanoparticles in Multi-drug Resistant (MDR) Cancer Cells: Influence of Poly(ethylene-*b*-propylene oxide) copolymer shells

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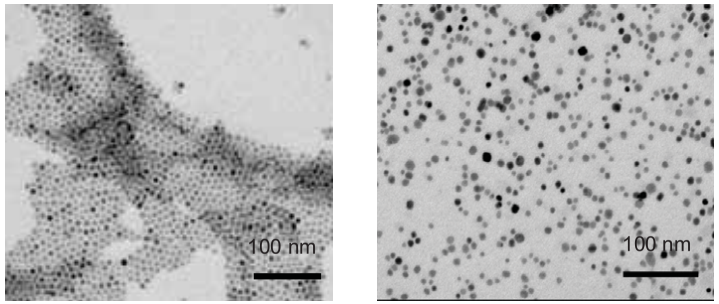
Biocompatible magnetite nanoparticles have been extensively studied over the past decade due to their potential use in many biomedical applications, such as in drug delivery, imaging, and cell separations. For these diverse biomedical applications, it is essential to have magnetic nanoparticles dispersed in physiological media that maintain high magnetic susceptibility. Our interest is in the potential for mediating cellular response by internalizing the magnetite nanoparticles within cells, then applying an external magnetic field.

Here we report an approach toward well-defined magnetite (Fe_3O_4) nanoparticles with amphiphilic poly(ethylene oxide-*b*-propylene oxide) shells. The hydrophilic-hydrophobic ratio was varied by controlling the block lengths of the poly(ethylene oxide) and poly(propylene oxide) segments respectively.

The doxorubicin (ADR)-selected human breast cancer cell line (MCF-7/ADR-RES) was use as a model for MDR cancer cells. The amphiphilic nanoparticles were incubated with the cells and the uptake of iron was quantified with a ferrozine fluorimetric assay. It is clear that these magnetite complexes enter the cell but the mechanism of internalization is still unclear.

Furthermore the interactions of these well-define nanoparticles with cellular compartments and the effect of magnetic fields on nanoparticle behavior are currently under investigation.



TEM images of A) 8 nm magnetite nanoparticles coated with oleic acid from their chloroform dispersions B) nanoparticles after coated with PPO-PEO represent a well-dispersed nanoparticle in physiological media

Preparation and characterization of the BSA/magnetic-nanoparticles for synergic application in Photodynamic Therapy and Hyperthermia Cellular

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The interest in nanosized drug delivery systems (NDDS) has grown exponentially in the last decade. The goals in the present study were to prepare nanoparticles from serum albumin (BSA) containing biocompatible magnetic fluid of maghemite citrate derivative (MF) in the presence of Silicon (IV) phthalocyanine (NzPc) as a photosensitizer compound. This dye belongs to the second generation of photoactive drug used in Photodynamic Therapy (PDT). MF consists in a magnetic suspension (γ-Fe_2O_3 maghemite core) surrounding with external citrate layer leading to a nanocarrier device. This material represent a new class of NDDS that combined action of PDT and Hyperthermia for a synergic application an represent an interesting strategy which can introduce new concepts and optimization in the traditional cancer therapies [1]. The entrapped material, in this case NzPc and/or MF, was developed by the method of heat denaturation using mechanical stirring process at high-speed from to optimize the preparation of the nanometric scale[2]. The samples were characterized by photocorrelation spectroscopy (PCS) from light-scattering technique with the determination of the hydrodynamic diameter and the zeta potential properties (Table I).

Table I- Physical-chemistry parameters of the NDDS

Samples	Size (nm)	Zeta potential (mV)	polidispersity
MF	172 ±38.9	-23.6 ± 5.86	0.389
NzPc	452 ±76.7	-22.3 ± 5.09	0.587
NzPc/MF	369 ±55.9	-23.7 ± 6.33	0.785

Zeta potential was calculated from the electrophoretic mobility using the Smoluchowski equation. The time resolved fluorescence analyses were also obtained for the studied systems. Magnitude of the Zeta potential indicates the physical-chemistry stability of the system. The fluorescence lifetime of the sample of NzPc/MF and of NzPc presented a classical biexponential decay. For NzPc/MF were founded a lifetime of 4.88 ns and 1.70 ns with population of 85.1% and 14.9%, respectively and for sample of NzPc itself the value was of 5.19 ns and 1.99 ns with population of 63.9% and 36.1%, respectively. The morphology analysis of the particles (NzPc, MF and the complex NzPc/MF) was examined by scanning electron microscopy (SEM) and their micrographics showed a spherical and smooth regular shape. It was also evaluated the kinetic release of NzPC in human plasma serum from a comparative study between the nanoparticles associated to the NzPc and the nanoparticles containing the complex NzPc/MF. It was observed that NzPc loaded nanoparticles have small burst release profile (at 1 h) compared with the same system containing the magnetic material. All these properties will allowed the design and development of *in vitro* and *in vivo* studies, necessary to clinical trial studies in human

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[2] A.R. Simioni, O.P. Martins, Z.G.M. Lacava, R.B. Azevedo, E.C.D. Lima, B.M. Lacava, P.C. Morais, A.C. Tedesco *J. Nanosci. Nanotechnol.* **6** (2006) 2413.

MAGNETIC NANOSPHERES FOR TARGETED THROMBOLYSIS: FABRICATION, DRUG RELEASE AND *IN VITRO* MAGNETIC TRAPPING

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A biodegradable, biocompatible carrier for the shielded transport of the clot lysis medication tPA (tissue plasminogen activator) which allows magnetic carrier guidance within the vasculature employing external magnetic fields as well as triggered drug burst release to achieve targeted, on-demand lysis at the clot site would be of great interest for many biomedical applications such as the treatment of heart attacks and strokes. Here we describe the synthesis and performance testing of the first tPA-encapsulated, magnetic nanospheres suitable for medical applications. Concentrated tPA aqueous solution (internal water phase (W1)) and DCM-dissolved poly(D,L-lactide-co-glycolide) and hydrophobic magnetite (oil phase (O)) were emulsified by probe sonication to form a W1/O primary emulsion. The primary emulsion was then added into an aqueous solution containing 0.5% poly(vinyl alcohol) (PVA) and further emulsified by homogenization. After rapid evaporation of the organic solvent, solid medicated magnetic nanospheres formed which were then characterized in terms of morphology, size, drug entrapment efficiency, *in vitro* ultrasound triggered release and magnetic trapping in a physiologically simulated model. The results identified that a) magnetic nanocarriers loaded tPA can be prepared by such a double emulsion technique; b) the tPA entrapment efficiency was 90%; c) particle size about 390~400nm in mean diameter; and d) the magnetization reached 25 emu/g. The tPA release from the carriers showed an initial burst release of 15% when placed into a fluid medium; however, brief ultrasound exposure trigged ~70% tPA release. The in vitro magnetic trapping efficiency was 65% in water at physiological flow rate employing a zero power magnetic of 0.3~0.4T. These results demonstrate for the first time the successful fabrication of magnetically- and ultrasound-responsive, biocompatible tPA nanocarriers.

Formulation Development and Evaluation of Metronidazole Magnetic Nanosuspensions as a Novel Magnetic Targeted and Polymeric Controlled Drug Delivery System with Improved Therapeutic Properties

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Poorly-water-soluble compounds are difficult to develop as drug products using conventional formulation techniques and causes inaccurate dose delivery, low stability and poor bioavailability. The use of nanotechnology to formulate poorly-water-soluble drugs as nanosuspensions offers the opportunity to address many of the deficiencies associated with this class of molecules. Nanosuspension of magnetically tagged metronidazole was developed and evaluated for the oral-controlled and targeted delivery. The metronidazole magnetic nanosuspension was formulated by using solvent displacement method coupled with ultrasonication; with magnetite as the targeting agent; polyvinyl pyrollidine and methyl cellulose as polymers; span 60 as surfactant and methyl paraben as preservative. The particle size of the dispersion of metronidazole incorporated with magnetite and polymer was reduced to nanoscale by ultrasonication, and thereby the suspension stability, solubility, absorption, release and bioavailability was improved. Preformulation studies were performed for the evaluation of drug-excipient compatibility; other formulation parameters such as concentration of surfactant, pH, temperature, sonication amplitude and time were optimized.

The metronidazole magnetic nanosuspension was evaluated (i) by photon correlation spectroscopy for its average particle size - 345 nm, size distribution - 68nm to 1480nm (Fig.-1), zeta potential -15.2 mV and polydispersity index - 0.680; (ii) by pyknometer for its density - 0.804 gm/cc; (iii) by open end reciprocating dialysis tube in USP dissolution apparatus for its *in-vitro* drug release at pH 1.2 under the influence of external magnetic field and normal conditions and its kinetics was found to follow Korsmeyer:Peppas equation and drug release is by diffusion mechanism; at pH 7.0 under the influence of external magnetic field and normal conditions and its kinetics was found to follow Higuchi equation and drug release is by diffusion and erosion mechanism; (iv) by magnetic susceptibility meter for its inclination towards magnetic field - 4 x 10^{-5}; (v) surface morphology was evaluated by atomic force microscopy - smooth texture (Fig.- 2); (vi) no change was observed in the above parameters upon stability studies carried out at 25℃ and 65% relative humidity for 60 days; (vii) redisperability – the magnetic nanosuspension was stable and could be easily redispersed on shaking; (viii) assay - drug content was found to be 99.7% when analysed spectrophotometrically at 277 nm; (ix) other properties such as viscosity - 0.887 cP, pH – 6.8, density – 0.80 gm/cc and conductivity - 0.127 mS/cm (x) anthelmentic activity – the time taken for the paralysis or death of adult Indian earthworm *Pheretima poi* at a concentration of 10 mg/ml and 50 mg/ml was 120 and 30 minutes respectively.

The metronidazole magnetic nanosuspension was developed and optimized; further the *in-vitro* and *in-vivo* evaluation results were very much promising with a better controlled and targeted drug action and will be a better therapeutic avenue in combating the GI disorders such as helmenthiasis.

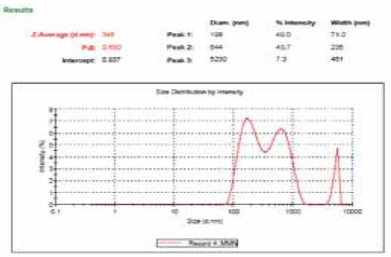

Fig.1 Size distribution of metronidazole magnetic nanosuspension by photon correlation spectroscopy

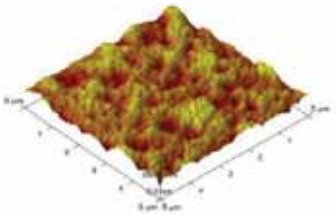

Fig.2 AFM photograph of metronidazole magnetic nanosuspension

DEVELOPMENT OF A MAGNETIC SYSTEM FOR TREATMENT OF *Helicobacter pilory* INFECTIONS

Érica L. Silva[1], Juliana F. Carvalho[1], Thales Renan F. Pontes[1], Elquio E. Oliveira[1], Bárbara L. M. F. Carriço[2], Aldo C. Medeiros[3], E. Sócrates T. do Egito[1, 3], Artur da Silva Carriço[4].

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Helicobacter pylori has been shown to cause peptic ulcers. The treatment of choice has commonly been based upon a triple therapy combining two antibiotics and an anti-secretory agent. Furthermore, an extended-release profile is of utmost importance for these formulations. As magnetite particles may be retained in the target by the action of a magnetic field, their use has been proposed as a promising approach to local drug delivery. The aim of this work was to develop Eudragit-coated magnetic system containing the antibiotic amoxicillin.

First, magnetite particles were produced by coprecipitation of iron salts in alkaline medium. The second step consisted of coating the particles and amoxicillin with Eudragit® S-100 by the spray-drying technique. The sample characterization was performed by scanning electron microscopy, optical microscopy (Ferret´s diameter principle), thermogravimetric analysis and vibrating sample magnetometry.

According to the scanning electron microscopy, the magnetic particles were successfully coated by Eudragit, resulting in nearly spherical polymeric particles with a mean diameter of 14.32 ± 2.46 μm. The magnetite and amoxicillin content in the polymeric microparticles was assessed by thermogravimetric analysis. The microparticles were found to be superparamagnetic, with an initial susceptibility controllable by the magnetite content in the suspension feeding the sprayer.

Our results suggest a possible way to treat *Helicobacter pillory* infections, using an oral drug delivery system, consisting in magnetite microparticles and amoxicillin, in a polymeric matrix which is resistant to low pH values. Another important finding in this work is that it opens new prospects to coat magnetic microparticles by the technique of spray dryer.

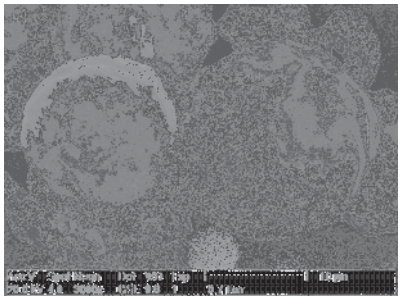

Fig.1. Scanning electron microscopy images of polymeric magnetic particles (with amoxicillin).

ADVANCES IN PARAMAGNETIC SENSOR APPLICATION FOR MAGNETIC CARRIERS STUDYING

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One of the main problems of direct drug delivery is the selection of optimal parameters for the drug adsorption on the carrier and its desorption in organism. In this work we suggeste to use paramagnetic sensor technique to study this question. This approach is based on the analysis of the ESR spectra of paramagnetic particles in the presence of magnetic species in diamagnetic medium. Stable nitroxide radicals have been used as paramagnetic sensors. ESR spectrum of stable radical is sensitive to the magnetic particles presence and to the radical immobilization on the particle surface. Radical immobilization results in the changing of ESR spectrum shape whereas the magnetic particles presence leads to the significant broadening of spectrum lines and their position in magnetic field (g-value).

Investigation of ESR spectrum parameters of non-immobilized radical in magnetic liquid was carried out. Experimental results were obtained using magnetite nanoparticles (17 nm) in water buffer. Stable nitroxide radical TEMPOL was used as paramagnetic sensor.

It was shown that the direction of radical spectrum shift and its magnitude depends on magnetic properties of nanoparticles and their concentration, as well as on the shape of ampoule with the sample and its orientation in magnetic field of spectrometer. Using the magnitude of the radical spectrum shift it is possible to calculate average magnetic moment of the particles and their saturation magnetization [1].

The main reason for the linewidth changes is the inhomogeneous broadening. The equation obtained in the frameworks of Anderson theory for NMR line broadening in rigid matrix containing magnetic particles [2] was used to calculate radical line broadening in magnetic liquid. According to this equation the width of Lorenzian line $\Delta H_L = 16\pi^2/(9\sqrt{3}) \langle\mu_z\rangle n$. The width of the sensor ESR line calculated from this equation was 9 Oe that is 1.5 times larger than the experimental line broadening (5.5 Oe).

It was suggested previously that anisotropic structures formation from nanoparticles under magnetic field is the main reason of the lack of coincidence of theoretically calculated and experimental linewidth. However recent experiments showed that linewidth broadening does not depend on the linear structures formation. Another reason of the observed discrepancy might be the fact that the experimental line is not Lorenzian but close to Gaussian and the used equation should be modified in accordance with the experimental lineshape.

This problem is the aim of the further research in the framework of general problem of radical adsorption on the particles surface.

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CEREBRAL PERFUSION ANALYSIS OF MAGNETICALLY-GUIDED NANOSPHERES

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The blood-brain barrier (BBB) embodies a tight blockade function excluding most medications from their effective delivery to the brain parenchyma. We were interested to study the interactions of freely circulating nanospheres on the BBB function during magnetic guidance to a predefined brain target region utilizing external magnetic fields. We employed an *in situ* rat brain perfusion method to study the BBB integrity using PLA-PLGA magnetic nanospheres. The nanospheres were synthesized using a double emulsion method creating a biodegradable surface with encapsulated magnetite in the core. Three groups of experiments, each with four animals, were performed: perfusion with nanospheres alone, exposure to a magnet filed alone, and treatment with both nanospheres and an external magnetic field. Blood flow results using the ^{3}H-diazepam result indicated both the nanospheres alone and the magnet alone gave normal flow values of 0.0375 (p=0.7844) and 0.0428 mL/s/g (p=0.4461), respectively. However, the combination of nanospheres and an external magnetic force led to a relative capillary flow increase to 0.0684 mL/s/g (p=0.0856) which, though higher, was still within the normal range (0.04-0.07 mL/s/g). Furthermore, BBB integrity measurements using ^{14}C-sucrose resulted in higher than control (0.0137 ml/g) volume values which were 0.0217 (p=0.04), 0.0237 (p=0.428), and 0.0203 ml/g (p=0.0273) for nanospheres alone, magnet alone, and nanospheres with exposure to magnetic fields, respectively. In summary, these results indicate that magnetic guidance of freely circulating, magnetic nanospheres to the brain (and across the BBB) seemed to have no abnormal impact of the intricate BBB function and hence, magnetic guidance of medications to the brain may be a feasible technology for drug delivery.

IN VITRO BIOCOMPATIBILITY OF DRUG CARRIERS FOR MEDICAL APPLICATIONS

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The development of drug carriers for biomedical applications which are minute in size, just enough for unrestricted systemic circulation, and possess the capability to evade the intricate immune defense system is of great biomedical interest and research focus. However, despite the impressive advances of 'nanopharmaceutics' current delivery strategies have yet to overcome two inherent challenges: First, appropriate pharmacokinetics allowing the drug carrier to concentrate at therapeutic levels; and, second, biocompatibility which is the ability of a carrier to evoke an appropriate host response but also the quality of not having toxic or injurious effects. We were interested in designing and validating a comprehensive test battery to evaluate the immunogenicity and cellular toxicity of a drug delivery platform under study in order to predict at an early, preclinical stage the ensuing clinical properties. For our biological assessment, the test-battery components were based on 3 criteria: a) biological compatibility (protein absorbance, complement activation, macrophage uptake, cell toxicity, cell viability/proliferation); b) correlation and validity of the test results with respect to *in vivo* performance; and c) relative ease and inexpensiveness of the test battery, i.e., for large scale screening. Magnetic PLGA/PLA-PEG-based nanocarriers and their individual components were tested for induced immune response though macrophage uptake and stimulation, cellular membrane damage, cell toxicity, and cellular propagation and proliferation. The function and impact of the primary components of carrier under study (PLGA, mPEG, magnetic iron) on immune response though the mononuclear phagocyte system (MPS), cellular membrane damage (compliment activation), direct cytotoxicity, and overall cellular viability are reported and a viable, biological assessments were obtained based on the carrier physio-chemical properties. The presented studies and methods provide the researcher with a practical, relative inexpensive and bioclinically logical method for validated *in vitro* testing of drug carriers.

Magnetically Guided Targeted Drug Delivery Across the Blood Brain Barrier

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Drug delivery to the brain is difficult due to the blood-brain barrier (BBB) which prevents transport into the brain. Targeted drug delivery that can cross the BBB would drastically reduce side effects and offer better treatment to patients. We are studying and investigating a magnetically guided and targeted carrier system for delivery across the blood brain barrier (BBB) based on nanoscale technology. The BBB consists of the capillary wall within the brain composed of specialized endothelial cells with tight intercellular junctions which make the brain impermeable to water-soluble molecules lacking specific transporters hence most therapeutic agents are excluded from the brain. The BBB properties are useful because they can prevent toxins from entering into the brain therefore, it is of vital importance that while the drug must cross it cannot damage the BBB.

We have proposed a novel delivery system utilizing designer magnetic nanoparticles which are biodegradable and non-toxic. The *in vitro* BBB model we are using in our laboratory consists of two layers of cells, rat brain endothelial and rat astrocyte cells, grown on collagen coated filters for BBB transport studies. We investigated the feasibility of collection of magnetic nanoparticles across this *in vitro* model while using two different size nanoparticles, two different size magnets, and two different concentrations of the particles. The magnetic nanoparticles were synthesized using a double emulsion technique. Both particle batches have a PLA-PLGA surface with encapsulated magnetite, but were different sizes. The two magnets were NdFeB cylindrical magnets with diameters: 9 mm and 22 mm. The nanoparticles and ^{14}C-sucrose were used to determine what crosses the BBB model over a 2-hour time period. The experiments revealed that the lower concentration at 20 ug/ml with the 9 mm diameter magnet and smaller particles provided the best results. These results will be used in an *in-situ* perfusion analysis across the rat BBB to verify the *in vitro* results. The overall results from the *in vivo* and *in vitro* results will provide evidence to determine how magnetically guided drug delivery affects the BBB.

Poly(PEGMA) modified superparamagnetic nanogels: preparation via photochemical method and application in drug carrier

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Magnetic nanogels are nanosized particles with core/shell structure, which are composed of Fe_3O_4 (or γ-Fe_2O_3) as the core and coated with cross-linked polymer as the shell. With the properties of magnetic response, biocompatibility, swelling property and surface functionality, magnetic nanogels are widely used as carriers in biochemistry and biomedicine fields such as targeted drugs, protein immobilization, DNA detection and magnetic resonance imaging(MRI).

Several methods have been developed to prepare magnetic micro- and nanogels, such as microemulsion polymerization, emulsion polymerization and in-situ polymerization. However, the processes usually involve surfactants and initiators, which pose limitations to practical biomedical applications. Due to adsorption of monomer and cross-linker on the surface of Fe_3O_4 nanoparticles, and based on relative literatures[5,6], we point out that free of initiators and additives, magnetic nanogels can be synthesized in one step by *in-situ* copolymerization of monomer and cross-linker in aqueous suspension under UV irradiation, and have made serious reports for this novel method.

Poly(PEGMA) modified superparamagnetic nanogels were synthesized by in-situ polymerization using poly(ethylene glycol) methacrylate(PEGMA) as the monomer and *N, N'*-Methylene-bis-(acrylamide) (MBA) as the cross-linking agent in magnetite aqueous suspension under UV irradiation. The TGA results indicated that the magnetic nanogels contained above 50 wt.% magnetite. The magnetic nanogels were of regularly spherical shape, and behaved superparamagnetic with saturated magnetization of 58.6 emu/g, the Zeta potential was 16.3~－17.3mV at physiological pH (pH=6.8~7.4). The preliminary application in drug carrier was carried and the nanogels adsorbing doxorubicin had excellent property in slow-release.

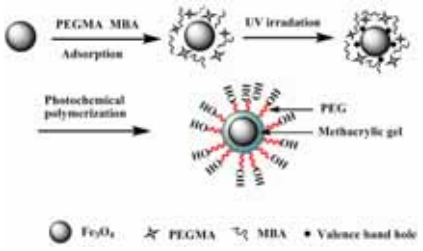

Fig.1 Schematic illustration of photochemical polymerization by UV irradiation

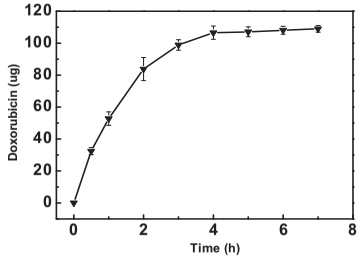

Fig.2 Adsorbtion curve of doxorubicin on the magnetic nanoge

Quantification of magnetic nanoparticles for cancer therapy

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Magnetic nanoparticles (MNP) are used in medicine both in vitro and in vivo. These particles are applied routinely for biomagnetic cell separation and in vivo as contrast agents for MRI. Magnetic drug targeting means the implementation of coated magnetic nanoparticles as carriers for cytostatic drugs, being able to be enriched after intravascular application with focused external magnetic fields in certain body compartments.This therapy led to complete tumor remissions in an experimental animal model without negative side effects. The aim of the present study was to analyse the biodistribution of the magnetic nanoparticles and to quantify this non invasively.

Magnetic nanoparticles bound to chemotherapeutic agents were applicated intravenous and/or intraarterial into tumor bearing rabbits (VX2-squamous cell carcinoma) and simultaneously an external magnetic field was focused to the tumor region. After sacrifying the animals the individual organ structures (tumor, liver, lung, spleen etc.) were asservated and quantified with the method of magnetorelaxometry on its amount of MNP. Hereby highly sensitive superconducting quantum interference devices (SQUIDS) served as sensors detecting the time varying magnetic induction generated by the relaxing magnetisation of the super paramagnetic iron oxide shortly after the application of the magnetic field.

After intravenous application the relaxation amplitude was substantially smaller in the respective tumor with 0.08 pT-0.81 pT (pico Tesla), compared to liver (72 pT-94 pT), lung (1.8 pT-5.5 pT) and spleen (3.7 pT-23.9 pT) (fig. 1). After intraarterial application the relaxation amplitude was significantly higher in the tumor than after intravenous application (fig.1).

Magnetorelaxometry offers the unique opportunity to quantify magnetic nanoparticles non invasively in different body compartments. This can be beneficial for human trials in the future, especially to control MNP-based cancer therapy.

Figure 1

Tissues	i.a.		i.v.			
Tumor	23,75	5,58	0,81	0,13	0,08	0,22
Spleen	15	9,4	23,95	8,84	5,36	3,72
Lung	15	10,88	2,74	4,69	5,46	1,86
Kidneys	<1,25	0	<0,16	0,51	0	0
Heart	<1,25	1,47	1,29	0	0	0
Liver	44,37	72,94	72,02	85,82	89,11	94,27

Percentage of iron detected with Magnetorelaxometry in different tissues after i.a./i.v. Magnetic Drug Targeting
i.a.= intraarterial
i.v.= intravenous

Stimuli-responsive hybrid core-shell nanocapsules

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Microgels are soft and porous systems with a high surface-to-volume ratio, hence, high loading capacity. Poly(N-isopropylacrylamide), PNIPAM, is a thermoresponsive microgel characterized by a lower critical solution temperature around 32 °C. Recently our group reported [1, 2] successful layer-by-layer (LbL) adsorption of polyelectrolyte multilayers onto PNIPAM microgels to tune the thermoresponsivity of the coated microgels. To achieve heating by an external trigger such as an alternating magnetic field, magnetic nanoparticles (MNP) were incorporated within the polyelectrolyte shell using the LbL technique [3, 4]. MNP were synthesized using the usual coprecipitation technique. In situ (during precipitation in the presence of a polyanion) as well as post modification (using LbL assembly) [5] were undertaken to modify the surface charge of MNP. X-ray diffraction of the modified MNP exhibits characteristic peaks of magnetite and maghemite with a core of around 5 to 7 nm, showing that the presence of the polyelectrolytes do not alter the crystalline structure of the particles. The size is in agreement with that obtained by transmission electron microscopy, indicating that these MNP are superparamagnetic in behavior, as further confirmed by magnetometry. Modified MNP have slightly lower magnetization than unmodified one, and the decrease is consistent with the polymeric content as quantified by thermal gravimetric analysis. Further evidence of the presence of polyelectrolytes is provided by the Fourier Transform Infrared spectroscopy. Characterizations of the hybrid core-shell structure using dynamic light scattering and electrophoretic measurements shows that system is still thermoresponsive and that the MNP do not get detached during the volume phase transition. Induction heating experiment demonstrates that modified MNP shows a specific absorption rate twice that of the unmodified one, and would produce sufficient heat to cause the collapse of the microgel core. This unique combination of thermoresponsivity and magnetism could open up novel perspectives towards remotely controlled drug released carriers combining hyperthermia with chemotherapy.

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Magnetic Nanoparticles for Target Thrombolysis in a Rat Embolic Model

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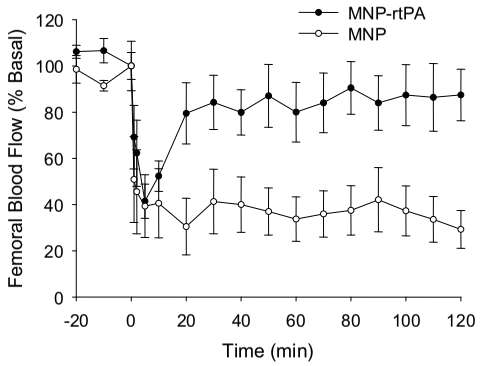
Recombinant tissue plasminogen activator (rtPA) has been used for thromboembolic diseases with hemorrhagic side effects. Magnetic nanoparticles (MNP) may serve as a carrier for target therapeutics to reduce the side effect and the amount of the drug used. We tested a hypothesis that under the guidance of an external magnet, reduced dose of rtPA bound to MNP may achieve similar thrombolytic effect as that induced by a full-dose of free rtPA in a rat embolic model. Polyacrylic acid-coated magnetite (PAA-MNP, 256 nm) was synthesized and characterized. Immobilization of the amino group of rtPA to the carboxyl group of MNP was conducted with carbodiimide mediated amide bond formation. The amount of carboxyl group on the surface of PAA-MNP was 1.03 ± 0.01 μmole/mg MNP, which is 10-fold of that on a dextran-coated MNP (Nanomag®-D COOH, 279 nm; dextran-MNP), as revealed by a toluidine blue O assay. The amount of rtPA immobilized to PAA- and dextran-MNP was measured 76 ± 2 and 59 ± 4 μg/mg MNP by a protein assay, respectively. In addition, the enzyme activities of rtPA bound to PAA-MNP, as measured by S-2288™ assay and ^{125}I-fribrinolysis assay, were 85 ± 1% and 86 ± 3% of that of the free rtPA, respectively, suggesting that most of the enzyme activity of rtPA was preserved after covalent immobilization to the PAA-MNP. However, the enzyme activities of rtPA immobilized to the dextran-MNP were only 9 ± 2% and 13 ± 2%, respectively. The thrombolytic activity of rt-PA bond to PAA-MNP was further determined with a rat embolic model (JMMM 311:342-346, 2007). In this rat model, a functional measurement of the hind limb blood flow with laser Doppler flowmetry revealed the effect of rtPA on a clot lodged in the iliac artery of the rat. The clot lodging dramatically attenuated the downstream blood flow to 41 ± 6%, and reversal of the flow was induced by free rtPA at 1 but not 0.5 mg/kg. With the NdFeB magnet placed by the iliac artery during intra-arterial administration of PAA-MNP-rtPA (with bound rtPA equivalent to 0.2 mg/kg of free rtPA), followed by moving the magnet back and forth along the iliac artery upstream to the clot, the downstream flow was reversed to 80 ± 13% of that before the clot lodging in 20 min (n=7) as illustrated in the figure, whereas the PAA-MNP exerted no effect on the reduced flow (n=4). In conclusion, immobilization of rtPA to PAA-MNP with covalent binding may result in a stable drug preparation, consistent drug concentration around the target site, and subsequently a reproducible and effective target thrombolysis in the rat embolic model; with this approach, 20% rtPA may achieve similar thrombolysis induced by a full dose of the drug.



Fe_3O_4-Au Dumbbell Nanoparticle as a Dual Imaging Agent and Drug Delivery System

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Synthesis of dumbbell-like nanoparticles e.g. Au-Fe_3O_4 nanoparticle has attracted much attention recently. It contains both Au and Fe_3O_4 nanoparticles that are known to be biocompatible and have been used extensively for optical and magnetic applications in biomedicine. Compared with the conventional single component Au or Fe_3O_4 nanoparticles, the dumbbell-like Au-Fe_3O_4 has the following distinct advantages: (1) the structure contains both a magnetic (Fe_3O_4) and an optically active plasmonic (Au) unit and is suitable for simultaneous optical and magnetic detection; (2) the presence of Fe_3O_4 and Au surfaces facilitates the attachment of different chemical functionalities for target-specific imaging and delivery applications.

Here, we firstly studied the relation between Au size and the imaging ability. We found out that the bigger the gold particle, the better signal we could get in optical imaging. However, the magnetic imaging ability was reduced when gold particle became bigger. The as a demo, anti-EGFR(epidermal growth factor receptor) modified Au-Fe_3O_4 was used to imaging A431 cell, which has excessive EGFR. During the optical imaging process, the floating cells could be controlled by the magnet field.

After the dual imaging ability was confirmed, we began to investigate the possibility to selectively delivery a cancer therapy drug, cisplatin onto the cancer cells. Thus we conjugated cisplatin onto Au core with a designed ligand. The result was impressive. Under the same Pt concentration, cisplatin on the dumbbell nanoparticles killed more tumor cells compared with pure cisplatin. And mechanism study clearly showed that there was more tumor suppressor protein, P53 in the anti-EGFR-Fe_3O_4-Au-Pt treated cells.

In summary, Au-Fe_3O_4 nanoparticles have been demonstrated as a dual imaging agent and drug delivery system.

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The Synthesis and Characterization of Polymeric Spheres Loaded with Anticancer Drug Taxol and Magnetic Particles

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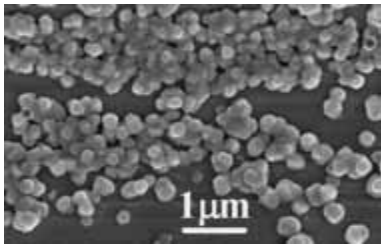
The aim of this study was to develop biodegradable and biocompatible Taxol loaded magnetic PLGA nanospheres (NPs) suitable for biomedical applications. Magnetic spheres with encapsulated Taxol were prepared by a modified nanoprecipitation technique which consists of adding the organic phase (drug and polymer solved in acetone) to the water suspension containing magnetic particles and Pluronic F68 as a stabilizing agent. After complete evaporation of the organic solvent Taxol loaded magnetic nanospheres were obtained. Biodegradable poly(D,L-lactic-*co*-glycolic acid) (PLGA) was used as a capsulation material. Taxol was chosen due to its therapeutic effects against various cancers (e. g. ovarian, breast, lung cancer). Magnetic fluid that was used as a magnetic carrier consisted of magnetite particles stabilized using sodium oleate as a first surfactant to prevent their agglomeration. To improve stability and increase the circulation half time of the particles, poly(ethylene glycol) as a second surfactant was added. Scanning electron microscopy confirmed the nearly spherical shape and size 200 – 250 nm of the Taxol loaded magnetic NPs (see figure). Measurements of magnetic properties of the prepared magnetic polymeric nanospheres using Superconducting Quantum Interference Device (SQUID) showed superparamagnetism with blocking temperature of 160 K and saturation magnetization 1.4 mT. Incorporation of magnetic particles and drug in the PLGA polymer matrix was confirmed by infrared spectroscopy and differential scanning calorimetry. The release of the drug from the prepared nanospheres to the surroundings under different conditions was also studied.



SEM image of Taxol loaded magnetic PLGA nanospheres

The prepared NPs have good stability in the presence of high NaCl concentration at 25°C, the toxicity of prepared samples declared 3 times higher value of lethal dose (LD_{50}) in comparison with pure Taxol (LD_{50} = 33 mg/kg) and showed a significant response to external magnetic field which is important from the point of view of achieving acceptable drug delivery systems for tumour treatment.

Acknowledgements This work was supported by the Slovak Academy of Sciences (grant No. 6166) and by the Slovak Research and Development Agency under the contracts No. APVV-26-026505 and APVV-0347-06.

Stabilization and Functionalization of Superparamagnetic Iron Oxide Nanoparticles (SPIONs)

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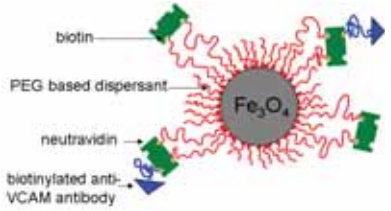

Figure 1: Layer-by-layer build-up of individually stabilized, functionalized SPIONs

Superparamagnetic iron oxide nanoparticles (SPIONs) are not only of scientific but also of commercial interest e.g. for contrast enhanced magnetic resonance imaging (MRI). Commercially available iron oxide based MR contrast agents are often stabilized with high molecular weight surfactants such as dextran or siloxanes. The comparably low binding affinity of these surfactants towards iron oxide leads to poor particle stability and limited control over the interfacial chemistry. The latter point is important for efficient targeting which is thought to lead to cellular or even molecular resolution in MRI. The stabilization of individual iron oxide cores with low molecular weight dispersants, which have one high affinity binding group per molecule, is an attractive alternative to the commercially used stabilization method. It not only leads to higher particle stability but also allows for a smaller hydrodynamic diameter and a controlled interfacial chemistry.

Superparamagnetic iron oxide nanoparticles have been synthesized by an aqueous precipitation route[1]. PEG-gallol, a low molecular weight, single chain, one foot, dispersant, was used to individually stabilize these SPIONs (fig. 1). Gallol shows a comparably high binding affinity towards iron oxide. It is a derivative of the amino acid DOPA, abundantly present in the mussel adhesive protein Mytilus edulis[2]. These PEG-gallol coated particles are stable under physiological conditions for more than 9 months. Furthermore, we could design the interfacial chemistry of these nanoparticles by adsorbing a mixture of methoxy terminated and biotinylated PEG-gallol on iron oxide nanoparticles. This allowed us to functionalize these nanoparticles with any biotinylated ligand using the neutravidin-biotin linkage. After having compared the size, size distribution and magnetic properties PEG-gallol stabilized SPIONs to Feridex, a commercially available dextran stabilized iron oxide based MR contrast agent, the amount of PEG-gallol and neutravidin adsorbed on one particle was quantified. Finally, biotinylated anti-human VCAM antibodies were bound to these individually stabilized neutravidin bearing SPIONs. VCAM is thought to be an early marker of atherosclerosis and a well suited receptor for targeted MR contrast agents[3]. Specific, fast and strong binding of so-functionalized particles was shown by *in vitro* quartz crystal microbalance with dissipation monitoring (QCM-D) measurements. The high particle stability, close control over the hydrodynamic diameter, narrow particle size distribution and high flexibility for further functionalization of these SPIONs opens up attractive possibilities for further studies mainly, but not exclusively, in the field of targeted MRI.

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Preparation Unimag During Dynamic Echohysterosalpingoscopy in The Experimental Study

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Imaging methods, including x-ray and contrast ultrasound examinations, have an important place in diagnosis of diseases of female reproductive organs. They are particularly useful in determining tubal infertility. Contrast sonography has advantages over x-ray examination in that it is literally harmless and gives opportunity of dynamic investigation. Yet this method needs to be refined. One of the important issues are searching, testing and implanting highly effective and cheaper contrast media.

The purpose of this experimental study was to investigate opacifying ability of Preparation "Unimag" (magnetic fluid) during dynamic hysterosalpingo contrast sonography, performed in sexually mature female rabbits. 2 ml of undiluted and diluted (10-fold or 20-fold) Unimag was slowly injected into the intact and amputated uterine tubes of experimental animals via a catheter. Dynamic observation was achieved with the aid of ultrasound machine – Siemens Sonoline Antares.

Observation revealed a marked opacifying ability of this magnetic fluid regardless its dilution. Injection of undiluted Unimag enabled to view uterine horns on ultrasound (Fig. 1). Rabbits having tied uterine horn demonstrated "amputated horn" on sonography (Fig. 2). During one of the experiments Unimag was accidentally injected into the urinary bladder. Visual effect was very impressive (Fig. 3). Unimag, even 20-fold diluted, displays marked opacifying ability during dynamic hysterosalpingo contrast sonography in adult female rabbits, that suggests high diagnostic potential of this substance during ultrasound examinations.

Results of this experimental study together with the recent experience of Unimag usage in surgical practice suggest that it is expedient to use this preparation during dynamic hysterosalpingo contrast sonography in women.

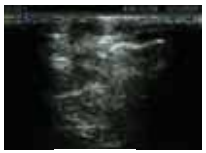

Fig. 1

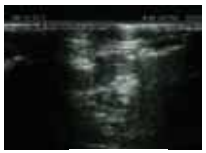

Fig. 2

Fig. 3

Magnetic Resonance Tomography using Ferromagnetic Spheres

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There has been a steady advance in the field of Magnetic Resonance Imaging (MRI) towards higher resolution, with the ultimate goal of atomic imaging capability. The attraction and intense research interest towards 3D MRI with higher resolution is driven by the well-known advantages of MRI as a three-dimensional, non-invasive, multi-contrast, and chemically specific imaging tool. The largest measurement challenges stem from weak signals typical of high-resolution magnetic resonance, and the limitation of available gradient field strengths from current carrying conductors.

We will present the methodology for obtaining two- and three-dimensional magnetic resonance images by using azimuthally symmetric dipolar magnetic fields from ferromagnetic nano-spheres [1]. We utilize the symmetric property of a geometric sphere in the presence of a large externally applied magnetic field to demonstrate that a complete two- or three-dimensional structured rendering of a sample can be obtained without the motion of the sample relative to the sphere. Sequential positioning of the integrated sample-sphere system in an external magnetic field at various angular orientations provides all the required imaging slices for successful computerized tomographic image reconstruction. The elimination of the requirement to scan the sample relative to the ferromagnetic tip in this imaging protocol is a potentially valuable simplification compared to previous scanning probe atomic resolution magnetic resonance imaging proposals, and a potentially significant new application of ferromagnetic spheres.

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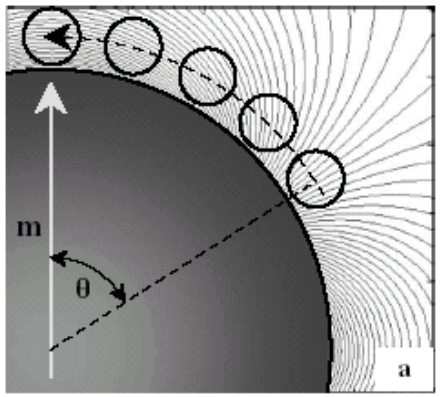

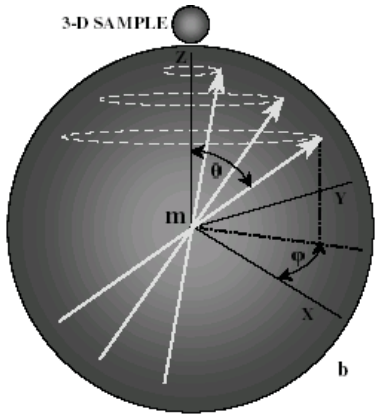

A new MR active Colloidal Amine-Functionalized Iron Oxide Nanoassembly

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We propose here a single-step simple process for the direct formation of nano-colloidal assemblies of amine-functionalized magnetic nanoparticles. High temperature thermal decomposition of Fe-chloride precursors was carried out in ethylene glycol medium in the presence of ethylenediamine molecules to achieve highly water-stable colloidal Fe_3O_4 nanoassemblies. The X-ray diffraction studies confirm the formation of single phase Fe_3O_4 and its nanocrystalline nature. From TEM (Fig. 1a), it has been observed that the size of assemblies is ~ 50 nm where as the individual nanoparticles in the assemblies are of the size about 6 nm. The surface functionalization of magnetic nanoparticles with amine molecules is evident from FTIR, thermal and CHN analysis. Amine groups on the individual nanoparticles acts as the driving source for the formation of nanoassemblies as well as their strong stabilization in water. In order to find out the potential applications of these nanoassemblies in magnetic resonance imaging (MRI), we investigated the magnetic properties and induced MR signals. Fig. 1b shows the T1-weighted MR, T2-weighted MR and T2 color map of Fe_3O_4 nanoassemblies at various concentration of Fe ions. The saturation magnetization and spin-echo r_2 of these nanoassemblies were found to be 49 emu/gm and 314.6 mM^{-1} s^{-1} respectively. The relaxivity ratio, r_2/r_1 of Fe_3O_4 nanoassemblies is found to be 143 which is much higher than the commercial contrast agent (Feromoxytol). The high value of relaxivity ratio indicates that these nanoassemblies are very promising high efficiency T2 contrast agents. In future, these surface functionalized nanoassemblies could be explored for targeted drug delivery.

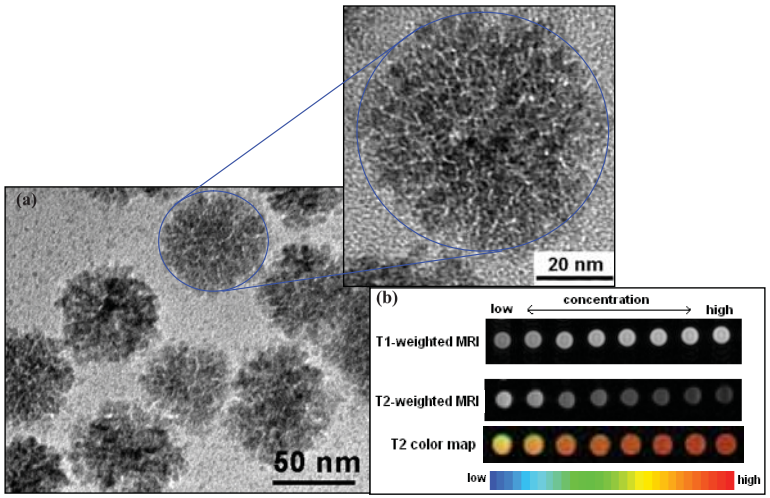

Fig. 1. (a) TEM image of Fe_3O_4 nanoassemblies and (b) its T1-weighted MR, T2-weighted MR images and T2 color map at various concentrations of Fe ions (from left: 0.006, 0.012, 0.025, 0.032, 0.039, 0.052, 0.065, 0.097 mM)

FERRITE NANOPARTICLES AS NEGATIVE CONTRAST AGENTS FOR MAGNETIC RESONANCE IMAGING

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Magnetic Resonance Imaging (MRI) is one of the most important non-invasive techniques for the diagnosis of diseases, especially in soft tissues of the human body like internal organs, muscles, tendons, etc. The contrast in MRI is based on the density distribution of protons or other nuclei in different parts of the tissue, weighted on their nuclear relaxation spin-spin (T_2) and spin-lattice (T_1) times. The aim of a more widespread use of this diagnostic tool has inspired intense research focused on the preparation of contrast agents (CA) which may increase the natural contrast. Superparamagnetic nanoparticles are good candidates, as they have been found to be effective in modifying the T_2 relaxation, inducing a local decrease of the signal intensity (negative CA).

In this work we investigated monodisperse ferrite nanoparticles, MFe_2O_4, M = Fe, Mn and Co, prepared by rapid decomposition of metalcarbonyl into a hot solvent in the presence of a coordinating surfactant, followed by an oxidation step. The size of the particles was tuned in the 4-12 nm range, by varying the metalcarbonyl/surfactant molar ratio. This allowed a systematic investigation of the dependence of the relaxometric efficacy on the magnetic properties, which in turn, depends on the particles dimension and composition. In particular, the comparison between the magnetic behaviour of the three systems considered is useful to clarify the role of magnetic anisotropy in the shortening of the relaxation times. A full characterization of AC and DC magnetic properties was carried out and compared to ^{1}H Nuclear Magnetic Resonance Dispersion (NMR-D) data, in order to correlate the properties of these novel CAs as a function of size, shape, coating and kind of magnetic ion, thus optimizing the MRI efficiency through a feedback between synthesis and NMR results.

MRI Contrast Enhancement using Polymer Stabilised Magnetic Nanoparticles

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Since its development in the mid 1970s Magnetic Resonance Imaging (MRI) has become one of the most widely used diagnostic tools, particularly for soft tissue imaging where the technique generates excellent anatomical detail with sub-centimetre resolution. Contrast agents, which are used in up to 50% of MRI procedures, provide the opportunity to extend the versatility of this technique by enhancing the contrast between different tissue types. This can be done to improve its effectiveness as both a tool for scientific studies (molecular and cellular tracking and imaging) and as a diagnostic tool.

In most cases the variation in signal intensity developed by the MRI contrast agents is due to their magnetic properties. The contrast agents produce a variation in the local magnetic field, which acts to increase both the longitudinal (R_1) and transverse (R_2) relaxation rates of the protons. In a very simplified picture the greater the variation in the local magnetic field the greater the change in the relaxation rates. MRI contrast agents take 2 basic forms, the first is paramagnetic ions such as gadolinium, the second is magnetic nanoparticles. The R_2 related contrast from magnetic nanoparticles is generally higher due to their higher magnetic susceptibilities.

Stabilising the magnetic nanoparticles against aggregation is a critical factor in the development of contrast agents. While ionic stabilisation can effectively prevent aggregation, it is not suitable for biological systems, leaving sterically stabilised nanoparticles as the preferred option.

In this paper we will introduce a series of design considerations for the optimisation of magnetic nanoparticles for use as contrast agents in MRI. We will then report on a series of potential MRI contrast agents based on iron oxide nanoparticles coated with polypropylene oxide (PPO) and polyethylene oxide (PEO) that have been developed from previous work on these systems. These nanoparticles have been characterised by a variety of techniques to determine their structure and magnetic properties. These techniques include transmission electron microscopy, SQuID magnetometry, dynamic light scattering, small angle x-Ray scattering, thermogravimetric analysis and elemental analysis.

Proton relaxation measurements on the PPO and PEO stabilized particles demonstrate a significant increase in the R_2 relaxivity as compared with Feridex® (a commercially available contrast agent) (Figure 1).

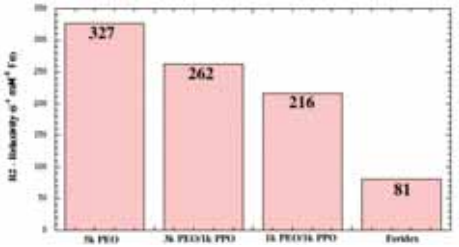

Figure 1. R_2 relaxivities of iron oxide nanoparticles with varying amount of PEO and PPO (e.g. 5k PEO = 5000 g/mol Polyethylene oxide), compared with Feridex®.

Theoretical modelling of the relaxation rates as a function of size and magnetization lead to predictions that differ from the measured values. Modifying these models to account for the presence of polymer on the surface leads to the general principle that the stabilizing layer should be as thin as possible to increase R_2, while acknowledging the competing need to have a sufficient quantity of polymer coating to maintain the stability of the suspension. While the general trends in the experimental measurements match the behaviour predicted by the model, there are variations in the contrast that are not predicted, suggesting the need for extensions to the model to describe polymer stabilised contrast agents.

DENDRONISED MAGNETITE NANOPARTICLES AS CONTRAST AGENT FOR MRI

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Magnetic resonance imaging (MRI) is one of the most powerful techniques in diagnostic clinical medicine and biomedical research. To enhance the contrast between normal and diseased tissues or to indicate organ functions or blood flow, contrast agents based on paramagnetic gadolinium or manganese ions, or superparamagnetic particles, consisting of colloidal magnetic iron-based nanoparticles, are intravenously administered to patients. The latter field of iron nanoparticles have attracted an increasing interest in the last 10 years due. However the main difficulty is to obtain homogeneous and stable aqueous suspensions of iron oxide nanoparticles without aggregates. Indeed sizes smaller than 200 nm are required to avoid toxicity and smaller than 20nm to improve tissular diffusion. Iron oxide nanoparticles, mainly maghemite, are in general coated with dextran (sugar-based polymer) and prepared by a method leading to a wide particle size distribution. In this work, very stable aqueous suspensions of magnetite nanoparticles with an average size of 10 nm have been prepared by co-precipitation of iron chlorides by a base.[1] These nanoparticles have then been covalently coated with a hydrophilic polyethyleneglycol-based dendron having a phosphonic acid as a focal point.[2] The suspension stability has been studied as a function of the grafting rate and optimisation of grafting conditions has conducted to very stable suspensions of magnetite nanoparticles in water. The functionalized nanoparticles have been carefully characterized and the magnetic and relaxation properties of the colloidal suspensions have been studied in order to evaluate the possible use of these materials as MRI contrast agents.

[1] T.J. Daou, S. Bégin, G. Pourroy, J.M. Grenèche, C. Ulhacq, C. leuvrey, P. Legaré, G. Rogez, Chem. Mater., 18(18) (2006) 4399

[2] T. J. Daou, S. Buathong, D. Ung, B. Donnio, G. Pourroy, D. Guillon, S. Bégin, Sensor and actuator B, 126 (2007) 159

SYNTHESIS AND CHARACTERIZATION OF XYLAN-COATED MAGNETITE MICROPARTICLES, AN ALTERNATIVE FOR THE ORAL USE OF MAGNETIC SYSTEMS

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Magnetic systems with magnetite particles have been proposed for oral use as magnetic resonance contrast agents and magnetic markers for monitoring gastrointestinal motility. As magnetite is soluble in acid, particles may undergo dissolution at gastric pH. Regarding pharmaceutical technology, protecting compounds from gastric environment is a key issue. In fact, many approaches have been proposed, namely a strategy that relies on the resistance of some polysaccharides to the digestive action of gastrointestinal enzymes. The matrices of polysaccharides are assumed to remain intact in the physiological environment of stomach and small intestine. Once they reach the colon, bacterial polysaccharidases come into play, and degradation of the matrices takes place. Such group of polysaccharides is comprised of amylase, chitosan, pectin, dextran, inulin, chondoitrin, xylan, etc. The aim of this work was to develop xylan-coated magnetic microparticles in order to protect magnetite from gastric dissolution.

Such polymer-based magnetic microparticles were produced by emulsification/crosslinking method. The sample characterization was performed by laser scattering particle size analysis, scanning electron microscopy, thermogravimetric analysis and vibrating sample magnetometry.

Characterization data showed that polymeric superparamagnetic particles were successfully produced. Polymer/magnetite ratio was 75:25. In vitro dissolution tests at gastric pH for 2h were evaluated for both magnetic particles (MP) and polymeric magnetic particles (PMP). Dissolution rate was nearly 30% and 3% for MP and PMP, respectively (Fig.1).

In conclusion, the obtained results have demonstrated the feasibility of the presented method to coat, and protect magnetite particles from gastric dissolution. Such systems may be very promising for oral administration.

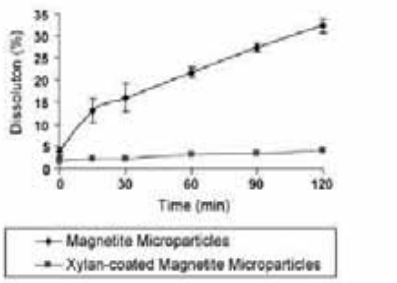

Fig.1. Dissolution profile of magnetic particles and polymeric magnetic particles.

Optimized nanomagnetic molecular probes (NMPs) for Magnetic Particle Imaging

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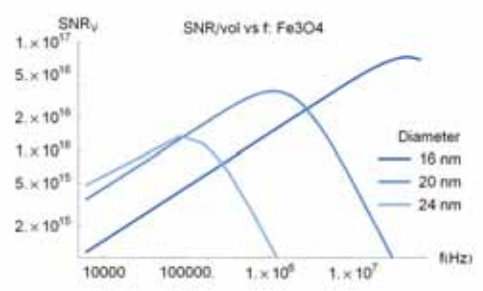
Magnetic Particle Imaging (MPI) is an attractive alternative to Magnetic Resonance Imaging for applications that require highly sensitive detection of nanomagnetic molecular probes (NMPs). Common examples in recent literature include cancer detection, hyperthermia treatment and drug delivery. With MPI, a recently invented technique, superparamagnetic NMPs can be directly imaged with excellent sensitivity and it is possible to generate full-body images in humans and animals. Promising initial results suggest that MPI can offer improvements over MR imaging by enabling high spatial resolution and extremely high sensitivity with rapid acquisition, provided appropriate molecular probes are employed.

We have developed a comprehensive model to analyze NMP magnetic cores and identify those best suited for MP Imaging. We use our model to discuss why MPI sensitivity and spatial resolution depend on NMP-core physical properties, their frequency-dependent AC magnetization, as well as their size distribution in a given sample. We also discuss how we measure relevant NMP properties in our lab. We show that monodisperse magnetite NMP cores, such as those synthesized in our laboratory by organometallic decomposition,[1,2] offer excellent potential for MPI performance due to their regular, controllable size and narrow size distribution. In addition, we show that MPI performance can surpass previous estimates if appropriate NMP cores are employed, and picomolar sensitivity and sub-millimeter spatial resolution are achievable. Finally, we present preliminary SNR results, consistent with our models, for various NMP core samples measured with a test MPI transceiver constructed in our laboratory.

Our results support the feasibility of MPI as an imaging platform and encourage further research.



SNR per volume of NMP for several core sizes

[1] M. Gonzales, K. M. Krishnan. Synthesis of magnetoliposomes with monodisperse iron oxide nanocrystal cores for hyperthermia. *JMMM*. vol. 293 pp. 265-270, 2005
[2] M. Gonzales, K. M. Krishnan. Phase transfer of highly monodisperse iron oxide nanocrystals with pluronic F127 for biological applications. *JMMM*. vol. 311 pp. 59-62, 2007

Size Evaluation of Lymph Node is protocol Dependence in MRI Using Ultrasmall Superparamagnetic Iron Oxide Nanoparticles

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Ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles have a great potential to detect lymph nodes and their metastases. These nanoparticles are uptaken by macrophages in normal lymph nodes and produce susceptibility artifact. No nanoparticles uptake is seen in metastatic nodes but these regions may be missed by susceptibility artifact, obtained in surrounding area. The size of susceptibility artifact depends on the type of MRI protocols and their imaging parameters. Accordingly, the study of protocol's effects on artifact size and problems for detection of lymph nodes are essential.

In this study, USPIO nanoparticles were used as MRI contrast agent and their produced susceptibility artifact size in axillary lymph nodes were measured with various MRI protocols. For this purpose in vivo studies were performed in rats using subcutaneously injection of 20 nm dextran coated iron oxide nanoparticles. MRI T1 and T2 weighted images were obtained with a 1.5 T MRI system before and 6 hrs after nanoparticles injection using Spin Echo (SE) and Gradient Echo (GRE) pulse sequences.

The axillary lymph nodes artifact sizes produced by these protocols were determined applying registration and subtraction methods. Comparing the anatomical delineation obtained by SE methods with the artifact sizes found in GRE pulse sequences, the size differences and various physical and physiological parameters effecting susceptibility artifact extension in GRE pulse sequences was determined. The parameter setting and fundamental considerations for an optimum imaging method is recommended.

Novel magnetic carrier encapsulating a fluorescent substance and ferrite nanoparticles in the functional polymers for biomolecular recognition and imaging

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[#]These authors contributed equally to this work.

We have developed a magnetic affinity carrier which includes the ferrite nanoparticles in the core and are covered with poly-(styrene-*co*-glycidyl methacrylate)[1]. The diameter of the carrier was about 200 nm, and was smaller than that of commercial magnetic affinity carriers. Furthermore it is possible to separate and purify a slight amount of target materials efficiently because our magnetic affinity carrier has the superior characteristic feature that the surface of the carrier, covered with the functional polymers, exhibits minimal non-specific adsorption of proteins. Therefore, the carrier is expected to apply for fluorescently- labeled carriers which visualize a slight amount of target molecules and are collected magnetically.

In this study, we prepared novel magnetic carrier encapsulating a fluorescent substance and ferrite nanoparticles in the functional polymers for biomolecular recognition and imaging. We selected europium iron (Eu^{3+}) complex for a fluorescent substance which have maximal excitation wavelength at ultraviolet region and maximal fluorescence wavelength at 615 nm, because our carrier had absorbance region at 400 - 600 nm of wavelength derived from ferrite nanoparticles and absorbance region of the carrier did not crossover excitation and fluorescent regions of Eu^{3+}-complex. We succeeded in encapsulating Eu^{3+}-complex into our magnetic affinity carrier by utilizing reversible swelling property of the carrier in organic solvent. Eu^{3+}-complex encapsulated in the carrier did not leak from within and obtained fluorescent magnetic carrier maintained the high dispersibility in aqueous solution as shown in Figure 1. Finally, we also succeeded in visualizing this fluorescent magnetic carrier on basal plate.

1) K. Nishio et al, *Colloids and Surfaces B: Biointerfaces*, in press.

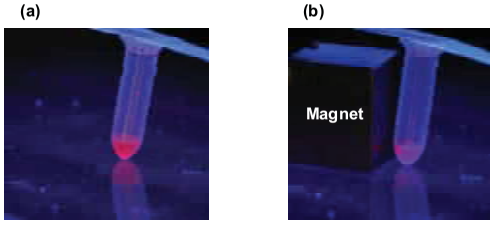

Figure 1. Magnetic separation of our novel carrier under UV irradiation.
(a) Before separation. (b) Separated by a magnet after a minute from left side.

Her2-Conjugated Magnetic Nanoparticles for Detection of Breast Cancer by Superconducting Quantum Interference Device (SQUID)

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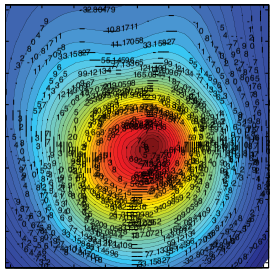
A major goal in emerging technology to enhance detection of cancer is the development of methods to identify tumors in the earliest stages of disease. For breast cancer, the current method of choice for screening and detection is mammography. While mammography has led to a significant improvement in our ability to detect breast cancer earlier, it still suffers from the inability to distinguish between benign and malignant lesions, and mammography fails to detect 10-30% of breast cancers. The use of magnetic nanoparticles conjugated to tumor-specific reagents represents a promising new technology that has the potential to improve our ability to detect tumors earlier. Furthermore, detection of targeted magnetic nanoparticles using SQUID imaging is fast, and is theoretically more sensitive than MRI detection, because only particles bound (to their target cells) would be detected. Our group is developing conjugated magnetic nanoparticles targeted to breast cancer cells that express the Her2 antigen, which is amplified/overexpressed on ~ 30% of human breast cancers.

We quantified Her2 antigen binding sites on several human breast cancer cell lines using a fluorescent-conjugated Her2 monoclonal antibody in a flow cytometry assay. Next, Her2 antibody was conjugated to SiMAG-TCL superparamagnetic iron oxide nanoparticles (SPIONs; Chemicell, Germany), and labeled SPIONs were incubated with breast cancer cell lines with high (MCF7/Her2-18) or low (MDA-MB-231) levels of Her2 expression. Labeled cells were analyzed by microscopy after Prussian blue staining for iron, and by SQUID magnetometry. Microscopy and SQUID measurements indicated an antigen concentration-dependent increase in the number of nanoparticles bound to cells. Finally, we inoculated breast cancer cells subcutaneously into nude mice, and injected Her2 antibody-conjugated SPIONs into the tumors. One hour later, mice were imaged in vivo by SQUID magnetometry. As shown in the figure below, the measurements indicate that the Her2 antibody-labeled SPIONs are retained in the tumor. Prussian blue labeling was detected in tumor tissue by histology. These results suggest that antibody-labeled SPIONs have the potential to label breast tumor cells in vivo, and thus show promise as a new tool in breast cancer detection. Ongoing efforts are aimed at quantitative analysis of Her2 antibody-conjugated SPION sensitivity, and the ability of Her2 antibody-conjugated SPIONs to "find" tumors in vivo, when injected intravenously or intraperitoneally.



Detection and localization of SPIONs *in vivo* by SQUID magnetometry. A nude mouse bearing a human MCF7/Her2-18 xenograft tumor was imaged 1 hour after intratumoral injection with Her2 antibody-conjugated SPIONs. The contours depict the strength of the SQUID-detected magnetic fields (in picoTesla) as a function of position in a horizontal plane (x and y axes are in centimeters). The contours indicate that the Her2 antibody-conjugated SPIONs are retained in the tumor, which was centered under the sensor system.

We acknowledge the support of the NIH (R44 CA096154, to ERF). This work was performed, in part, at the Center for Integrated Nanotechnologies, a U.S. Department of Energy, Office of Basic Energy Sciences user facility at Los Alamos National Laboratory (Contract DE-AC52-06NA25396) and Sandia National Laboratories (Contract DE-AC04-94AL85000).

Synthesis and Characterization of Magnetite Nanoparticles Stabilized Via Amphiphilic Diblock Copolymers for Use in MR Imaging

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Abstract

Magnetic resonance imaging (MRI) has been one of the most non-invasive imaging tools used in the detection of various diseased tissues.[1,2] The enhancement of contrast in this imaging technique comes from the use of contrast agents. The most common contrast agents are gadolinium-based chelates[1], but there is significant interest in the development of iron oxide nanoparticles due to their capacity to generate larger contrast enhancement.[1,3] Superparamagnetic magnetite-based contrast agents are already used for a variety of applications, particularly for image enhancement of the liver, spleen and lymphatic system. However, there is increased interest in developing cell specific contrast agents for early diagnosis and detection of disease for various forms of cancer.[2,3] The basis for contrast enhancement is the high magnetic susceptibility of iron oxide nanoparticles. Localized field gradients generated by these particles in applied magnetic fields result in rapid local de-phasing of the proton spins, thus increasing transverse relaxivities (R_2).

In this study, magnetite nanoparticles were synthesized and stabilized with a series of amphiphilic PPO-*b*-PEO amphiphilic copolymers with systematically varied compositions. The complexes consist of a magnetite core surrounded by a hydrophobic PPO segment and an outer hydrophilic PEO brush. The size and aggregation characteristics of the complexes in water were measured via DLS and compared to colloidal predictions in the absence of a field. The transverse relaxations of these nanoparticle systems as well as commercially available materials were probed in light of the chemical and colloidal properties. The resulting magnetic nanoparticles were shown to have much higher transverse relaxations, 2.7 times greater, than those of commercial materials.

[1] Shellate, F.G., Kard, E., *J. Magn. Reson. Imag.* **1999**, *10*, 477.
[2] Bulte, I.W.M.,Kraitchman, D.L., *NMR in Biomedicine.* **2004**, *17*, 484.
[3] Semelka, R.C,, Helmberger, T.K.G., *Radiology.* **2001**, *218*, 27.

Functionalized nanomaghemite from iron(II) acetate for medical and biochemical applications

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Due to the spinel structure with two magnetically nonequivalent interpenetrating sublattices, maghemite, γ-Fe_2O_3, exhibits strong ferrimagnetic behavior which has been used practically in various biomedical and biological applications such as increase of MRI contrast, biomagnetic separations or magnetic drug targeting.

We have prepared crystalline maghemite nanopowder by thermal decomposition of iron(II) acetate in air at a temperature of 400 °C for 1 hour. The average size of the prepared nanoparticles was approximately 25 nm. Relatively highly monodisperse nanoparticles of maghemite with the specific surface area of 150 m^2/g exhibited the saturation magnetization of 53 emu/g at 2 K. These magnetic, size and surface properties of the as-prepared maghemite nanoparticles thus make them very promising in the field of biomedical applications.

The use of maghemite nanoparticles as negative contrast agents in MRI constitutes one of the most frequent applications in medicine. In general, they are proton enhancers and they especially enhance the T2-relaxation which then enables better resolution of the healthy tissue from the pathological one.

In our work, we have focused on the preparation of the superparamagnetic maghemite contrast agent for the medical diagnostics of the gastrointestinal tract via MRI. To obtain biocompatible and uniformly dispersed maghemite nanoparticles, maghemite was incorporated into bentonite matrix. By optimization of the synthesis condition, we have confirmed an excellent contrast effect of this suspension in in-vitro phantoms by MRI. Currently, in-vivo tests of our contrast agents for gastrointestinal tract imaging are performed.

Magnetic nanoparticles coated with chitosan are also used as suitable carriers for immobilization of enzymes. Oligosaccharide-modified trypsin, exhibiting high thermostability and resistence to autolysis, was covalently immobilized onto chitosan-modified maghemite nanoparticles with a high efficiency. Immobilized trypsin can be used for specific proteolysis of studied proteins, followed by MALDI-TOF mass spectrometry of the released peptides.

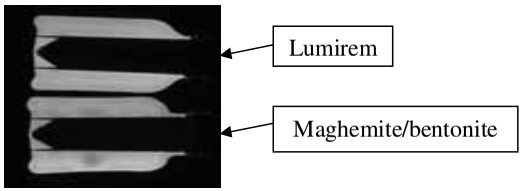

Figure 1. MRI contrast effect of maghemite modified with bentonite in comparison with commercial Lumirem in T2-weighted images

Dendrimer-Protected Superparamagnetic Iron Oxide Nanoparticles as Targeted MRI Contrast Agents

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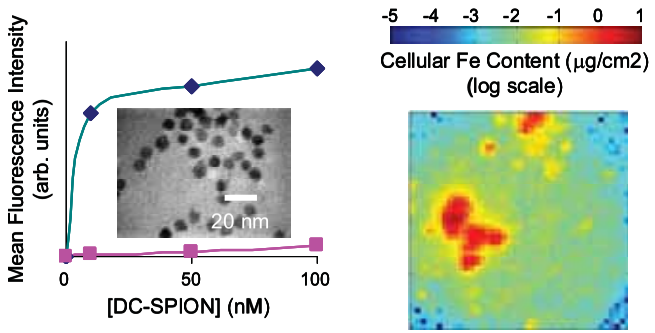
Organic-coated superparamagnetic iron oxide nanoparticles (OC-SPIONs) were synthesized and characterized by transmission electron microscopy and X-ray photoelectron spectroscopy. OC-SPIONs were transferred from organic media into water using poly(amidoamine) dendrimers modified with 6-TAMRA fluorescent dye and folic acid molecules. The saturation magnetization of the resulting dendrimer-coated SPIONs (DC-SPIONs) was determined, using a superconducting quantum interference device, to be 60 emu/g Fe versus 90 emu/g Fe for bulk magnetite. Selective targeting of the DC-SPIONs to KB cancer cells in vitro was demonstrated and quantified using the two distinct and complementary imaging modalities—UV-visible and X-ray fluorescence; confocal microscopy confirmed internalization. The results were consistent between the uptake distribution quantified by flow cytometry using 6-TAMRA UV-visible fluorescence intensity and the cellular iron content determined using X-ray fluorescence microscopy.



WATER STABLE CORE-SHELL MAGNETITE NANOPARTICLES AS MRI CONTRAST AGENTS

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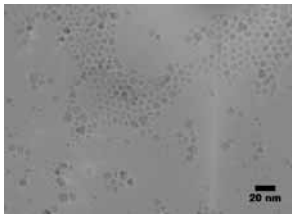
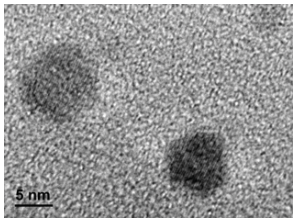
Magnetic Resonance Imaging (MRI) is one of the most powerful diagnostic tools in medicine, due to its non-invasive nature and high spatial resolution. Although enormous progress has been achieved in the improvement of the technique itself the development of MRI contrast agents is still a wide research field. Superparamagnetic nanoparticles are being used as MRI contrast agents due to their capability of enhancing image contrast by means a dramatic reduction in T_2 of the water protons in their surroundings. In this work we present the synthesis of water-stable superparamagnetic magnetite nanoparticles and their potential use as magnetic resonance imaging (MRI) contrast agents.

Monodisperse Fe_3O_4 nanoparticles with size around 5 nm have been synthesized by thermal decomposition of Fe(III)acetylacetonate in phenyl ether in the presence of oleic acid and oleylamine following the procedure described by Sun and co-workers [1,2]. The synthesis method give us the possibility of varying the size of the magnetic core, up to 12 nm. These nanoparticles are only soluble in hexane and other non-polar or weakly polar organic solvent. Following a previously reported strategy [3] they are subsequently coated with poly[maleic anhydride alt-1-octadecene] in order to disperse them in water (or any other physiological buffer), forming a very stable suspension. The polymeric shell provides also the biocompatibility required for *in-vivo* applications, and opens up a wide range of functionalization alternatives. This makes our nanoparticles suitable to be used as smart MRI contrast agents for cancer diagnosis and/or treatment, after their linking the polymeric shell to the appropriate tumor marker receptor and/or chemotherapy drug.



TEM image of the 5 nm Fe_3O_4 nanoparticles



HRTEM image of the 12 nm Fe_3O_4 nanoparticles

Magnetization measurements confirm the superparamagnetic character of our nanocrystals in a wide temperature range (from room temperature down to 30 K). Their size has been measured by transmission electron microscopy (TEM) and dynamic light scattering (DLS). The results confirm the high monodispersity of the particle size-distribution. The presence of the polymer outer shell was confirmed by Fourier Transform Infra-Red spectroscopy (FTIR), X-ray Photoelectron spectroscopy (XPS) and Thermogravimetric analysis (TGA).

NMR relaxation measurements were performed in a top-bench equipment, operating a 1.5 Tesla and 37 °C, for several magnetite concentrations in water-stable suspension, in order to determine T_1 and T_2 relaxation times. Both spin-lattice and spin-spin relaxation show a monoexponential decay. The obtained value of the r_2 relaxivity makes our biocompatible water ferrofluid a promising candidate to be used as MRI contrast agent. A systematic study has been undertaken in order to evaluate the dependence of the transversal relaxivity on the magnetite content, depending on the size of the core.

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[2] Sun, S., Zeng, H., Robinson, D. B., Raoux, S., Rice, P. M., Wang, S. X., and Li, G. "Monodisperse MFe2O4 (M= Fe, Co, Mn) nanoparticles" *J. Am. Chem. Soc.* 2004, 126, 273-279.
[3] Pellegrino, T., Manna, L., Kudera, S., Liedl, T., Kokysh, D., Rogach, A. L., Keller, S., Rädler, J., Natile, G., and Parak, W. J. "Hydrophobyc nanocrystals coated with an amphiphilic polymer shell: a general route to water soluble nanocrystals" *Nanoletters* 2004, 4(4), 703-707.

Method of synchronous ultrasonic Doppler imaging of magnetic microparticles *in vivo*.

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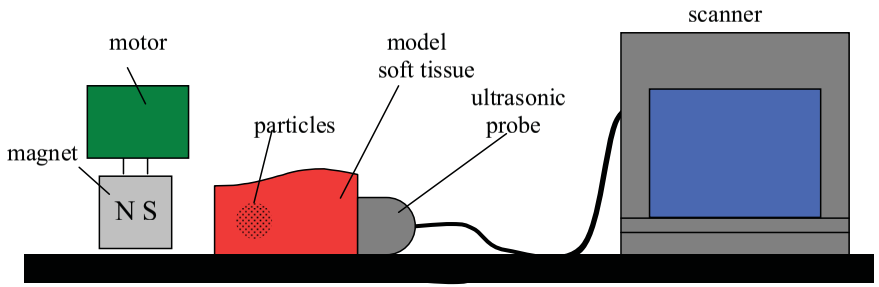
Ultrasonic medical imaging technology is non-invasive, widely available, safe, easy to use, and inexpensive. Keeping these advantages in mind, it would be highly desirable to use the acoustic imaging technology for *in vivo* detection of magnetic microparticles, which are used for magnetically targeted drug delivery and magnetic hyperthermia. However, the microparticles are much smaller then the resolution of conventional medical ultrasonic imaging techniques, and cannot be detected by these methods directly.

We propose a method for detection and imaging of magnetic microparticles and their congregations inside soft tissues *in vivo*. When the particles inside a tissue are subjected to a low frequency alternating magnetic field, they oscillate, and these vibrations are transferred to the tissue. Using the A and B-scanning modes it is possible to register Doppler signals from the moving tissue, and these signals can indicate the localization of the magnetic particles. To improve the sensitivity of the detection, the Doppler signal can be detected at the frequency of the modulating magnetic field, and purified with a low frequency filter.

The feasibility of the method was tested using a model of a soft tissue made of pig liver (ca. 5x7x10 cm). Suspension of ferrocarbon microparticles was injected into the tissue using a syringe. The tissue area containing magnetic particles was positioned inside the field of view of an ultrasonic medical scanner УДС-08-ЦМА (Medical Acoustic Imaging Science and Technology Center, Ltd.), operating at 4.0 MHz, with a custom software designed for this technique.

Alternating magnetic field was generated by a rectangular (40x40x20 mm) NdFeB alloy magnet, which was rotated by a step motor at the rate of 10 revolutions per second. Peak magnetic field in the area with the particles was about 1500 Gauss. Magnetic field vector was perpendicular to the direction of rotation, and was almost parallel to acoustic scanning plane.

Doppler signals from the area of soft tissue containing magnetic microparticles were observed, demonstrating the feasibility of imaging magnetic particles congregations inside a soft biological tissue using acoustic imaging.



Polymer Coated Superparamagnetic Nanoparticles for Cellular Uptake and MRI Detection

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Magnetic resonance imaging (MRI) has been recognized as one of the best noninvasive imaging modalities in both clinical and research fields due to its ability to provide a wealth of spatial and temporal information with excellent resolution. Recently, development of MRI contrast agents based on superparamagnetic nanoparticles is of growing interest due to the requirement of manipulating the imaging contrast and labeling of certain cells, pathological tissues or organs.

Superparamagnetic iron oxide nanoparticles (SPION) synthesized in high boiling organic solution are promising candidates for T_2 contrast agents owing to their narrow size distribution and high crystallinity. SPION are subsequently transferred to an aqueous solution with the assistance of Pluronic F127 (PF127) copolymers. The PF127-coated SPION (POA@SPION) show remarkable performance as T_2 contrast agents underlined by high values of relaxivity ratios (r_2/r_1).

The composite nanoparticles of POA@SPION with different concentrations are incubated with osteosarcoma MG-63 cell line for predetermined periods. Internalization of nanoparticles is observed under microscope after Prussian blue staining. MTT assay shows that the viability of cells was not significantly perturbed after endocytosis of composite nanoparticles, indicating that POA@SPION is biocompatible. Negative contrast enhancement is observed and quantified with magnetic resonance (MR) phantom imaging for MG-63 cells.

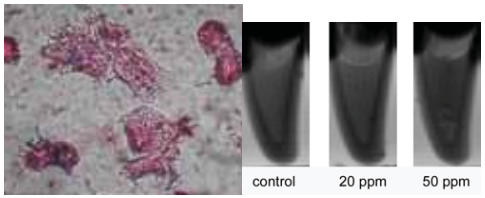

Figure 1. (Left) Particle internalization by MG-63 cells after Prussian blue staining; (Right) MR phantom imaging of MG-63 cells after one hour incubation with POA@SPION of different concentrations

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The synthesis of dendrimer-modified superparamagnetic contrast agent and its application in enhancing MR Imaging of hepatocellular carcinoma

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Magnetic resonance imaging(MRI) is the most promising non-invasive diagnostic technique of hepatocellular carcinoma. Currently, more than 35% of all MRI examinations are accompanied by administration of contrast agents. Up to now, two kinds of contrast agents, namely paramagnetic and superparamagnetic agents, have been used for intravenous administration. The paramagnetic agent have been used successfully to enhance the imaging of brain and central nervous system. However, these small hydrophilic complexes are nonspecific extracellular contrast agents and are excreted rapidly through the kidneys, and its renal toxicity has been documented recently. Therefore, it is a significant need for superparamagnetic agents with better hydrophilicity, biocompatibility and targeting specific organs such as dendrimer-modified superparamagnetic contrast to gain the greatest diagnostic value.

Based on our previous work, a project is presented that the synthesis of dendrimer-modified superparamagnetic contrast agent and its application in enhancing MR Imaging of hepatocellular carcinoma. The relationship between generations of dendrimer-modified superparamagnetic contrast agent and targeting in hepatocellular carcinoma, and the relationship between generations and enhancing MR Imaging will be discussed. The research will be promising in MRI contrast agent and its clinical application.

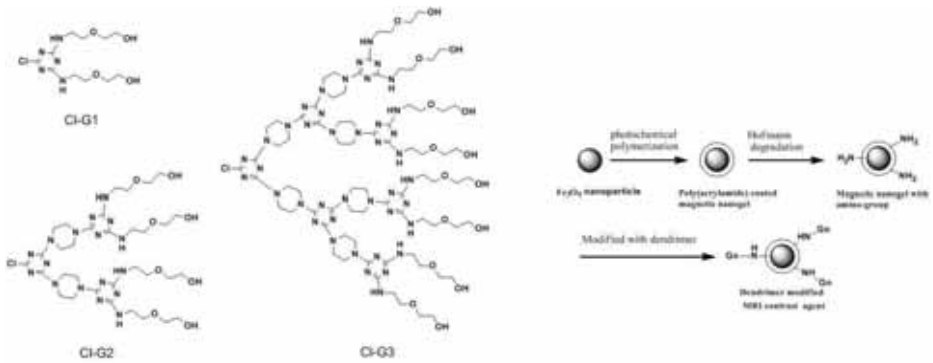

Fig.1 Schematic illustration for synthesis of dendrimer-modified superpamagnetic contrast agent

Carbocyanine Labeled BMPs for Optical Imaging in *vitro* and in *vivo*

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Bacterial magnetic particles (BMPs) synthesized by magnetotactic bacteria have drawn great interest as replacements for targeted drug carriers due to their unique features such as paramagnetism, nano-scale, narrow size distribution and membrane-bounded. Our group recently tested if BMs used as nucleic acids carrier, antibodies carrier and drug carrier for the anti-neoplastic agent doxorubicin (DOX). Incorporation of a fluorescent dye into BMPs enables the detection and monitoring of the movement of BMPs with a sensitive fluorescence detection technique such as confocal laser scanning microscopy. As BMPs have a negatively charged cytoplasmic membrane, one of the methods for the fluorescence labeling is using a lipophilic carbocyanine dye such as DiI (1,1'-dioctadecyl-3,3,3',3' -tetramethylindocarbocyanine perchlorate, ex. 549nm, em. 564 nm).

Using BMPs labeled with DiI we performed cell uptake studies, confocal microscopy, and in vivo optical imaging. The labeling efficiency of DiI-BMPs was 76 ng DiI/μg BMPs when the DiI stock was added to the BMPs suspension to yield a final ratio of 300 μg DiI to 1 mg BMPs. Confocal microscopic images showed uptake of DiI-BMPs throughout the cytoplasm in the mouse embryonic fibroblast (MEF) cells when cells were incubated with 5 μg/ml DiI-BMPs at 37°C and the fluorescence of DiI-BMPs was still intense enough to be detected in cells after 118 h incubation.

After injecting DiI-BMPs we were able to trace BMPs fate for at least 8 days in nude mice using a highly sensitive CCD camera. Maximum intensity was found after injected for 2 days, but luminescence signals remained detectable throughout the entire experiment. Immediately after injection, DiI-BMPs started migrating from the application site. From day 2 to 8, luminescence appeared to have accumulated in the lungs indicating that DiI-BMPs had mainly accumulated in the lungs of nude mice. It could be explained that blood vessels in lungs were very profuseso that BMPs were apt to migrate to lungs with blood stream, but the BMPs retained in lungs for such a long time should be investigate in future.

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Spatial resolved quantification of magnetic nanoparticles by multi-channel magnetorelaxometry in pig lungs after magnetic drug targeting (aerosol application)

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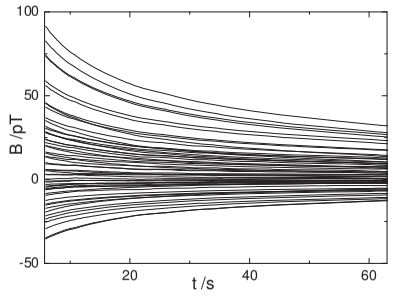
A novel way of Magnetic Drug Targeting is to guide tiny aerosol droplets containing magnetic nanoparticles to specific regions of the lung by an external magnetic field. With this technique, higher doses of drugs can be delivered to a cancerous lung region without increasing side effects. Recently, the method was transferred from a mouse model to a pig model being more similar to a human lung.

The development of this procedure requires detailed knowledge about the biodistribution of the nanoparticles in the different parts of the lung in dependence on the applied targeting magnetic field. Here, we suggest Magnetorelaxometry (MRX) as an effective and reliable technique for the quantitative and spatial resolved detection of magnetic nanoparticles in biological systems.

We developed a procedure that quantifies spatially resolved the uptake of magnetic nanoparticles by the pig lung. After switching off a magnetizing field of 1 mT, the magnetic field distribution caused by the relaxation of the magnetic nanoparticles in the ex-vivo pig lung was recorded for 60 s by the PTB 304 SQUID vector system. Subsequently the lung was divided into smaller pieces (individual lung lobes, ventral and dorsal parts) and the relaxation measurements were repeated. Finally, the lung was dissected into small specimen of about 1 cm^3, which were individually measured using our single channel MRX spectrometer.

A magnetic dipole model was used to describe the field generated by the relaxation signals, and the position and amplitude of the net magnetic moment of the individual lung sample was calculated by a least square pseudoinverse fitting algorithm. By comparison with measurements of a reference magnetic nanoparticle sample, the spatial distribution of the total magnetic nanoparticle amount over the lung was quantified and related to the reconstructed nanoparticle distribution determined by the single channel MRX measurements. Since only relaxation signals are taken into account, these quantification results are not influenced by any remanent background magnetization of the organ.



Butterfly plot showing the relaxation curves measured over the lung sample.

side	lobus	m_{MNP} / mg
right	cranialis	1.79
right	caudalis	4.42
right	accessarius	0.85
left	cranialis	2.04
left	caudalis	5.27
	complete	14.37 (~34 %)
	administered (1.25 ml 34.3 mg/ml)	42.88

Total MNP amount in individual lobes of a pig lung quantified by MRX.

Liophilic Superparamagnetic Iron Oxide Nanoparticles with Mixed Micellar System-Folate Conjugate

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In this study, we prepared folate-conjugated liophilic superparamagnetic iron oxide nanoparticles, SPION(-DA)$_{ex}$(-MUA-FA)$_5$ (DA = decylamine, MUA = mercaptoundecanoic acid, and FA = folic acid), and investigated their cellular uptake by KB cell (human epidermoid carcinoma cell), which is one of the representative folate receptor over-expressed cells, using magnetic resonance (MR) imaging. Folate-conjugation to imaging probes has been known to target and image breast cancer since some of the breast cancer cells over-express the foliate receptors.

Our SPION(-DA)$_{ex}$(-MUA-FA)$_5$ was soluble in non-polar solvents such as hexane, toluene, and chloroform. The TEM image analysis implied that each SPION(-DA)$_{ex}$(-MUA-FA)$_5$ was aggregation-free without its size change. The cellular tests with/without folic acid and their MR imaging showed efficient uptake of functional hydrophobic SPION(-DA)$_{ex}$(-MUA-FA)$_5$ to KB cells and about 10% decrease under the inhibition of excess folic acid.

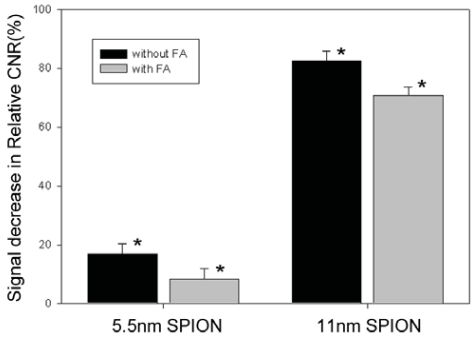

Figure 1. Signal decrease of cellular SPION(-DA)$_{ex}$(-MUA-FA)$_5$ in relative CNR (%), where CNR (%) = {T2*(cell alone)- T2*(cellular SPION)} x 100/T2*(cell alone).

Spherical and Cubic High Magnetic-Moment Nanoparticles as Highly Effective MRI Contrast Enhancement Agent

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Magnetic nanoparticles are promising candidates for magnetic resonance imaging (MRI) contrast agents. Superparamagnetic iron oxide particles are well studied and extensively used to enhance imaging contrast. However, the enhancement still needs improvement and the dosage of the nanoparticles is causing concerns. High magnetic moment FeCo nanoparticles with a composition of 70:30 were demonstrated in this report as a new MR agent to further enhance the MRI contrast and to reduce the dosage in future clinical diagnosis.

FeCo nanoparticles were synthesized using a sputtering gas condensation technique. Narrow size distribution of the nanoparticles is achieved by separating the nucleation and growth in both time and space during the synthesis. By introducing a modification of the magnetic field for sputtering, precise control of particle morphology and phase was realized. Two different FeCo nanoparticles were prepared, one with dominantly cubic shape (Fig. 1a), the other with dominantly spherical shape (Fig. 1b). For further bio-medical study, particles were directly deposited onto glass substrates, which were pre-coated with polyethelene glycol (PEG). The nanoparticles were then transferred into water.

Both the cubic and spherical nanoparticles were tested under a 9.4 Tesla magnetic field. The relaxivity of the agents reflects their interaction with surrounding protons and their detectability. Preliminary test showed that the rate of ΔR_2* change with respect to concentration of the FeCo nanoparticles was about two times higher than that of the commercial monocrystalline iron oxide (MION) particles. The effect of the shape of the magnetic nanoparticles on MRI contrast enhancement was studied. These results indicate that with low concentration FeCo nanoparticles are able to provide sufficient contrast enhancement. It opens the possibility of lowering overall dosage, targeting and imaging specific site without undesired high concentration.

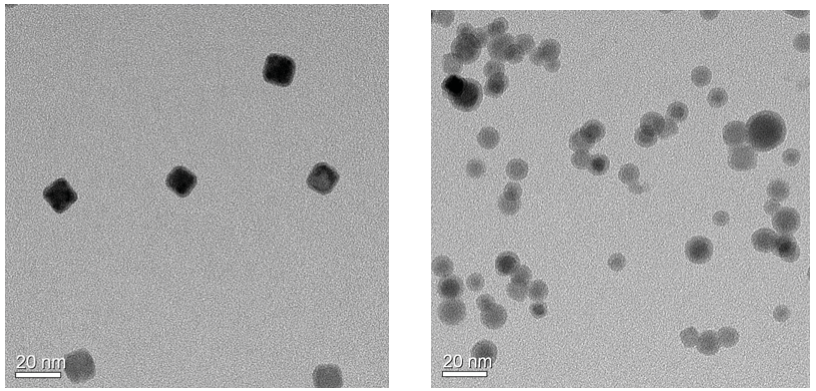

Figure 1. TEM bright field images of (a) FeCo cubic particles (b) FeCo spherical particles

The SQUID Window

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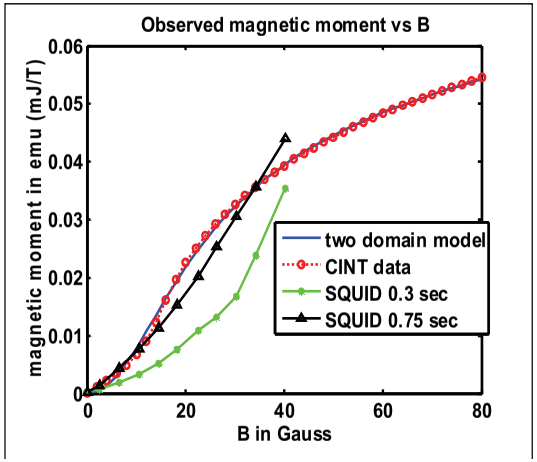
The use of SQUID (Superconducting Quantum Interference Device) array to localize objects of clinical interest (e.g. cancer cells, T cells, amyloid plaques) labeled with magnetic nanoparticles has proven very promising. The sensitivity of this technique depends both on the SQUIDs themselves and, critically, on the properties of the magnetic nanoparticles.

Our detection method consists of briefly magnetizing the nanoparticle moments using a pulsed magnetic field, followed by detection of the decaying remanence magnetization in zero field. However, we detect only those moments that decay with a relaxation time constant comparable to the "SQUID window" (measurement timescale = 50 ms to ~2 s). The Néel relaxation time constant (in zero-field) is given by $\tau_N = \tau_0 \exp(KV/kT)$, where τ_0 is approximately 1 ns, K is the anisotropy energy density of the magnetic material, and V is the volume of the magnetic particle. This sensitive dependence of this time constant on particle volume implies that highly mono-disperse particles will be required to maximize the detection sensitivity of our technique.

We have characterized the relaxation and other properties of some commercially-available iron oxide nanoparticles (Chemicell SiMAG-TCL and Ocean Nanotech SHP), using our SQUID array and standard susceptometry (which has a measurement timescale ranging from 0.001 - 100 s). The figure shows the difference between the excitation curve (M vs. B) measured by standard susceptometry (red symbols) and the SQUID array (green, black symbols), due to the difference in the measurement timescales. In general, our results demonstrate that existing nanoparticles (even those exhibiting relatively low polydispersity) exhibit a range of time constants much wider than the SQUID window, implying that we currently detect only a small fraction of the magnetic material. Additionally, we consider subtle effects due to the details of the distribution of particle relaxation times, the inclusion of anisotropies, and/or the existence of multiple-domains. For example, in the figure, the susceptometer results (red symbols) were fit using a two-domain modification of the Langevin function (solid blue line).



Ocean Nanotech SHP-30 nanoparticles characterized by a SQUID array (using pulsed field durations of 0.3 s and 0.75 s) and standard susceptometry (red symbols). The SQUID array data were multiplied by 100 to enable comparison of the shapes of the excitation curves.

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About application of a magnetic fluid for a sedimentation analysis of the size of particles

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A sedimentation analysis is often applied for the microparticle size measurement. In this paper, we report the improved method of sedimentation analysis of the degree of dispersion and sizes of microparticles.

The basic difference of this method is use of a magnetic fluid (ultra disperse colloid of ferromagnetics) as medium, in which the motion (sedimentation) of particles is explored. It allows studying features of a motion of non-magnetic bodies in such mediums by measuring the change of magnetic properties in different points of medium.

For this purpose the explored powdery substances got into the tube with a magnetic fluid. On the tube the inductance coil of small length was located. Then the change of inductance of the coil caused by the sedimentation of disperse particles was measured. The sedimentation curve $L(t)$ is constructed by results of measuring.

Analyzing the sedimentation curve, we can find a share of particles of some fraction from total number of particles in a sample:

$$\frac{N_i}{N} = \frac{\Delta L_i}{r_i^3} \Big/ \sum \frac{\Delta L_i}{r_i^3}, \qquad (1)$$

where r_i is the radius of particles of some fraction; ΔL_i is the change of inductance, happening when particles of this fraction reach the place of the tube with a magnetic fluid, where the measuring coil is located. The value of r_i is given by the following equation (Stoke's equation):

$$r_i = \sqrt{9\eta h / 2 g t_i (\rho_1 - \rho_2)}, \qquad (2)$$

where $\rho_{1,2}$ is the density of the particle and the medium; g is the acceleration of gravity; η is the dynamic viscosity of the medium (magnetic fluid); t_i is the time of the particle motion; h is the traversed path. Thus, measuring the change of inductance of the coil and the time relevant to this change and using (1) and (2), the particle size distribution curve may be obtained.

As an example, the sedimentation curves, measured for particles of a diamond powder (curve 1) and a sand (curve 2), are shown in Fig. 1*a*. The particle size distribution curves, determined using this data, are shown in Fig. 1*b*.

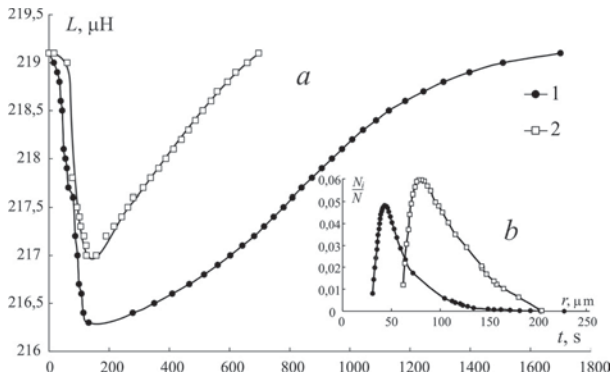

Fig. 1.

The authors have taken out a patent for this invention.

AC Susceptibility of Magnetic Markers in Suspension for Liquid Phase Immunoassay

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Recently, magnetic immunoassays utilizing magnetic markers have been developed to detect biological targets. One of the merits of the magnetic method is that we can perform immunoassay in the liquid phase, i.e., we can distinguish bound markers from unbound (free) ones without using the so-called bound/free (BF) separation process. This function can be realized by using Brownian rotation of the magnetic markers in a solution.

In order to develop the liquid phase immunoassay, we studied ac susceptibility of the magnetic markers in a solution. Distribution of the marker size and the degree of aggregation of the markers were estimated from the ac susceptibility. For this purpose, experimental result on the frequency dependence of the susceptibility was analyzed with the so-called singular value decomposition (SVD) method. The estimated size distribution was also compared with that obtained from dynamic light scattering (DLS) measurement.

As an example of the liquid phase immunoassay, we show the detection of biotin-labeled polymer beads in suspension. In this experiment, avidin-coated magnetic particles were used as markers, as schematically shown in Fig.1(a). The binding reaction between avidin and biotin can be detected with the change of the susceptibility. As shown in Fig. 1(b), real part of the susceptibility χ' decreased with the increase of the number N_p of the polymer beads. The size distribution estimated from the susceptibility is shown in Fig. 1(c), where the vertical axis corresponds to the number of the unbound (free) markers. As shown, the number of free markers decreased with the increase of N_p.

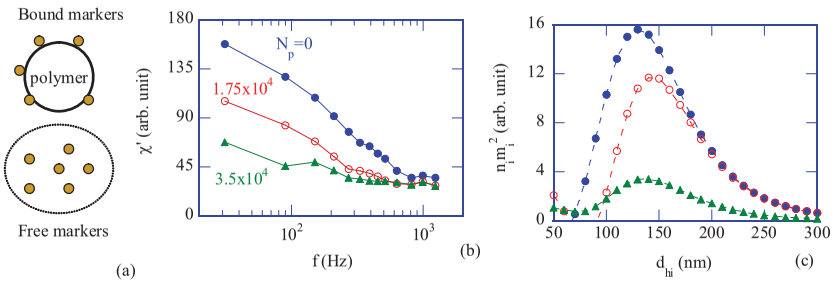

Fig. 1. (a) Detection of biotin-labeled polymer beads without bound/free (BF) separation. (b) Real part of the susceptibility when magnetic marker couples to the polymer beads with number N_p. (b) Change of the size distribution of the free markers.

Lattice spacing control in the triangle lattice of feeble magnetic materials formed by interactions among the induced magnetic dipoles

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Recently, the utilization of high magnetic fields in excess of several Teslas has attracted much attention as a novel method to control materials processing. This is because that a magnetic field has a mechanical effect on materials without any direct contact with the matter; in addition, magnetic field control is applicable to a wide range of materials because magnetism is a property shared by all materials. It was reported that feeble magnetic particles form a triangular lattice with some spacing under high magnetic fields[1)] as shown in Fig. 1 by arranging some conditions properly. The advantage of this physical phenomenon is that it can control a lattice structure formed by particles with diameter ranging from 10 to several hundreds micrometers. Control of the structure of this range of particles is difficult by other methods. Therefore, this technique seems promising.

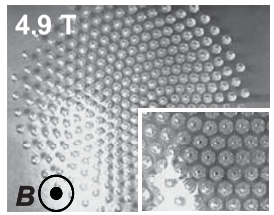

Fig. 1 Triangular lattice structure of gold particles (1 mm in diameter) formed by induced magnetic dipole interactions.

In this study, the way to control the lattice spacing in a triangular lattice of feeble magnetic materials was examined. The triangular lattice is formed by the balance between the magnetic force that is caused by the applied magnetic field and works as a centripetal force, and the induced magnetic dipole interaction as a repulsive force. It is expected that the distance between particles can be controlled by changing some experimental conditions such as the product of the volume and the magnetic susceptibility of particles, $\Delta\chi V$, the magnetic susceptibility of the surrounding medium (the concentration of the solution), and the intensity of applied magnetic fields. In this study, these three parameters were evaluated through experiments and numerical simulations as a way to control the lattice spacing

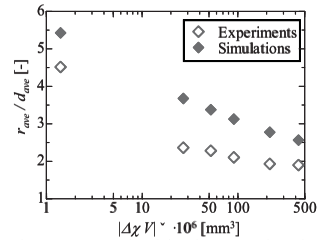

Fig. 2 Control of lattice spacing by changing the volume and the magnetic susceptibility of particles.

In the experiments, diamagnetic particles, such as glass, bronze, and gold, were used. A manganese dichloride aqueous solution was selected as a surronding medium to enhance the dipole interaction. The experiments were performed in a quasi-two-dimensional closed vessel to avoid the effect of the air-liquid interface, such as the surface tension. The parameters used in the numerical simulation were set to be the same as the experimental conditions. Figure 2 shows the results of the lattice spacing obtained with changing $\Delta\chi V$. The horizontal axis represents the absolute value of $\Delta\chi V$, and the vertical axis is the lattice spacing normalized by the diameter of particles. It was confirmed by both results of the experiment and the simulation that the lattice spacing could be controlled by changing $\Delta\chi V$. Furthermore, it was also confirmed that the lattice spacing could be controlled by another parameters. Details are reported in this presentation.

Reference: 1) T. Takayama et al., *Materials Transactions* **44** (2003) 2563-2566.

Specificity and sensitivity testing of immunomagnetic labeling reagent by VIS and UV Cell Tracking Velocimetry (CTV)

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Measurement of magnetophoretic mobility (MM) of magnetically labeled cell complexes plays an important role in the design, optimization, and operation of magnetic cell separators. MM is defined as the ratio of the field-induced velocity divided by the gradient of the local magnetic energy product, 1/2*HB*. The MM was measured using a visible light (VIS) and ultraviolet (UV) microscope with a digital camera, with a permanent magnet generating an isodynamic magnetic field in the region of interest, orthogonal to gravity (CTV). A typical sample size is on the order of a thousand cells. Jurkat cell line (ATCC, Manassas, VA) was used to determine specificity and sensitivity of anti CD45 antibody-fluorochrome conjugate (PE) and magnetic colloid. In principle, the CD45-expressing Jurkat cells are all labeled with the anti CD45-PE Ab and the magnetic colloid when the reagents are applied sequentially. In practice, due to non-ideal reaction kinetics and the reagent chemistries, one also observes cells that do not bind the Ab and the magnetic colloid (fluorescence negative, magnetic negative, F^-M^-) and cells that bind one reagents but not the other (F^-M^+ and F^+M^-). The combination of VIS and UV CTV provided a unique opportunity to investigate the size of each fraction. The VIS CTV was used to determine cell MM distribution after addition of the magnetic colloid alone. Subsequently, MMs of cells targeted with the anti CD45 PE conjugate (Invitrogen) and the magnetic colloid (Milteny Biotec) were determined by both VIS CTV and UV CTV. Only fluorescent cells in the labeled sample could be observed by UV CTV. Untreated cells were used as a control to determine cutoff MM (MM_1) that distinguished labeled from unlabeled cells. The specificity of the magnetic tagging reagent was equal to unity minus fraction of cells labeled with the magnetic colloid alone, whose VIS MM>MM_1; the mean MM of that cell fraction, MM_2, was used to determine non-specific binding following targeting antibody addition. The combination of VIS CTV and UV CTV analyses on test and control samples resulted in determination of five cell subpopulations: A_1:= {cells: VIS MM < MM_1} = ($F^-M^- + F^+M^-$); A_2:={cells: UV MM < MM_1} = F^+M^-; A_3:= {cells: MM_1 < UV MM< MM_2} = F^+M^+, A_4:= {cells: MM_1 < VIS MM < MM_2} = ($F^-M^+ + F^+M^+$), and A_5:={cells: VIS MM > MM_2} = F^+M^+. The fractional sizes of the sets, $\|A_1\| + \|A_4\| + \|A_5\| = 1$, and $\|A_2\| + \|A_3\| + \|A_5\| = x$, which is the fluorescently labeled cell fraction. The sensitivity of the magnetic tagging reagent was defined as $\|A_3\| + \|A_5\|$. For the particular combination of reagents used in this study, $\|A_1\| = 1.44\%$, $\|A_2\| = 0.34\%x$, $\|A_3\| = 0.77\%x$, $\|A_4\|=1.88\%$, and $\|A_5\| = 96.68\%$. Therefore, the sensitivity was equal to 97.43%. The specificity was 98.87%, as expected for a nearly homogeneous cell population. Other commercial magnetic colloids are investigated using the same procedure.

A MEMS-based bio-ferrograph: design, fabrication and testing

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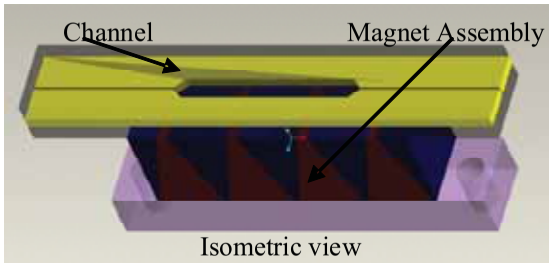
The design and fabrication of a MEMS-based analog of an existing macro-scale bio-ferrograph (BFG) will be presented. The micro-BFG consists of a microfluidic channel and magnetic assembly and allows magnetically-tagged cells to be isolated from the flow by entrapment in portions of the channel adjacent to four high-gradient zones. The entrapped cells can be fixed, stained and analyzed microscopically, combining sample enrichment and slide preparation in a single step.

The microchannels are fabricated of SU-8 (an epoxy-based negative photoresist) on Pyrex substrates. The fabrication process was composed of two major steps: 1) fabrication of 250 µm-thick open ducts on Pyrex wafers and 2) wafer bonding with SU-8 based adhesive to form enclosed microchannels. The permanent magnet assembly was designed - using the software Amperes™ - to give the highest mean gradient inside the channel. The separation was modeled to predict zonal capture ratios by numerical simulation of particle trajectories (Runge-Kutta method) from an input of magnetophoretic mobility data - acquired from an in-house technique called Cell Tracking Velocimetry (CTV).

A horizontally-positioned microscope with video camera and acquisition software was used to acquire real time deposition video images. This allowed, for the first time, visualization of the trailing gas-liquid interface and its deleterious scouring effect of the deposited magnetic microspheres. Monotonically declining capture ratio with increasing flow rate was observed from image analysis with ImagePro™ software and manual counting. Cell studies employed Jurkat cells tagged with a double antibody; the primary antibody was conjugated to the fluorochrome, phycoerythrin, and a secondary antibody was conjugated to magnetite-bearing nanospheres. Post-processing of the video images with ImagePro showed that these cells have monotonically decreasing mean fluorescence intensity with increasing zone number. The established relationship between cell surface marker expression and florescence intensity, suggests that the micro-BFG can separate cells into distinct fractions by their intrinsic properties. A second iteration of the micro-BFG designed for increased resolution could serve as a stand-alone analytical device.



DPPH/polystyrene nano-phantom for 3D MRFM imaging

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e-mail:

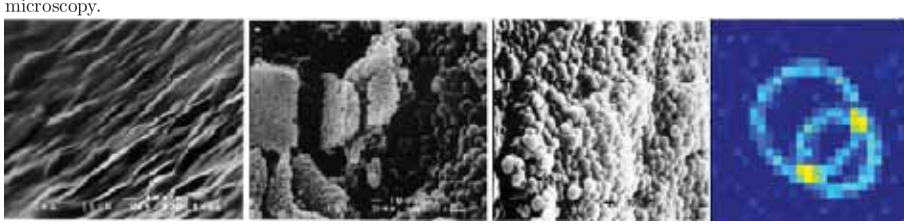
Insightful microscopy has always depended on interesting samples. Magnetic Resonance Force Microscopy (MRFM) promises to non-destructively image in three dimensions at nanoscales, but our first step is to prepare a phantom in a doped sphere of 200nm where rings of different size reflect the structure of the sphere as it is intersected by a resonance slice.

Magnetic Resonance Force Microscopy (MRFM) was first conceived by John Sidles in 1991 as a novel method for three-dimensional, non-destructive, in situ imaging with atomic-scale resolution. MRFM combines the non-invasiveness of MRI and the atomic resolution of the atomic force microscope (AFM) through the generation of localized, large gradient magnetic fields and mechanical detection of the resultant forces acting upon an AFM cantilever. A proof of concept experiment in 1992 demonstrated this mechanical detection of an electromagnetic force, and this experiment led to a concerted developmental effort with the first three-dimensional image demonstrated in 1996 with 3 *um* resolution. Current resolution is about 40-80 nm voxels. Engineering efforts have improved the single-nuclear-spin channel capacity by increases of 140 dB of signal-to-noise (SNR) (in 13 years), culminating in the detection of a single-electron-spin by Dan Rugar at IBM in 2004. Efforts are now focused upon attaining single-proton-imaging within the next two years.

Our phantom target sample will be a 200nm sphere initially doped with a free electron because of the greater force compared to proton spins. We have overcome commercial fabrication limits that tend to destroy the paramagnetic property of our phantom sample, and have created a biologically-equivalent, viral-scale target for validating MRFM imaging. Similar to the development of electron microscopy (scanning and transmission,) MRFM faces the challenge of appropriate sample preparation techniques as-well-as interpretation through image deconvolution. Our target is a validation sample to prove that MRFM can uncover the 3D structure of a nano-particle without harsh chemical preparation or interrogation by high-energy, and with a preserving environment no more harsh than cold and vacuum.

The paramagnetic substance, DPPH, is a free radical compound routinely used for ESR calibration. The free radical nature makes it a difficult compound in polymerization processes as it interferes with other free radical polymerization initiators, itself being used in certain polymerization processes as an initiator. Using pre-polymerized polystyrene dissolved in benzene as a starting material, however, confers some protection (mostly from light) to the compound as it becomes enmeshed among the polymer strands during mixing and is trapped upon removal of the solvent. We used an emulsion evaporation process on an organic phase mixture containing the polymer, DPPH and organic solvent. This mixture is added to an aqueous detergent solution and generally emulsified using a high speed stirrer or similar agent. Variables affecting the final particle size will be discussed, and these are verified with commercial polystyrene calibration beads. To disperse the particles suitable for imaging, we investigated gravity/vacuum filtration; tangential flow or diafiltration systems; centrifugation; capillary/gel electrophoresis; field flow fractionization; and column chromatography. Emulsification produces a "milky" solution, subsequent filtration produces aggregated particles, and final embedding was performed with gelatin blocks for sectioning (a raisin-cake,) and by using a particle-packing process in the way that opals are created. In the packed result, doped spheres were mixed with plain polystyrene calibration spheres to give a 20% distribution which allows the scanner to pick one sample with free electron force.

SEM images verified the opal surface and 100-200nm aggregate beads, the color purple and ESR verified the DPPH content, and the simulated image shows what we expect to see in the magnetic resonance force microscopy.



DynoMag – AC Susceptometer for Magnetic Characterization of Particles

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A portable AC-susceptometer, DynoMag for characterization of various magnetic materials has been developed at Imego. The principle of the instrumental method is based on the determination of the dynamic magnetic properties of magnetic particles by measuring the frequency dependent complex magnetic susceptibility, $\chi(\omega) = \chi'(\omega) - j\chi''(\omega)$.

The instrument comprises an induction coil system with integrated electronics for excitation and signal read-out. A software package is used for settings of the measurement parameters, instrument calibration, data visualization, storage of data and analysis. The measurement frequency interval is from 1 Hz to 250 kHz, with a resolution in magnetic moment of $3 \cdot 10^{-11}$ Am^2 or volume susceptibility $4 \cdot 10^{-7}$ (SI-units) at 1 kHz and excitation amplitude of 0.5 mT.

DynoMag can be used for dynamic magnetic characterization of for instance magnetic particles dispersed in liquid or magnetic particles in powder form. The different states of the samples (liquid or powder) determine the ability of the particles to magnetically relax trough Brownian (stochastic particle rotation in a viscous liquid) or Néel relaxation (internal relaxation of the single-domains).

As an example we can consider magnetic particles in a liquid. If the Néel relaxation time is longer than the Brownian relaxation time, the Brownian relaxation can be magnetically detected since the magnetic moment of the particle rotates with the same rate as the particle itself. By using these particles it is possible to determine the size distribution of the particle system (see the figure below) and we use this approach in a novel technique in order to detect bio-molecules in a liquid. When the particles are immobilized so that no particle rotation can take place (as in a magnetic particle system in powder form), the only possibility to relax at low fields is by the internal, Néel relaxation. This relaxation process is very sensitive to the size of the single-domains in the particles. In this case the frequency behaviour of the complex susceptibility is dependent on the size of the single-domains which results in magnetic remanence in the particle system. Thus, the frequency behaviour of the complex susceptibility can be used to indirectly measure magnetic remanence in the particle sample.

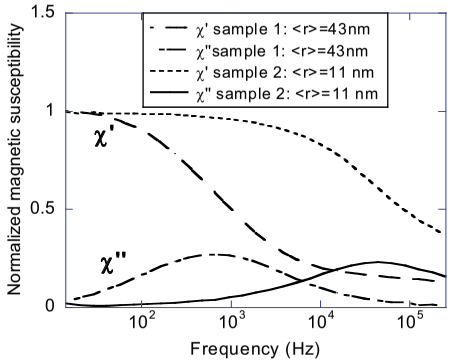

Figure: Measurement of the frequency dependent complex magnetic susceptibility for two magnetic nanoparticle samples dispersed in a carrier fluid.

Development of an Artificial Neural Network Correlation for Prediction of Rotating Magnetic Field Effects on Process Production of Disperse Systems Fe_3O_4 - Liquid

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Studies over the recent decades were concerned with application of magnetic fields in different areas of engineering processes. Static, rotating or alternating magnetic fields might be used to augment the intensity of processes instead of mechanically mixing. One advantage of rotating magnetic fields is the possibility to apply them to generation and control of hydrodynamic states for the magnetic particles disperse systems.

Under the action of a rotating magnetic field, the hydrodynamic conditions of the produced liquid-ferromagnetic particles (Fe_3O_4) disperse systems may be analysed. It is shown that complex structure of these systems may be successfully described on the basis of statistical characteristics. This proposition make it possible to formulate optimal hydrodynamic conditions for the liquid-ferromagnetic particles mixture. Experimental investigations of hydrodynamic behaviour of the liquid-magnetic particle mixture in rotating magnetic field have been carried out using a novel type of experimental apparatus. All experimental measurements were initiated by loading into glass container the magnetic particles (Fe_3O_4) with the longitudinal dimensions to the order of the Gaussian distribution. As follows from analysis of the obtained histograms, the distributions of magnetic particle becomes similar to the initial distribution with increasing magnetic induction as well as electrical conductivity of liquid phase. Therefore, hydrodynamic behaviour of the liquid-ferromagnetic particle mixture may be easy studied basing on the resultant particles size distributions.

The suspension process of ferromagnetic particles in the liquid phase may be effectively described using standard distribution moments. Then, this assessment of the complex behaviour of magnetic particle-liquid disperse system in the rotating magnetic field generator should be based on the average value as a simple numerical characteristic. As follows from the experimental investigations, this average value is based on the longitudinal dimension of magnetic particles. Obviously, the dispersion of magnetic particles in the liquid phased increase with increasing of the magnetic induction and the electrical conductivity of the carried liquid.

This paper presets the results of a study aimed at analysing spatial distribution of the ferromagnetic particles in liquid systems by using the rotating magnetic field. The experimental data were analyzed by means of multivariate statistical techniques, in order to identify the lifting ability of rotating magnetic field. The general trends of this approach should be well steered to apply neural network model. This fact is permitted to adopt this model to identify and describe the hydrodynamic behaviour of ferromagnetic disperse systems.

Acknowledgements

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Informational Assessment of Heating Effect of Ferrromagnetic Disperse Systems in Rotating Magnetic Field

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Ferromagnetic disperse systems are a very promising tool for technical or biomedical applications. Particularly, heat generation by these particles in a rotating magnetic field may be used to thermal control of heterogeneous chemical reaction. Obviously, the efficiency of sensitive chemical engineering processes increases with increasing of rotating magnetic field intensity.

Experimental database of temperature variation obtained for various ferromagnetic-liquid disperse systems and spatial distribution of magnetic induction vector were used to analytical describe of appearing thermal effect. In order to assessment of this effect in generated disperse systems was applied a novel laboratory set-up with the generator of electromagnetic field. Temperature fluctuation at time duration of process generation of the disperse systems were measured in many points of bulk using innovate very sensitivity device. The electric signals from microprocessor temperature sensors were transformed by special thermal transducers and passed through converter to personal computer for further mathematical analysis.

The obtained experimental database of temperature variations may be treated as discrete stochastic processes. Thus, the informational entropy may be perfectly used to assessment of the thermal state of generated disperse system with the ferromagnetic solid phase. This informational characteristic is defined by using statistical averaging operator. The approach presented in this work should be treated as interesting alternative to the description basing on classical methods. Moreover, the connected informational and statistical characteristics may be used for optimization problems of engineering process as well as adopted to dynamical description of heat transfer problems in area of the biomedical applications of the ferromagnetic disperse systems.

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Laccase-functionalized magnetic beads for biotechnology applications

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Enzyme laccase, a multi-copper enzyme, catalyzing the oxidation of *p*-diphenols with the concomitant reduction of molecular oxygen to water, has been already used for degradation of azodyes [1]. This way of degradation is not convenient in textile industry by reason of contamination and low stability of free form of enzyme in waste water. One of possible solution how to eliminate this failure is covalent binding of laccase on appropriate support [2]. The choice of a matrix and immobilization chemistry is key factors influencing quality of the enzyme reactor, the scope of final applications and/or process automation. To increase the operational and storage stability of immobilized bioactive enzyme molecules the group with relatively low occurrence in the protein molecule and in known locations away from active sites can be used for oriented immobilization [3,4].

Enzyme laccase (EC 1.10.3.2, p-diphenol: O_2 oxidoreductase) from white rot fungi *Trametes versicolor* (*T. v.*) and *Pycnoporus cinnabarinus* (*P. c.*), were immobilized onto various types of magnetic beads (differing in material, size, porosity, surface modification), either orientedly by glycosidic chain or randomly through amino- or carboxy- function moieties. The highest laccase activity was achieved on the magnetic macroporous bead cellulose and its hydrazide derivative form with final activity 0.22 ± 0.005 I.U./ml and 0.63 ± 0.11 I.U./ml of settled carrier, respectively. The activity of laccase was measured kinetically using two substrates: syringaldazine and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt. The higher activity of enzyme immobilized on hydrazide derivative of the carrier confirmed the profit from oriented immobilization.

Finally, model anthraquinone dye (ANTD) solution was degraded by laccase of more than 60% by formation of new products. Degradation of azo dye (Dye I) solution best proceeded at pH 3 and dye was decolorized from 10%. Degradation of Dye I was not quite effective although dyes of this type are easy oxidizable. It would be desirable to study further the effect of mediator, e.g. ABTS, on dye decolorization. Prepared laccase reactor is very stable, retains high enzyme activity for at least one month.

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The authors with to acknowledge the Ministry of Education (MSM0021627502) for financial support of research program.

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Extraction of Alkylphenols and Nonylphenol Mono and Diethoxylates from Water Using Magnetically Modified Adsorbents

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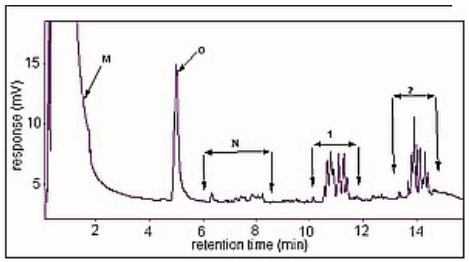
Alkylphenols are basic materials for the production of paints, pesticides and others. 4- Nonylphenol ethoxylates (NPnEO) synthesized from a technical nonylphenol NP (a very complex mixture of 22 isomers) are often used as nonionic surfactants in pharmaceutical, agrochemical, cosmetic, textile, petroleum and chemical industrial production. Residues of NPnEO and their biodegradation products can occur everywhere in the environment, especially in surface water, where they are partially converted to more persistent and toxic metabolites.

Concentrations of NP, NP1EO and NP2EO in surface water, and in water after biodegradation tests of NPnEO are low, therefore it is necessary to accomplish extraction, clean-up and extract concentration before chromatographic separation and determination. The aim of our work was to extract NP, NP1EO and NP2EO from different water samples using magnetic solid phase extraction and compare these results with liquid-liquid extraction (LLE).

Different types of adsorbents, such as Chromosorb, Tenax, Porapak and Chezacarb were magnetically modified and extraction parameters were optimized (sorption 2 min; elution by 1 ml methanol, 1 min). The target analytes were isolated from spiked distilled and tap water, real samples from river Labe and the reservoir Rozkos and from water phase after biodegradation tests of oxyethylated nonylphenols. Extracts of pollutants were analysed by capillary gas chromatography (CGC).

Magnetic solid phase extraction seems to be a suitable method for fast extraction of alkylphenols with middle length alkyls and oxyethylated nonylphenols with one or two oxyethylated groups from water samples. Magnetically modified Chezacarb B is appropriate for extraction NP1EO and NP2EO to achieve recovery 98%. For extraction of NP technical mixture, magnetic Chromosorb 103 was used with the recovery 96%. The results are similar to LLE with the recovery of NP1EO and NP2EO 93-95% and NP technical mixture 88%. The concentration of nonylphenols in real surface water samples was under the detection limit, but NP1EO and NP2EO were detected (1.5 - 2.2 µg/l) by both methods. It is obvious, that LLE can be superseded by MSPE, besides, MSPE is more economical, faster and less demanding than LLE and it is especially suitable for emulsion forming samples.



Chromatogram of technical mixture of 4-nonylphenols and 4-nonylphenol mono- and diethoxylates. M = Methanol; O = 4-tert-octylphenol; N = NPtech.; 1 = NP1EO; 2 = NP2EO

The Biobarcode-Assay
An automated approach for the ultrasensitive detection of proteins

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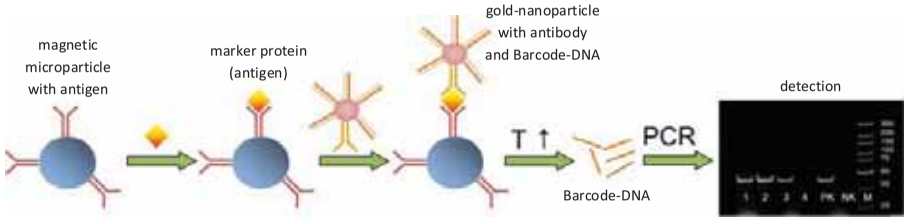
Several severe diseases are associated to marker proteins which can be found in the patient's serum at very low concentrations. The detection of these proteins requires automated assays, which are characterised by their quickness and high sensitivity. The Biobarcode-Assay is a powerful new method for the detection of biomolecules (disease markers) down to attomolar concentrations (Mirkin *et al.*, 2003). Despite its attractiveness, the Biobarcode-Assay has not been implemented on a commercial platform up to now. Applying the magnetic separation module "*chemagic prepito*" *(chemagen Biopolymer-Technologie AG)* five out of six steps of this magnetic bead-based assay have been automated. The aim of this work is to demonstrate its high sensitivity and quickness by the detection of new secretory prostate cancer markers.



The assay (see scheme above) is based on the mutual recognition of an antigen (marker protein) by two antibodies, in which one antibody is coupled to a gold-nanoparticle and the other one to a magnetic microparticle. In the presence of a target protein a magnetic microparticle and a gold-nanoparticle form a complex. All non-magnetic components like excess gold-nanoparticles or serum components are removed by magnetic separation. Detection occurs indirectly via specific double stranded DNA-sequences bound to the gold-nanoparticle. The Barcode-DNA is then amplified and quantified by polymerase chain reaction (PCR).

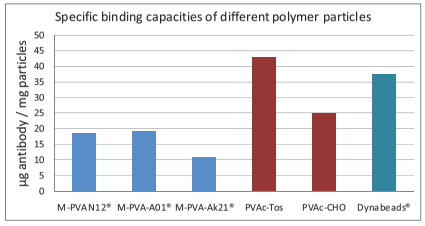


Fig.1: **Polyvinyl acetate** (PVAc) and **polystyrene particles** with **tosyl-activation** show higher antibody binding capacities than polyvinyl alcohol particles.

The specificity and sensitivity of the assay depend on both, antibody binding capacities of the magnetic particles and surface functionalisation of the beads to reduce nonspecific or false positive signals. By experimental comparison of commercial microparticles with self-made polyvinyl acetate particles (PVAc), comprising encapsulated magnetite nanocrystals, PVAc-particles were found to generate less unspecific background signals.

As a model system we work with the well established antigen PSA (prostate specific antigen), which subsequently will be replaced by new secretory prostate cancer markers, recently found at the Institute for Toxicology and Genetics at the research center *Forschungszentrum Karlsruhe*. These new cancer markers are promising candidates to replace the ambiguous prostate cancer marker PSA, which may lead to both false negative and false positive diagnoses (Dearnaley *et al.* 1999, Klotz 1997).

Measuring polymer density distribution around magnetic nanoparticles

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Virtually all existing biomedical applications of magnetic nanoparticles rely on the modification of the surface properties of the particles. The most common form of surface modification is using either synthetic polymer surfactants such as poly (ethylene oxide) or biological based coatings such as dextran. The surface modification of the nanoparticles plays several key roles

(i) reduce the immune response of the body,
(ii) suspend the nanoparticles in the intended medium,
(iii) provide a stabilizing layer that prevents agglomeration of the particles and
(iv) provide a structure for potential functionalisation.

In addition these surface modifications may affect the biological response, for instance altering the ability of the particle to pass through the blood brain barrier, or physical response, for example changing the variation in contrast produced in MR imaging. These changes may not always be positive and so in order to tailor the surface modification to specific applications it is important to be able to understand the structure and morphology of the surface modification.

Critical to questions of both stability and performance of the magnetic nanoparticles is the issue of polymer morphology in solution. What is the polymer density distribution on the surface of the magnetic nanoparticles? Is the coating a condensed solid phase around the nanoparticle or is it a dilute brush of polymer standing out from the surface of the particle? It is possible to theoretically model the polymer density distribution on a particle using the blob model developed by Vagberg et al [1]. However, in recent work on MRI contrast agents (based on PEO-PPO stabilized magnetite) we have found that this model is a poor predictor of contrast enhancement. We believe that this is due to inadequacies in either the models for the polymer density distribution or the models for MRI contrast enhancement.

Itis very difficult to image or determine the morphology of the surfactants even in the 'dry' state, as most techniques rely on electron density for contrast which for polymer and biological surfactants is very low. Measurements in solution are even more difficult. However, neutron scattering is dependent on the neutron scattering cross section, which varies significantly even for isotopes of the same element. Small angle neutron scattering (SANS) provides one of the few experimental techniques for examining the polymer density distribution in solution. Using a technique called contrast variation a series of neutron scattering patterns can be obtained by varying the neutron contrast of the solvent.

In this work we will describe how small angle neutron scattering can be used to visualise the polymer density distribution around magnetic nanoparticles, and show results from a recent experiment on the measurement of the structure of polydimethylsiloxane (PDMS) stabilizers on the surface of iron oxide nanoparticles. In this experiment we measured the SANS in which the solvent contrast was varied by varying the proton to deuteron ratio of the solvent(Figure 1). We compare the fitted polymer density distributions to the theoretical values obtained using the blob model.

[1] L.J.M. Vagberg, K.A. Cogan, A.P. Gast, Macromolecules, 24, 1670-1677, 1991

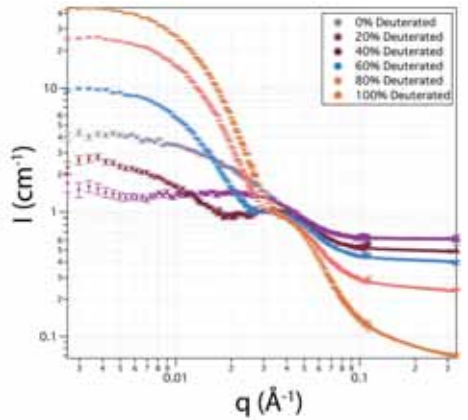


Figure 1. SANS scattering pattern obtained using contrast variation technique for a single sample of PDMS coated magnetite nanoparticles

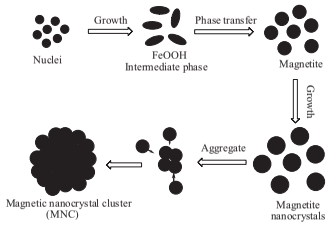
One pot synthesis of water-soluble carboxyl functionalized magnetic nanocrystal cluster via polyol method

Changming Cheng, Yinhui Wen, Ning Sun, Xuefei Xu and Hongchen.Gu*

Nano biomedicine research center, Med-X research institute, Shanghai JiaoTong university, Shanghai, 200240, P.R.China

Abstract

The magnetism and surface property of magnetic nanomaterials is essential for its application in biomedicine, such as in immunoassays, bioseparation, drug or gene delivery, and magnetic resonance imaging [1]. Herein, we report a facile strategy to synthesize carboxyl functionalized water-soluble magnetic nanocrystal cluster (MNC) via polyol method. In a typical synthesis, $FeCl_3 \cdot 6H_2O$ (iron resource) was completely dissolved in 30mL ethylene glycol to form a uniform solution under ultrasonic and vigorous stirring, followed by addition a amount of sodium citrate as surfactant and urea as base. Then, the uniform mixture solution was sealed in a Teflon lined stainless-steel autoclave (50 mL capacity) and heated at 200℃ for 6~8h, then cool to room temperature. The black precipitation was washed several times with ethanol and deionized water to remove the impurities. Finally, the product was dispersed and stored in deionized water. The effects of the surfactant on the properties of MNC were carefully investigated. The MNC was proved to be magnetite by XRD and its grain sizes of MNC calculated from (311) facet by Scherrer formula were varied from 14.6nm to 9.5nm with the increase of surfactant. Furthermore, by simply changing the amount of the surfactant in the reaction, the size of MNCs were varied in the range from 80~200nm. The surface charge of MNC was also investigated by measuring its zeta potentials. The MNC shows superparamagnetic property at room temperature and its magnetism can be tuned from 40emu/g to 68emu/g with the decrease of surfactant. Therefore, with excellent magnetic property, the water-soluble MNC has good potentials application in bioseperation, drug delivery and molecular imaging.



Scheme 1. Formation of MNC in polyol reaction.

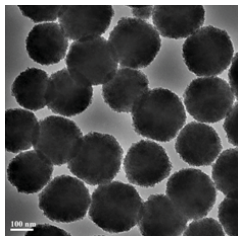


Fig.1. A representative TEM images of MNCs

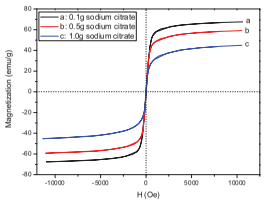


Fig.2. Hysteresis loops of the MNCs measured at 300 K. MNCs were synthesized with 0.1g (a), 0.5g (b) and 1.0g (c) sodium citrate, respectively.

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Reference

[1] Ste´phane Mornet, Se´bastien Vasseur, Fabien Grasset and Etienne Duguet. J. Mater. Chem., 2004,14, 2161-2175

Use of SDS to Enhance the Penetration of Magnetic Nanoparticles into Swollen Polystyrene for the Preparation of Magnetic Polymer Microspheres

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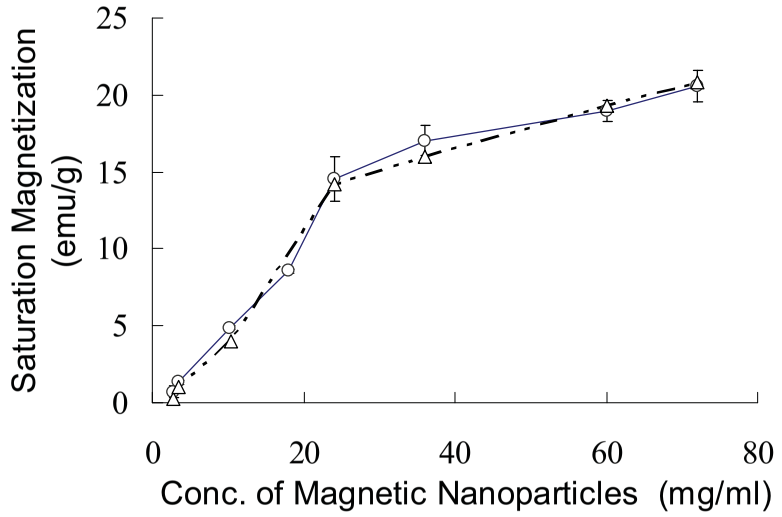
Magnetic polymer microspheres could be prepared by a swelling and penetrating process with polystyrene (PS) particles of micron size as the seeds. An aqueous solution of N-methyl-2-pyrrolidone (NMP) was employed for the swelling of PS seeds, and in a following incubation, superparamagnetic iron oxide nanoparticles were allowed to penetrate into the swollen particles. The higher the concentration of magnetic nanoparticles incubated with swollen PS particles, the higher the saturation magnetization of the resultant polymer magnetic microspheres. The selection of a proper proportion of NMP to water was crucial since NMP could also partially dissolve the polystyrene particles. The presence of sodium dodecyl sulfate (SDS) in the NMP aqueous solution could significantly enhance the swelling and penetrating process. Since it could facilitate the diffusion of NMP into the polymer particles, the use of SDS reduced the process time so that the product yield was raised with a lower concentration of NMP.



Influence of applied magnetic nanoparticles concentration on the saturation magnetization of the resultant microspheres prepared with SDS.

A Versatile Pathway to Surface Modified Magnetite Nanoparticles

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Superparamagnetic nanoparticles are of great interest in various fields of fundamental and applied research due to their unique interaction with static or dynamic electromagnetic fields. By this, they give rise to the development of more complex soft materials with magnetic properties by the incorporation of magnetic nanoparticles into organic or polymeric matrices. In order to achieve a good compatibility and a defined interface, it is desirable to modify the particles surface accordingly by the introduction of different functional moieties.

In the present study, we demonstrate the surface modification of Fe_3O_4 nanoparticles with several organosilanes. The synthetic pathway is composed of alkaline precipitation of Fe_3O_4 followed by the electrostatic stabilisation. These particles serve as basis material for the following organosilane modification. By selecting the functionality of organosilanes, the colloidal properties of the particles can be adjusted. Depending on the surface functionality, the modified particles can be used as macrocomonomers or -crosslinkers or as macroinitiators for surface initiated polymerization.

By using methacryloxypropyltrimethoxysilane (TPM) as surface-modifying agent, we obtain particle surface functionalization with monomer units that can participate in radical polymerisations. On the one hand, we polymerize methylmethacrylate (MMA) in presence of these TPM-particles in bulk to achieve composite materials. The particles act as crosslinker leading to swellable networks. On the other hand we synthesize poly(N-isopropylacrylamide) (PNIPAAm) core-shell particles in dispersion to obtain nanohydrogels. The PNIPAAm coated particles are well dispersible in water showing a LCST behavior which is well known for PNIPAAm in water.

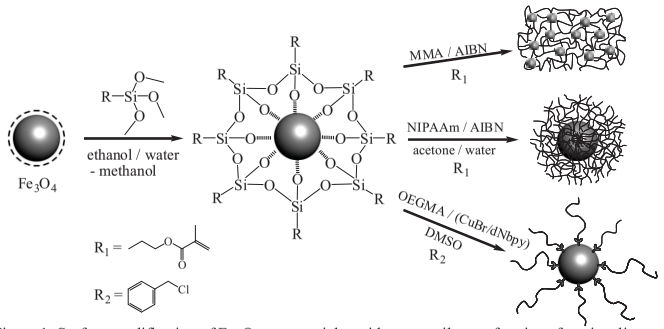

Figure 1: Surface modification of Fe_3O_4 nanoparticles with organosilanes of various functionality

By modification of the particle surface with 4-(chlormethyl)propyl-trimethoxysilane (ClPTS), the particles serve as macroinitiators for the surface initiated atom transfer radical polymerization (SI-ATRP). This way we are able to synthesize magnetic hybrid particles with a brush architecture. By grafting a polymeric shell of poly(oligo(ethyleneglycol)-methylether methacrylate) (POEGMA), we obtain magnetic polymer brushes instantly dispersible in polar solvents like DMF, DMSO and water[1].

The great manifoldness of the applied modification reagents makes these particles accessible for a wide range of applications like technical devices or biomedical approaches.

[1]T. Gelbrich, M. Feyen, and A. M. Schmidt, *Macromolecules* **39**, 3469-3472, 2006;
T. Gelbrich, M. Feyen, A. M. Schmidt, *Zeitschr. Phys. Chem.* **220**, 41-49, 2006.

Superparamagnetic Nanoparticles In The Hyperthermia Treatment Of Cancer And Other Medical Applications

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The potential value of superparamagnetic nanoparticles for medical applications such as the hyperthermia treatment of cancer has long been recognized. However, this potential has not been fully realized in the past because of the difficulties in achieving unaggregated dispersions of the very high specific surface area superparamagnetic nanoparticles at adequate concentrations within polymer matrix particles. In this work we have designed stabilizing polymers based on reversible addition fragmentation chain transfer (RAFT) controlled radical polymerization processes to largely overcome these problems.

The anatomy of a liver cancer tumour is such that 32 micron particles injected into the hepatic artery will become lodged in the blood vessels of the tumour. Sirtex Medical Limited has successfully made use of this behaviour in their radioactive SIR-Spheres® product, which enables the highest intensity of the radioactive dose to be confined to the tumour itself. Superparamagnetic particles subjected to an oscillating magnetic field of appropriate strength and frequency will generate heat. If a sufficient concentration of superparamagnetic nanoparticles can be incorporated into polymer microparticles of appropriate size they can be make to accumulate within the tumour by the same mechanism. The patient can then be put into an oscillating magnetic field so that the particles generate heat and kill the tumour with little collateral damage. 32 micron particles have been prepared, containing approximately 10^9 individually stabilized magnetic nanoparticles/micro particle that, in vitro, have generated the amount of heat calculated to be required using therapeutically acceptable field strength and frequency.

Superparamagnetic nanoparticles also have recognized potential as imaging agents for MRI. However, their full potential has not yet been realized because of difficulties in adequately stabilizing sufficiently small particles without appreciably adding to their size due to the stabilizing corona. In separate work 5 nm superparamagnetic particles have been individually stabilized in concentrated salt solution at concentrations of approximately 10^{21} particles/L. These particles are stable to dilution and can even be dialysed without loss of stability. NMR studies have shown that they have potential as positive contrast agents for MRI.

Grain size effect MR on Polycrystalline $La_{1-x}A_xMnO_3$ (where A=Ca, Sr Ba and X=0.2,0.15)

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Polycrystalline $La_{1-x}A_xMnO_3$ where A=Ca, Sr and Ba X=0.20 and 0.15 were prepared by standard solid state reaction technique. Stoichiometric amounts of mixed powders were calcined at high temperature (1050) for 24 hours. After calcinations pellets were prepared and sintered at 1573K, 1673K and 1773K for 5h in air.

X −ray diffraction was carried out to check the sample homogeneity and status of the samples. Microstructural properties and average grain size of the sample were investigated from high resolution optical microscopical picture. The DC electrical resistivity of the samples were measured by standard four point probe and van der pauw technique from room temperature down to liquid nitrogen temperature in zero applied field and in presence of magnetic field. A liquid nitrogen cryostat and an electromagnet were used for this magnetoresistance (MR) measurement. Magnetoresistance as a function of applied magnetic field (0-0.86T) at 77K and at room temperature for all samples were compared.

It was found that the T increases as the average ionic radius of the A-site cation increases all $La_{1-x}A_xMnO_3$ where A=Ca, Sr and Ba X=0.20 and 0.15 Polycrystalline samples except Ca doped sample show a semiconductor- metal transition in the measured temperature range. Resistivity of all samples decreases as sintering temperature increases. All the samples show MR both at room temperature and 77K. Smaller grain size sample exhibit larger MR compared to larger grain size of the same composition .The $La_{0.80}0Sr_{0.20}MnO_3$ sample sintered at 1573 K in air show highest room temperature MR in presence of 0.86T magnetic field. Moreover, the MR(H) curves show a linear behavior up to 0.86T applied field . This important result is very useful for device fabrication.

New Methods of Iron-Based Microparticles' Modification and their Application for Hemoglobin Adsorption

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It is known that modified magnetic microparticles are widely used in various biomedical and diagnostic applications. Besides, they can be used for cleaning of biological liquids (blood, plasma, liquor, donor blood and plasma) from different xenobiotics and metabolites, e.g. free hemoglobin.

We have modified the known methods and have worked out new methods of restored-iron, magnetite and iron-carbon microparticles surfaces' modification. The restored iron sized 0.06-0.2 mkm with 90% metallic iron content had an saturation magnetization up to 160 emu/g. Carboxilate-modified magnetic particles were obtained by albumin or gelatin coating with the following glutar- or form-aldehydes processing, aldehyde-modified - by dextran coating with $NaJO_4$ activation. $TiCl_4$-modified composites were obtained by $TiCl_4$ activating of gelatin-coated microparticles. We have also got iron particles coated by denaturated by heating albumin.

Adsorption of hemoglobin and other substances on modified particles was carried out in a physiological solution and in a model biological liquid (0.6% albumin in physiological solution) at 20^0C (pH 7.2) at different mass rations of adsorbent to hemoglobin, during 30 sec. The hemoglobin sorption efficiencies at the ratio of mass adsorbent to hemoglobin equaling 10, reached by restored iron modified by denaturated albumin and by activated dextran, were 47.9 % and 32.6% versus 44.6% and 25.0% in physiological solution and in the model biological liquid, respectively. The high sorption efficiency of coated by denatured albumin restored iron (49.7%) was shown for barbiturates. It was found out that the sorption efficiency of hemoglobin and other substances can be much increased by raising adsorbent mass or by multiple adsorption. The mechanism of hemoglobin and barbiturates' absorption at different modifications of iron particles is discussed.

COBALT VERSUS MAGNETITE NANOPARTICLES: CELL TOXICITY AND EFFICIENCY AS COMPONENTS OF GENE DELIVERY VECTORS IN VITRO

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Metallic Co-, Fe-, or Fe/Co-nanoparticles exhibit a higher saturation magnetization compared to iron oxide base nanoparticles. Therefore, they are considered to have a potential for numerous biomedical applications as cell separation, site-specific drug targeting, tumour hyperthermia, purification of biomolecules.

In the present work a set of Co-based and magnetite based nanoparticles with similar core size of ca. 9-10 nm and coatings or surface modification were synthesized, characterized and tested regarding their cell toxicity (MTT-test) and efficiency as components of gene delivery vectors for magnetic force assisted transfection in vitro.

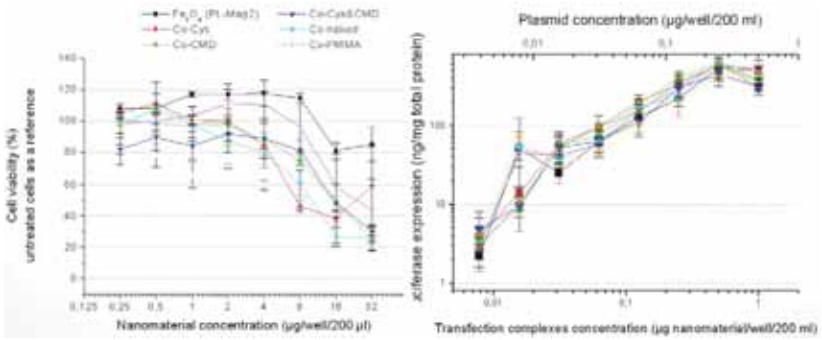

Figure 1. 3T3 mouse fibroblasts viability (MTT-test) and Luciferase gene expression after 24 h exposure to Co-based magnetic nanomaterials and magnetic transfection complexes.

The results show that toxicity of surface modified metallic particles strongly depends on the mode and type of surface modification as well as on the cell type. Toxicity of the transfection complexes is acceptable for in vitro applications in a concentration range that is of interest for gene delivery in vitro.

Hydrodynamic Flow Focusing: Applications in Pharmaceutical Sciences

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Hydrodynamic flow focusing is a promising method for the generation of microspheres for controlled drug delivery. The present study was conducted to show the feasibility of flow focusing (**Figure 1**) to encapsulate the anticancer drug camptothecin in biodegradable polymer microspheres using a one-step approach. Poly(D,L-lactide-co-glycolide) (PLGA) and poly(L-lactide) (PLA) were used as the matrix materials. Camptothecin was dissolved in the disperse phase and microspheres with a mean size between 2 and 3 µm generated using hydrodynamic flow focusing. When up to 1 wt.% of the drug was added to PLA, the drug encapsulation efficiency was 64%. For PLGA, the drug encapsulation efficiency was between 39 and 46%. Drug release from PLA particles was rapid and complete within 6 h, while drug release from PLGA particles showed no burst effect and followed a first order release profile. The encapsulated camptothecin stayed in its active lactone form, as shown by HPLC, and was able to exert cell toxic effects as shown by a cell viability assay.

The encapsulation of a highly active drug in a polymer matrix by hydrodynamic flow focusing is the first step towards the larger goal of generating biodegradable, drug loaded, magnetic microparticles in a contiuous mode. Ongoing research is focused on the optimization of the particle size distribution and the addition of superparamagnetic iron oxide (SPIO) nanoparticles to the disperse phase.

Biodegradable drug-loaded magnetic microparticles generated by hydrodynamic flow focusing may overcome challenges by the traditional particle generation methods (e.g., spray drying, freeze drying) that use high-energy physical approaches for the particle or droplet generation.

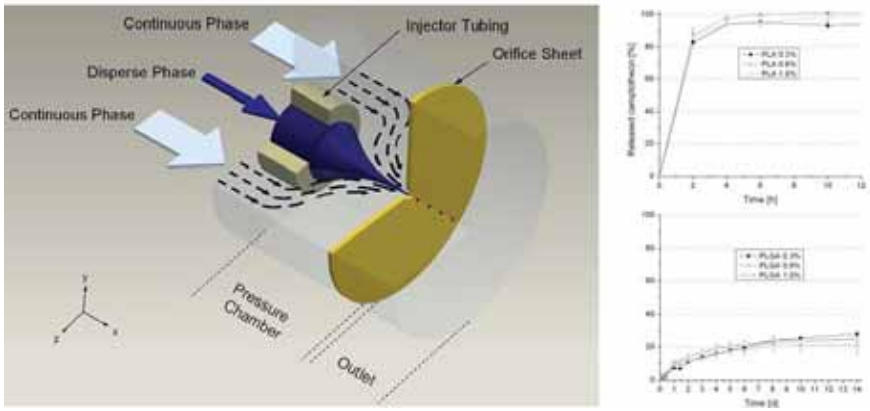

Figure 1. Principle of flow focusing with *in vitro* release curve of camptothecin from generated PLA and PLGA microspheres.

The structural Properties of Magnetite/porous silica nanocomposite and its applications in cosmetics

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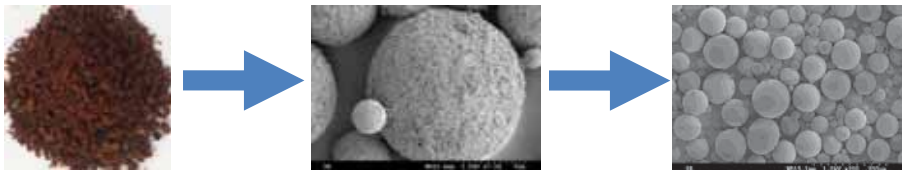
This scientific literature demonstrates a growing interest in magnet-support composite, driven primarily by their application in cosmetic products as an enhancer of blood-circulation and a volumizing agent of hair.

The properties of polymer-encapsulated magnetite nanoparticle / porous silica nanocomposite, which have the potential to be used as enhancer of blood-circulation and volumizing agent of hair in the cosmetic products of anti-darkening cream and mascara, have been studied.

Magnetite/porous silica nanocomposite(MSN) is composed of spherical magnetite nanoparticle with mean diameter of about 10nm or less and porous silica pigment with mean diameter of 5㎛. Magnetite/porous silica nanocomposite(MSN) was prepared by the method of a reduction-precipitation with ferric chloride as starting material. It was partially reduced to ferrous salts by Na_2SO_3 before alkalinizing with ammonia. The uniform distribution of magnetite nanoparticles on the all volumes of nanocomposite is shown. The mean diameter of magnetite / porous silica nanocomposite(MSN) was 5㎛. In SEM(Scanning Electron Microscope) observation, it is shown that the mean size of nanoparticles, before and after introducing porous silica matrix, was not changed.

Then for the control of particles and convenience into cosmetic products, Magnetite/porous silica nanocomposite(MSN) was encapsulated with PMMA(M_w = 15,000) by being absorbed on to pore of porous silica surface.

The structural properties of the magnetite / porous silica, and PMMA-encapsulated magnetite / porous silica were characterized by X-ray diffraction, BET measurement, as well as by vibrating sample magnetometry.



Magnetite particle — magnetite/porous silica nanocomposite(MSN) — PMMA-encapsulated MSN

Electro-Magnetooptics of Emulsions with Magnetic Droplets

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Emulsion of microdrops of magnetic fluid in nonmagnetic media are very interesting system. Spherical magnetic drop can be deformate by an electric as well as magnetic fields and, i.e. in external fields in emulsion of these drops anisotropy of physical properties can be registrated.

We investigate the effect of change of optical density of emulsions of magnetic droplets in mineral oil. Object of investigation is emulsion of magnetic fluid drops in mineral oil AMG-10, diameter of droplets are 2-5 μm. Change of optical density of emulsion we estimate by the value $\delta D=(D_F-D_0)/D_0$, where D_F и D_0 – optical densities of emulsion when magnetic field is applied and is absent respectively. If the transmittance of a system is increase change of optical density is negative ($\delta D<0$) at contrary – positive ($\delta D>0$).

Change of intensity of light occur under the action of a magnetic as well as an electric fields (fig. 1). Sign of the effect of change of optical density in constant and alternate magnetic fields depend on orientation of a field and laser beam. In AC electric field quantity and sign of effect depend on frequency of field. In low frequency field (under 200Hz) sign of effect is positive and in high frequency field (upwards of 200 Hz) sign of effect is negative (fig. 2). In simultaneous action of collinear electric and magnetic fields compensation of a magnetooptical and electrooptical effects is possible.

Estimation of value change of optical density of emulsions in anomalous diffraction approximation (ADA) shows that electrooptical and magnetooptical effects in magnetic emulsion can be interpreted by a deformation of a droplets under the action of fields.

This work was partially supported by Russian Federal Agency of Education in scientific program “Development of a scientific potential of high education”.

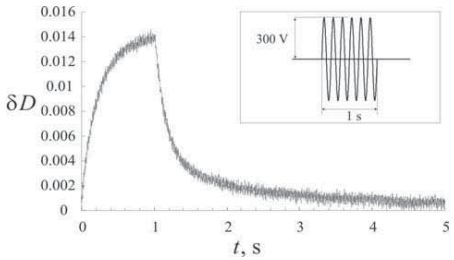

Fig . 1. Change of optical density of emulsion in pulsed AC electric field (on the insert picture - the pulse form).

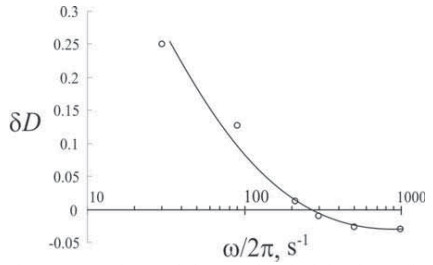

Fig . 2. Dependence of change of optical density on the frequency of alternate electric field.

Synthesis and magnetic properties of magnetite clusters with various sub-structures and potential application in hyperthermia

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Hydrophilic magnetite clusters with high saturation magnetization and various sub-structures were synthesized by hydrolysis and reduction of iron(III) cations in ethylene glycol. The surface of clusters can be modified by amino group and coated by silica. TEM investigation reveals that the clusters are monodispersed and the size of the clusters is ranged from 100 to 200 nm, whereas, the size of the crystallites composed of clusters varies from 10 to 50 nm, and the morphologies are from sphere to flake. Selected area electron diffraction taken on individual cluster indicates that the clusters have nearly single crystal structures. Magnetic hysteresis measurements demonstrate that the clusters have different hysteresis properties from superparamagnetism to ferromagnetism. The superparamagnetic clusters can be well dispersed in water after coated with silica or PEI, which may have potential biomedical applications. All of these clusters have higher saturation magnetization up to 80 emu/g compared with magnetic poly microsphere. The large specific absorption rate of 85 W/g.Fe_3O_4 was obtained under an alternating magnetic field of 200 Oe at 55 kHz, which can be used in magnetic hyperthermia.

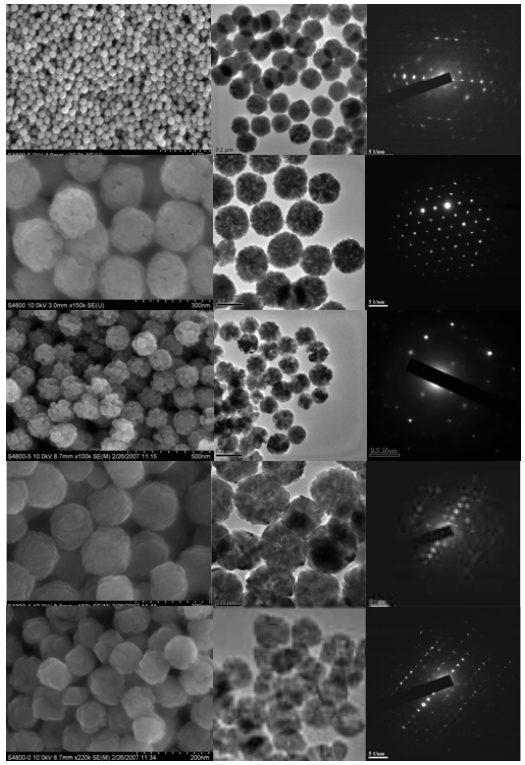

Fig. TEM images and selected area electron diffraction patterns of Fe_3O_4 nano-clusters with different sub-structures.

Controlling size and shape of ferrite nanoparticles and elucidating chemical bonds that conjugate biomolecules to ferrite surfaces

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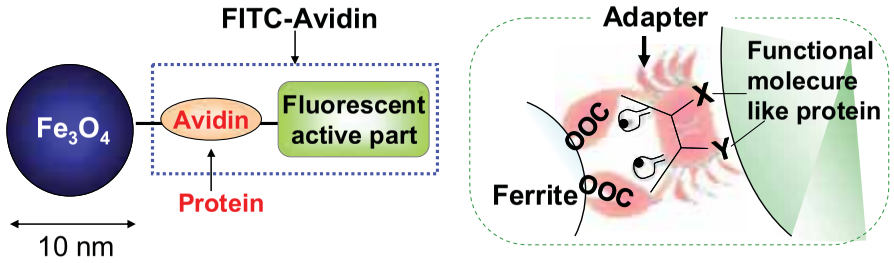
In order to use ferrite nanoparticles (FNPs) as medical magnetic carriers, strictly controlling size and shape as well as stably conjugating functional biomolecules are vital. Here we describe some of our current studies on magnetite FNPs prepared by aqueous routes which enabled accurate size/shape control and stable immobilization of biomolecules to the ferrite surfaces by specific chemical bonds as follows:

(1) Adding seed ferrite crystals to a reaction solution **narrowed size distribution**.[1)]
(2) **Spherical shape** (which improves homogeneity of particle surfaces and enhances dispersibility) was attained by adding a disaccharide to the reaction solution. [2)]
(3) **Hollow structure** (usable for DDS and ultrasound contrast agent) was attained by coating silica spheres (a template) with ferrite and dissolving the template. [3)]
(4) Specific **proteins were immobilized** onto FNPs **directly during their syntheses** from the reaction solutions containing the proteins. This opened the door to immobilize protein-tagged molecules on FNPs during their synthesis (left figure).
(5) The proteins were **strongly bonded** to FNPs **intermediated by specific amino acids and the analogues** composing the proteins. Such molecules have **two carboxyl groups, which make chelate bonds** onto ferrite surfaces (right figure) as FT-IR analyses revealed. The molecules work as **adapter molecules** for conjugating specific biomolecules to FNPs and will be utilized as **tags to recognize FNPs**.[4)]

[1] R. Shimazu et al., Joit MMM-Intermag Conf., Baltimore, USA, (2007), FF-05.
[2] T. Tanaka et al., The 1st Int. Symp. Advanced Magn. Mater., Jeju, Korea (2007), QD03.
[3] T. Kanemaru et al., MMM. Conf., Tampa, USA (2007), AE-10.
[4] K.Nishio et al., Colloids and Surfaces B, 54 (2007), 249.



Avidin-tagged fluorescent material was immobilized onto magnetite particles during their synthesis from the aqueous solution added with the fluorescent material (left). Biomolecules are immobilized onto ferrite surfaces strongly intermidiated by the adapter molecules via chelate bonds (right).

Fabrication of a thin Fe_3O_4 magnetic nanofilm on silica titania for bioapplications

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Among the nanoparticles, iron oxide nanoparticles have attracted a considerable attention during the last decade and they have been of great interest in many important technological applications. The use of magnetite nanoparticles in clinical medicine is important and has considerable promise for applications in the biomedical and diagnostic fields. Also magnetic nanoparticles may help to resolve many separations problems.

Therefore we synthesized stable Fe_3O_4 nanoparticles by a chemical method and a layer of Fe_3O_4 magnetic nanoparticles was deposited on a hydrophilic $Si(Ti)O_2$ surface. The kinetics of desorption of the stable magnetic nanoparticles was measured on the controlled conditions using optical waveguide lightmode spectroscopy (OWLS), with which the number of deposited particles could be accurately calculated.

Whereas previous investigations were restricted to a very narrow range of solution conditions in which the particles were stable, here we have directed our attention to particles whose suspension is robustly stable in practically useful buffered of salt solutions.

Size Regulation Effect in Stabilization of Non-Polar Organic Ferrofluids with Mixtures of Mono-Carboxylic Acids

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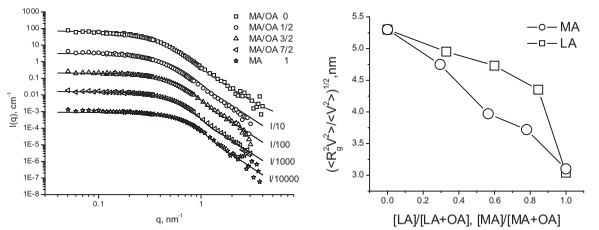
As we have shown previously [1] short chain length mono-carboxylic acids, such as lauric (LA) and myristic (MA) acids, can be effectively used for the synthesis of highly stable magnetic fluids in organic non-polar media. In comparison with the classical oleic acid (OA) the shorter surfactants stabilize magnetite particles of smaller size and reduced polydispersity. Taking into account that the considered surfactants are well miscible in bulk liquids, we expected that a similar property would take place at the interface with magnetite particles.

Ferrofluids with stabilization based on single coating of magnetite in several non-polar organic carriers (cyclohexane, decahydronaphtalene DHN) were prepared like in the previous work [1] with the only difference that instead of one surfactant, mixtures LA/OA and MA/OA were used. The procedure results in highly stable ferrofluids, which were characterized by the magnetization analysis and small-angle neutron scattering (SANS). Changes in the size and polydispersity for the stabilized magnetite were observed when varying the content of LA and MA in the mixtures. Example is given in Fig.1, where SANS curves (non-polarized neutrons) reflect the change in the characteristic magnetite radius (comprising both the mean radius and polydispersity) for 3 % ferrofluids in DHN. One can conclude that it can be varied within interval of about 3-5.5 nm. In the case of the MA/OA stabilization a linear dependence of this radius on the MA relative content shows full miscibility of MA and OA at the magnetite surface, while for LA/OA worse miscibility is detected.



(*a*) (*b*)

Fig.1. Size regulation effect from SANS data for ferrofluids with LA/OA and MA/OA mixtures. (a) Example of the scattering curves for MA/OA samples with different MA content. For convenient view curves are divided by some coefficients indicated at the right; (b) Characteristic particle radius for LA/OA and MA/OA samples as a function of the LA and MA contents.

The work is done in the frame of the project Helmholtz-Russian Joint Research Group (RFBR-Helmholtz - HRJRG-016).
This research project has been also supported by the European Commission under the 6th Framework Program through the Key Action: Strengthening the European Research Area, Research Infrastructures. Contract nr: RII3-CT-2003-505925, GKSS, Germany, as well as by the CEEX research program of the Romanian Ministry of Education and Research, contract NanoMagneFluidSeal nr.83/2006.

[1] M.V.Avdeev, D.Bica, L.Vékás, et al., *J. Mag. Mag. Mater.* 311 (2007) 6-9.

Surface Modification of Monodisperse Magnetic Nanoparticles for Biomedical Applications

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We studied the preparation of surface modified magnetic nanoparticles using poly(ethylene glycol) (PEG) or carboxymethyl-dextran (CMDx) chains covalently attached to the particle surface using carbodiimide chemistry, preventing polymer de-sorption in subsequent applications. In this method, particles are synthesized by thermal decomposition using oleic acid and oleylamine as surfactants that are later exchanged by 3-aminopropyl trimethoxysilane (APS) to render particles with reactive amine groups (-NH_2) on their surface. Amines are then reacted with carboxyl groups (-COOH) in mPEG-COOH or in the CMDx using 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) and N-hydroxysuccinimide (NHS) in water. mPEG-COOH was obtained by converting the hydroxyl end group in methoxy-PEG into carboxylic acids using Jone's Reagent. CMDx was obtained from a commercial source. Particles size was characterized by Dynamic Light Scattering (DLS), SQUID magnetic measurements, and Transmission Electron Microscopy (TEM). Stability of the particles in water was studied as a function of pH and ionic strength using Zeta Potential Measurements. The method produces highly stable suspensions of magnetic nanoparticles in water based systems that make them suitable for biomedical applications such as magnetic fluid hyperthermia and magnetic resonance imaging.

Functionalization of magnetic nanoparticles with organosilanes

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For biomedical applications, the surfaces of magnetic nanoparticles need to be functionalised, i.e., covered with organic molecules that enable the attachment of different biomolecules to their surfaces. At the same time, a layer of functionalisation molecules on the surfaces of the nanoparticles ensures their stability in a colloidal suspension. The organic functionalisation molecules should bind with strong covalent bonds to the surface of the nanoparticles.

For amino functionalisation aminopropyltriethoxysilane (APS) can be used. In the presence of water the silane ethoxy groups of the APS molecules hydrolyse to reactive silanol species, which can react with the surfaces of the nanoparticles or between themselves thus forming oligomers. The procession of these two competing reactions, which decisively defines the nature of the functionalisation layer, depends on the chemical nature of the nanoparticle surfaces and on the experimental conditions used during the silanization. The surfaces of the oxide nanoparticles are chemically relatively inert and usually do not allow strong covalent bonding. In order to enable the strong surface bonding of organosilane molecules, the nanoparticles are usually coated with a thin layer of silica. Silica provides the surface OH-groups, which can condensate with the silanol groups of the silane molecules to form strong covalent Si-O-Si bonds.

In this study, the reactions occurring during the functionalisation of superparamagnetic maghemite particles with APS in aqueous suspensions were systematically studied as a function of different experimental conditions, such as the concentrations of the reactants, pH, temperature, etc. The APS molecules were grafted directly onto the surfaces of the maghemite nanoparticles, or onto the nanoparticles embedded in a thin (~ 2 nm) silica shell. The surface properties of the nanoparticles were characterized with FTIR, electro-kinetic measurements, and with measurements of the surface amine concentration using conductometric titrations.

Characterization of the dipolar chains formed by cobalt nanoparticles

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Chemically synthesized magnetic nanoparticles with controllable size and shape have found a range of biological applications from imaging to cancer treatment. Due to their strong dipolar interaction, these nanoparticles tend to assemble with and without an external magnetic field. A better understanding their assemblies would facilitate their applications. Here, we present the synthesis of 10 nm sphere-shaped and 50 nm cube-shaped cobalt (Co) nanoparticles using thermo-decomposition in 1,2-dicholorbenenze (DCB) and the formation of the dipolar chains by these nanoparticles with and without an external magnetic field. The results from x-ray powder diffraction (XRD) show that these two different shaped nanoparticles adopt the same epsilon Co crystalline structure. Transmission electron microscopy (TEM) was used to characterize the morphologies of these nanoparticles and their dipolar chains. A superconducting quantum interference device (SQUID) magnetometer was used to measure the magnetic properties of the Co nanoparticles in DCB. A series of sequential magnetic moment vs. temperature measurements were performed to understand how the dipolar chains formed by 10 nm spherical Co nanoparticles in DCB change under the influence of external magnetic fields of different magnitudes. When Co nanoparticles synthesized in DCB are purified and re-dispersed in ethanol or toluene, the results from the magnetic measurements and high-resolution TEM show the formation of cobalt (II) oxide (CoO) at the surface of Co nanoparticles. Thus the dipolar chains formed by Co core/CoO shell nanoparticles were observed in the colloidal solution.

Fabrication of Fluorescent-Magnetic Bifunctional Nanoparticles

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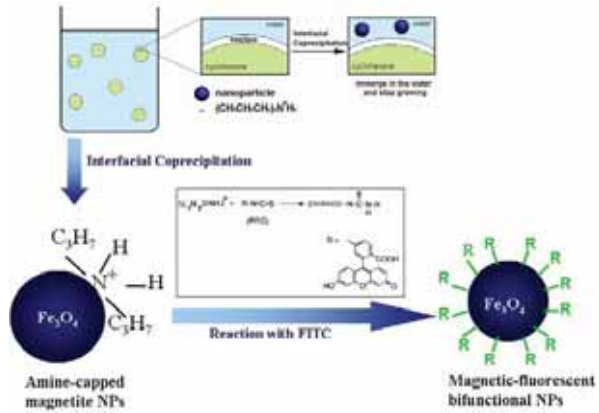
Magnetic-fluorescent bifunctional nanoparticles (NPs) have attracted much intension recently because they are supposed to possess wider potential biomedical applications than mono-functional nanoparticles. Considerable efforts have been devoted to synthesizing variable kinds of bifunctional nanoparticles, among which magnetic/quantum dot biphase nanoparticles is the most popular one. However, another strategy to prepare bifunctional nanoparticles, which is based on the binding of organic fluorescent dye with magnetic nanoparticles, should not be neglected. The combination of organic fluorescent dye and magnetic nanoparticles has been performed by many researchers. For example, the binding between iron oxide and FITC can be obtained by dextran labeled dye or APS modified magnetic nanoparticles.

Recently we proposed a facile interfacial coprecipitation method to prepare magnetite nanoparticles (MNPs), on which ammonium salt molecules were anchored. We herein present a novel fabrication take advantages of these ammonium salt molecules on the MNPs to fabricate the magnetic-fluorescent bifunctional NPs. The approach includes two steps: the synthesis of amine-capped MNPs by interfacial coprecipitation, and the combination of MNPs and FITC molecules. By this approach, MNPs/FITC bifunctional nanoparticles were prepared by utilizing ammonium salt as a coupling agent. The results can confirm not only the existence of amine molecules on the surface of nanoparticles, but also the possibility to modify MNPs via these amine molecules. In addition, the fluorescence intensity of resultant bifunctional nanoparticls is direct related to the amount of ammonium salt molecules capped on MNPs.



Schematic representation of fabricating fluorescent-magnetic bifunctional nanoparticles

Magnetic properties of water based magnetite-citrate-ferrofluids with high particle concentrations

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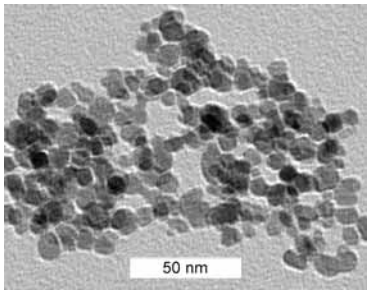
Water based ferrofluids are used for a variety of different medical and technical applications. For a lot of them it is advantageous when the fluids show a high magnetic particle concentration. Due to the increasing particle interactions in higher concentrated ferrofluids the agglomeration rate increases and this causes a decrease of the stability against sedimentation. To solve this problem, in the present investigation a ferrofluid with a high particle concentration as well as a good sedimentation stability was prepared and characterized.

The primary particles (maghemite/magnetite) were prepared by means of co-precipitation in alkaline medium (ammonia) starting from Fe^{2+}- and Fe^{3+}-solutions. These particles were complected by citric acid and stabilized as a magnetite/maghemite-hydrosol in the pH range from 6.5 to 8. By-products and excessive citrate were washed out with distilled water.

During the preparation procedure two different fractions were formed: a highly concentrated and partially aggregated sediment and a lower concentrated supernatant ferrofluid. The volume concentration of particles in the supernatant was increased up to 10 vol.% by removal of water. Present aggregates in the fluid were partially redispersed by ultrasonic treatment and the remainig aggregates were removed by filtering with glass wool and centrifugation at 3000g .



Typical TEM image of a prepared sample.

In TEM investigations the core size of the particles was determined in the range from 4 to 15 nm which is in good agreement with a mean diameter of 9 nm measured by XRD. The first peak of the mean volume weighted hydrodynamic diameter was calculated at 10 nm from PCS data by means of non-invasive back-scatter technology (NIBS®).

For the saturation magnetisation of the fluid a value of about 31 Am^2/kg was determined by VSM. From this value a mass weighted particle concentration of about 41 m.% was derived. Due to the very low coercity of the immobilized particles (H_C = 0.07 kA/m) and a low relativ remanence (M_R/M_S = 0.0011) superparamagnetic behaviour of the particles could be expected. This allowed the determination of a mean particle diameter of 7.5 nm from the magnetic data by using the Chantrell method. The specific heating power of the particles for different volume concentrations (from 10 vol.% to 0.5 vol.% through dilution by pure water) was constant 50 W/g (at f = 400 kHz and H = 10 kA/m). This fact confirms the assumption that the particles are very well dispersed in the fluid and therefore, a very low amount of agglomerates only is present in the ferrofluid.

Potential applications of the prepared high concentrated, colloidal stable water based ferrofluid are drug targeting, hyperthermia, particle separation for diagnostics and the float-sink-technology, respectively. However, for magnetic heating applications the specific heating power of the fluids has to be increased in future work.

Biomineralized and Bio-inspired Magnetic Nanoparticles

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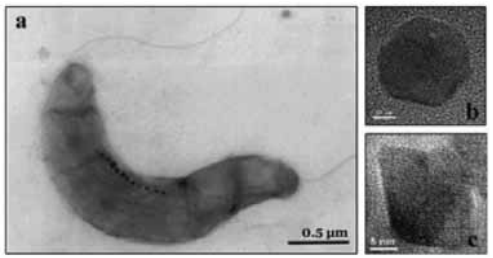
Magnetic nanoparticles (MNP) are key components in the development of many novel bio- and nanotechnological applications. One of the most intriguing natural examples of MNP is provided by magnetosomes, which are magnetic nanocrystals embedded in a membrane produced by magnetotactic bacteria. Magnetosomes have an entire set of unique properties that them superior to abiotic particles. Firstly, they have uniform dimensions (40 to 100 nm depending on the bacterial strain) encompassing magnetic single-domain size, and unique elongated shapes highlighting their unique magnetic properties, i.e., specifically unmatched remanence and coercivity. Secondly, magnetosomes are chemically pure, and embedded in a membrane, which assure stabilization, even at the nanoscale, and which enables self-organization in a chain. Thus, magnetosomes represent MNP with ideal characteristics and have therefore a long list of potential uses.

The properties of magnetosomes are characteristic of a process called biomineralization, in which the organism exerts a strict control over the nucleation and growth of the mineral. This control is mainly due to a set of specific magnetosome proteins encoded by genes clustered within the so-called genomic magnetosome island. Thus, biomineralization of magnetosomes is a unique process, which until now could not be replicated by purely inorganic means. However, despite the fact that more than 30 years have elapsed since their discovery, there is a lack of convergence between interdisciplinary fields related to magnetotactic bacteria that needs to be overcome in order to better understand how these organisms function, so that we can develop methods to produce more magnetosomes, or to find alternative routes to produce magnetosome-like nanoparticles.

Specifically, we present a new route for the biomimetic synthesis of magnetosomes-like MNP in a high-yield low-cost and controlled manner. It includes the use of synthetic key peptides derived from biological determinants of magnetosome formation in magnetotactic bacteria. This technique enables the formation of size- and shape-tailored MNP comparable to magnetosomes and significantly differing from the MNP obtained by purely inorganic syntheses or syntheses using non-specific proteins and / or peptides. Thus, we believe that this technique will enable the use of those MNP in ferrofluids with application in bio- and nanotechnologies.



Micrograph images of a- a magnetotactic bacterium, b- an isolated magnetosome, and c- a purely inorganic magnetite nanocrystals. Our synthetic route enables to reduce the size difference between biogenic and abiogenic nanoparticles.

Magnetic nanoparticle encapsulated within a biocompatible thermoresponsive polymer

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Magnetic nanoparticles (MNP) have unique size-dependent properties and MNP based on iron oxides are attractive candidates in the field of biomedical applications [1]. For *in vivo* applications, MNP should not form any agglomerates; and to prevent this, their surface is usually modified by either coating or encapsulating them in organic (polyelectrolytes [2]) or inorganic (silica [3]) materials. Thermoresponsive polymers have attracted much attention as they possess a release-trigger mechanism when they undergo fast, reversible structural changes from a swollen to a collapsed state by expulsing the solvent, and have recently been exploited as remote controlled drug delivery vehicles [4].

This study describes a facile two-step approach to modify the surface of nanoparticles with a coating of thermoresponsive biocompatible polymer, hydroxypropyl cellulose (HPC) [5], using a coupling agent to covalently bind the core to the shell. HPC is known for its biocompatibility and biodegradability, and its thermoresponsive properties make it an excellent candidate for fabricating biocompatible stimuli-responsive MNP. We report the synthesis of MNP and the successful binding of the polymer to them. X-ray diffraction studies show that the surface modification of the MNP does not result in any phase change and the size of the magnetic core calculated (7 nm) reveals that such hybrid core-shell system is superparamagnetic in nature, as further confirmed by magnetization measurements. The size obtained by X-ray diffraction is in good agreement with that obtained by transmission electron microscope. Evidence of binding is provided by Fourier Transform Infrared spectroscopy and a quantitative analysis of the polymeric content obtained by thermogravimetry analysis. Dynamic light scattering as a function of the temperature shows the thermoresponsive behaviour of the particles [6] with a lower critical solution temperature around 41 °C, which is also the temperature at which cellulose undergoes a coil-to-globule transition.

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Tuning of the magnetic moment of nanoparticles in microcompartments

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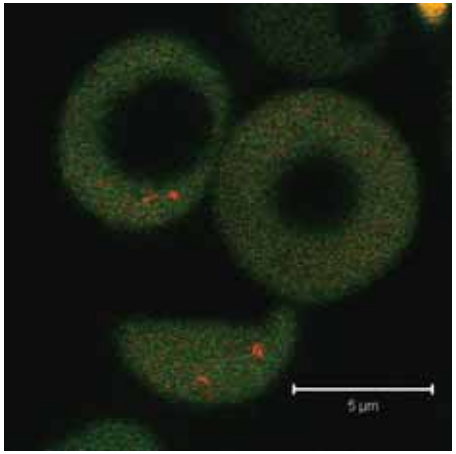
Red blood cells (RBC) are an ideal model system to investigate cell membrane properties. Recently they also gained increasing interest as natural carriers for drugs, enzymes and nanoparticles. Additionally, their surfaces can be modified with specific antibodies for targeting.



Taking advantage of the tuneable membrane permeability erythrocytes were loaded with nanosuspensions of biocompatible, surface tuneable, luminescent, and magnetic nanoparticles. The simplicity of these cells allows to observe the behaviour of nanoparticles in confined compartments at different conditions of the environment or in the presence of external fields. For example nanoparticles coated with stimuli responsive polymers can be forced to form reversible aggregates by changing the temperature.

Acknowledgements: The financial support of the European Union (EFRE–ProFIT–10134275) is acknowledged.

Facile Synthesis of Multifunctional Magnetic Nanoparticles for Drug Delivery, Targeting, and Imaging

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Nanoparticle formulations of nanocrystalline materials present unique opportunities for improved medical imaging and diagnostics via optical, fluorescence, and magnetic resonance imaging modalities. The common requirements of these applications are biocompatibility, high payload of contrast agent, precise control of particle size and surface functionality, which allow for selective localization of contrast agents at levels sufficient to provide enhanced detection in vivo. In this work, we present a novel technology - Flash NanoPrecipitation - a controlled precipitation process that produces stable nanoparticles at high concentrations of encapsulated components using amphiphilic block copolymers to direct self-assembly. Uniform particles with tunable sizes from 50-500 nm can be prepared in an economical and scalable manner. The key to the process is the control of time scales for micromixing, self-assembly, and nucleation and growth. The diffusion-limited assembly enables particles of complex composition to be formed. Using this technology, pre-formed inorganic nanocrystals can be incorporated within biocompatible block copolymer core/shell-type Composite Nanoparticles (CNPs). Because the CNPs assemble spontaneously from solution by simultaneous desolvation of nanocrytals and amphiphilic copolymer components, explicit surface functionalization of the nanocrystals is not required, and the method can be applied to a variety of nanocrystals that lack appropriate conjugate surface chemistry. Examples of CNP formulations with superparamagnetic iron oxide nanocrystals as enhanced T_2 contrast agents for magnetic resonance imaging will be presented. In addition, the combined incorporation of nanocrystals with drugs into a single nanoparticle will be demonstrated, enabling simultaneous drug delivery and medical imaging. Finally, the functionalization CNP surfaces with disease-specific targeting ligands allows for directed delivery of the carriers. The technology provides a comprehensive and highly flexible platform for tailored preparation of multifunctional nanomaterials.

Synthesis and Properties of Hydrophobic Magnetite Nanoparticle Fluids for Use in Biomaterials

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Magnetic, biocompatible, polydimethylsiloxane (PDMS) ferrofluids are under investigation as internal tamponades for treating ocular disorders such as detached retinas.[1] Previous reports have described ferrofluids comprised of PDMS-magnetite nanoparticle complexes dispersed in PDMS carrier fluids.[2] However, concern for the long-term stability of these magnetic nanoparticle dispersions has lead to research efforts to improve the design of the hydrophobic magnetic fluids.

Advancements in the synthesis of PDMS-magnetite complexes has allowed for the formation of one-part fluids, where the well-defined magnetite complexes do not require the incorporation of a PDMS carrier fluid. These PDMS-magnetite nanoparticle complexes were prepared by adsorbing tricarboxylate-functional PDMS onto cationic magnetite surfaces via a high-shear process. Rapid agitation of the coprecipitation/interfacial adsorption reaction has been shown to decrease the initial particle size distributions of the complexes. Magnetic separation techniques have been developed to further narrow the particle size distributions of the magnetite complexes while retaining control of the surface concentration of PDMS. The particle sizes and distributions of the resulting one-part PDMS-magnetite ferrofluids have been examined using TEM. Different molecular weight PDMS-tricarboxylates (3,000 g mol^{-1} and 7,000 g mol^{-1}) have been studied as stabilizers for the PDMS-magnetite complex fluids.

Recently, novel hydrophobic magnetite nanoparticle fluids have been synthesized using a poly(1,2-butylene oxide)-tricarboxylate stabilizer. These ferrofluids were synthesized using the approach developed for the PDMS-magnetite nanoparticle fluids. DLS and TEM characterization of these fluids show narrow particle size distributions. The hydroxyl endgroups on the poly(1,2-butylene oxide) stabilizer chains may allow for further functionalization or crosslinking of the hydrophobic magnetite nanoparticle fluids.

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Magnetic Nanoparticles as Imaging Agents for Magnetic Particle Imaging

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Iron oxide based nanoparticles with different sizes and surface coatings are frequently used as in-vivo contrast agents in Magnetic Resonance Imaging (MRI). Today, several different formulations of superparamagnetic iron oxide nanoparticles are approved for human use such as Resovist® or Endorem® for e.g. liver cancer imaging. Magnetic particles can also be used for targeted imaging of pathologies or for tracking of labeled cells in-vivo using MRI. The disadvantage of MRI is its poor sensitivity and the lack of providing quantitative information on the amount of iron oxide present. Recently a new imaging method was invented by Philips, called Magnetic Particle Imaging (MPI), that allows to visualize magnetic particles directly with a superior sensitivity [1,2]. This new imaging methods may be much better suited for targeted imaging or stem cell tracking than MRI. While MRI detects iron oxide based contrast agents only indirectly by visualizing the contrast agents induced changes of the magnetic properties of water, magnetic particle imaging allows visualizing magnetic particles directly. The signal-to-noise ratio in MPI depends to a big extend on the magnetic properties of the used particles.

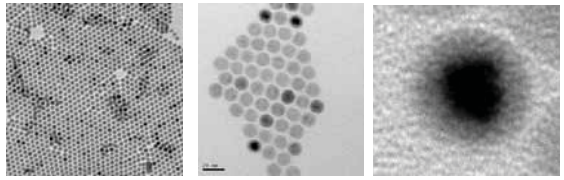

Figure: TEM and high resolution TEM of magnetic particles. Particle diameter is around 14 nm. A core /shell structure is visible.

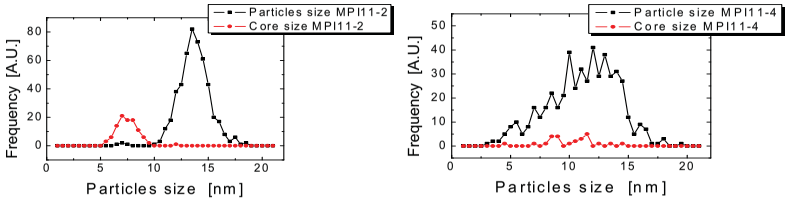

Figure: Particle and core size determination of magnetic particles from TEM images before and after oxidation with trimethylamine N-oxide.

Superparamagnetic iron oxide nanoparticles are synthesized through thermal decomposition of iron(*II) or (III*) oleates and myristates respectively at temperatures 300 < T/ °C < 350 in organic solvents with high boiling points (e.g. 1-octadecene, n-eicosane or docosane). During this synthesis iron oxide particles with a core/shell structure are formed. The core consists of magnetic Fe_3O_4 or γ-Fe_2O_3 while the shell is a non-magnetic amorphous iron hydroxide. The overall particle diameter, the core diameter as well as the respective crystal structures were determined by TEM. Particle sizes determined by fitting the Langevin equation to data obtained with vibrating sample magnetometer (VSM) are considerably smaller than the overall particle diameter and were found to correspond to the size of the core. After a post-synthesis oxidation step of the particles using trimethylamine N-oxide, core/shell structures are no longer observed and the particles appear homogeneous. MPI measurements show an improvement in the signal especially at high frequency harmonics of remagnetization response, which is linked to the increased size of the magnetic domain.

[1] B. Gleich, J. Weizenecker, Nature 435, 1214 (2005).
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Preparation of ferrite hollow spheres (340 nm diameter) encapsulating anticancer drug and fluorocarbon for DDS

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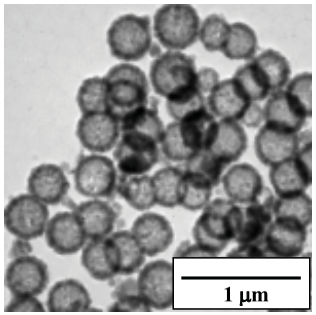
Ferrite hollow spheres (FHSs) are expected to be used as magnetic carriers for DDS and/or ultrasound contrast agencies when drug and/or gas are encapsulated in the hollows. FHSs so far reported were composed of ferrite nanoparticles which were bonded to each other by binders [1] or sintering process [2]. Recently we successfully prepared FHSs self-standing without binders or sintering [3]. Silica spheres were coated with a continuous ferrite layer by ferrite plating, an aqueous process which synthesizes crystalline spinel layers at $T < 90$ ºC [4], and the template of the silica spheres was dissolved.

In this study we improved yield and in-water dispersibility of FHSs by replacing the template from the conventional silica spheres conjugating OH group on their surfaces with those conjugating SH group. We deposited magnetite layer on the SH conjugated silica spheres (300 nm in diameter), which were dissolved by a NaOH solution. All processes were performed at 60 ºC. The resultant FHSs were completely spherical in shape and monodisperse, 340 ± 40 nm in diameter, as shown in the figure. As compared to our previous FHSs, defective FHSs decreased in number and in-water dispersibility improved. Utilizing vacuum exchange technique and liquefaction of fluorocarbon, we successfully introduced liquid $C_5H_2F_{10}$ (a fluorocarbon) with 53 ºC boiling temperature and cisplatin (an anticancer drug) into the hollows. The cisplatin was contained at 2.9 x 10^{-18} mol/FHS, which was released by heating the FHSs to evaporate $C_5H_2F_{10}$.

[1] C. C. Wang et al.: J. Magn. Magn. Mater. , **304** (2006), e451.
[2] F. Caruso et al.: Chem. Mater. , **13** (2001), 109.
[3] T. Kanemaru et al.: *MMM Conf., Tampa, USA* (2007), AE-10.
[4] M. Abe et al.: Jpn. J. Appl. Phys., **22** (1983), L511.



TEM image of ferrite hollow spheres

Synthesis of Thermal Seeds for Magnetic Fluid Hyperthermia by pH Stabilized Coprecipitation Technique

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The specific heat absorption rate (SAR) of the thermal seeds for magnetic fluid hyperthermia (MFH) depends on their size and size distribution. Though magnetite nanoparticles (MNPs) with considerable heating potential under ac magnetic field conditions have been attempted using thermal decomposition, oxidation and co-precipitation (CP) techniques, the control over their size and size distribution is yet to be achieved. Among the above techniques, CP is preferred over the others due to controllability of size over a wide range and scalability. In this study, MNPs were synthesized by pH stabilized CP technique using buffer solution composed of hydrogen carbonate and sodium hydroxide and their suitability for MFH was investigated by evaluating physical and DC and AC magnetic properties. The average particle diameter (Dm) decreased for any increase in the reaction pH and the magnetic properties such as saturation magnetization (Ms) and coercivity (Hc) values also confirmed the above trend (Fig. 1). The maximum heating rate was recorded for the MNPs synthesized at pH 10, measured in an AC magnetic field strength and frequency of 3.2 kA/m and 600 kHz. The Dm, Ms and Hc of the above sample were 13 nm, 63.7 emu/g and 6.4 Oe, respectively. The SAR of this sample was 9.1 W/g. The TEM photograph of the above sample suggested that large fraction consisted of particles with diameters larger than 13 nm (Fig. 2) and the size distribution was narrower than particles synthesized by the conventional CP technique. However, DC and AC magnetic properties suggested that the size distribution of the sample was bimodal and the average diameter of smaller particles is around 13 nm. The modification of the synthesis technique to inhibit the formation particles with diameters larger than 15 nm is in progress.

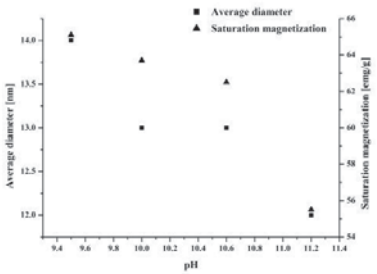

Fig. 1 Dm and Ms of magnetite particles synthesized under various pH.

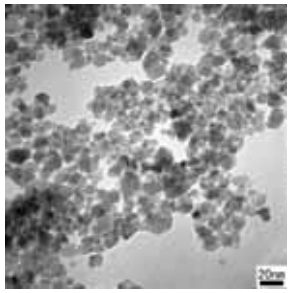

Fig. 2 TEM micrograph of magnetite particles synthesized by coprecipitation method at pH of 10.0

Structural defects induced magnetic anisotropy Fe over layer on Pt (110) surface.

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Recently many experimental reports on the magnetic films have shown that the film morphology including their structures strongly affect the magnetism of the system. Our study the magnetic properties on highly ordered equiatomic Fe over layer on Pt (110) 1x2 reconstruction surface. Most studies now agree that the clean Pt(110) surface shows the missing raw structure along the (1-10) direction, so that the size of the unit cell in the [001] direction is double that of the bulk lattice and film growth on top of this surface will results in the directional growth due to the strong lattice mismatch in a single direction which created thick microtwin along [110] direction and antiphase boundaries along [001] on Fe/Pt(110) systems due to external field, these can change the spins pinning site and deepening sites density. We observed low energy electrons diffraction (LEED), surface magneto optical Kerr effect (SMOKE) to show the interesting behavior of anisotropy of this Fe over layers on Pt (110). In plane SMOKE signal for different angles shows different coercivity. In this experiment shows large anisotropy energy along [110] direction and low anisotropy energy along [001] direction. It is remarkable that all direction, hysteresis loops are square shape. We also observed 1x1,2x1,1x2 phase using LEED on Fe/Pt(110) thin film due to annealing T<550K ,T>550K and T>600K. One of the interesting issue in this Fe/Pt(110) 2x1 system that the SMOKE signal has reflected low relative remanence (Mr) and high coercivity (Hc) than another two phase at easy axis low relative remanence (Mr) and low coercivity along hard axis. At low temperature Fe/Pt(110) system shows some exchange bias.

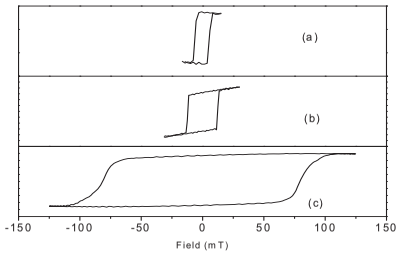

FIG.1.Fe10ML on Pt(110) surface (a) In plane SMOKE signal at RT 90 degree azimuthally from [110] direction (b) 70 degree (c) along [110]direction

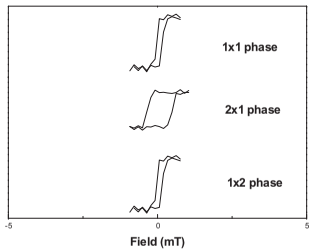

FiG.(2) Fe1.4ML along easy axis.

Structure and Magnetic Properties of γ-Fe_2O_3–SiO_2 Nanocomposites Obtained by Sol-Gel Approach

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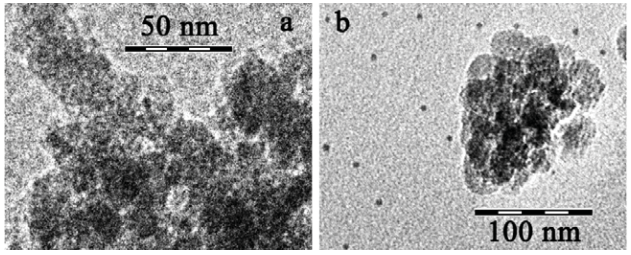
Films and xerogels of γ-Fe_2O_3–SiO_2 nanocomposites have been studied. The samples were obtained by mixing separately prepared sols of SiO_2 and $Fe_3O_4 \cdot nH_2O$. They represent clusters of Fe_3O_4 or γ-Fe_2O_3 or α-Fe_2O_3 in an amorphous SiO_2 matrix that depends on annealing temperature. Porous structure of SiO_2 was attained by adding highly dispersed SiO_2 powder into the SiO_2 sol.

X-Ray Diffraction (XRD), Transmission Electron Microscopy (TEM), IR-spectroscopy and Electron Paramagnetic Resonance (EPR) techniques were used to reveal the peculiarities of the Fe_2O_3–SiO_2 nanocomposite structure depending on Fe_2O_3 concentration (0.1-12 wt. %), annealing temperature and conditions of the SiO_2 sol preparation.

Magnetic properties of the γ-Fe_2O_3–SiO_2 thin films deposited onto a glass-ceramic substrate were studied by Superconducting Quantum Interference Device (SQUID) measurements in temperature range of 2-300 K in field cooling (FC, 100 Oe) and zero-field cooling (ZFC) modes. Temperature-dependent magnetization of the bulk γ-Fe_2O_3–SiO_2 samples (xerogels) was measured in filed cooling-heating mode by the Faraday's method.

The clusters of γ-Fe_2O_3 were found to locate loosely in the SiO_2 porous structure and do not interact with it within Fe_2O_3 concentration range of 5-8 wt. %. Clusters of γ-Fe_2O_3 in the γ-Fe_2O_3–SiO_2 (5-8 % of Fe_2O_3) composite grow from 2 to 5 nm with temperature increasing from 370 to 1170 K. Thermally stimulated Fe_3O_4 → γ-Fe_2O_3 → α-Fe_2O_3 phase transformations were studied. Magnetic transitions in the Fe_2O_3–SiO_2 composites were correlated with their structural peculiarities.



TEM images of the γ-Fe_2O_3–SiO_2 composite annealed at 370 K (a) and 1000 K (b).

Templated electrodeposition of aspect ratio adjusted Ni micro- and nanorods and their magnetically driven diffusion through an extracellular matrix

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Current methods in magnetic drug delivery utilize almost exclusively spherical particles. High aspect ratio magnetic particles may offer increased surface loading of therapeutics and also experience larger forces for a given field gradient, as compared to spherical particles of comparable diameters. We seek to study these potential advantages by using an in-house engineered high throughput magnetic micro-rheometer (Magnetic High Throughput System, MHTS) and applying forces to arrays of these particles suspended in an extracellular matrix phantom.

Templated electrodeposition is an inexpensive, convenient, and versatile method for fabricating magnetic particles, rods, and wires. Specifically, we use templated electrodeposition to grow Cu/Ni multilayered rods and selectively etch Cu portions of these rods, leaving only Ni nanorods. These rods have specific diameters, based on the template pore dimensions, and specific lengths, based on the deposition duration and potential. We use both Synkera, Inc. templates, as well as Whatman Anodisc AAO templates to grow rods ranging from 55nm to 300nm in diameter and aspect ratios from 1 to 20+. This method also allows for compositionally-varying rods and these have been demonstrated.

Novel to the field of magnetic micro- and nano-carrier research is our ability to observe single particles move through extrallular matrix (ECM) phantoms (20% Engelbreth-Holm-Swarm murine sarcoma in Dulbecco's Modified Eagle's Medium) as AC or DC magnetic field gradients are applied. The ECM, and particularly collagen, is cited as a primary inhibitor to particle transport within a tumor interstitium. Therefore, understanding how particulate transport occurs and what physical particle properties lead to more efficient drug carriers is essential for increasing the efficacy of magnetic drug targeting. In-house video spot tracking software is used to determine particle motion with respect to time and this information is then correlated to magnetic field action applied by the MHTS. Using these systems we apply a variety of AC and DC forces to these micro- and nano-rod Ni particles and observe how particle properties (diameter, aspect ratio) affect the efficacy of particulate motion.

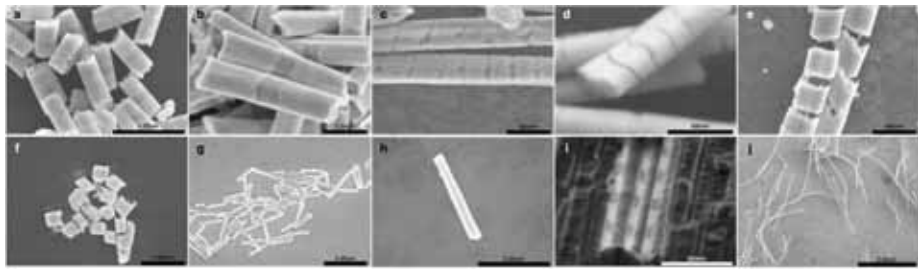

Figure 1: (a) and (b): short and long Au-Ni-Au rods, (c) and (d) Cu/Ni wires grown from a single electrolyte via pulsed electrodeposition, (e) and (f) Rods grown from a single electrolyte after 8M KOH Cu etching, (g) and (h) longer Ni rods, (i) and (j) 55nm diameter rods grown in AAO membranes (Synkera, Inc.). Rods in images (a) through (h) were grown in Whatman Anodisc 13 AAO membranes with nominal pore size of 200nm.

Silica-Coated and Amine-Derivatized Hybride Perovskite Nanoparticles for Medical Applications in MFH and MRI

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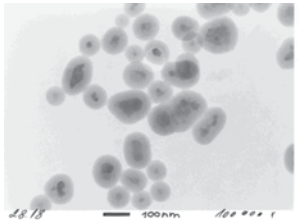
$La_{0.75}Sr_{0.25}MnO_3$ (LSMO) manganese perovskite appears to be new material for magnetic cores favourable for medical applications, particularly for Magnetic Fluid Hyperthermia (MFH) and in Magnetic Resonance Imaging (MRI). In contrast to the currently used iron oxides the synthesis is, however, more complicated. It includes sol-gel processing, thermal and mechanical treatment (rolling and milling), separation by centrifugation followed by a suitable encapsulation in order to ensure water colloidal stability of the cores, to suppress the toxicity caused by the presence of manganese ions and to enable labelling with appropriate targeting ligands.

Two approaches were used:

- nanoparticles stabilized in water by citric acid were coated by silica shell using tetraethoxysilane (TEOS), see Fig. 1
- nanoparticles stabilized in water by poly(vinylpyrrolidone) were coated by a complex shell using TEOS and 3-aminopropyltriethoxysilane (APS).

TEM evidenced well dispersed embedded particles, silica character of the shell was confirmed by IR spectra. The density of amine groups at the accesible surface was determined by the photometric analysis, concentration of nanoparticles in the stabilized suspension related to the content of Mn ions was measured by AAS. Comparative measurements of the heating efficiency of the coated and uncoated particles were carried out.



LSMO embedded by silica

Efficiency of the labelling procedure and viability of the cells in the presence of the LSMO@SiO_2 particles at concentrations around 0.1 mM_{Mn} were tested with the culture medium of rat mesenchymal stem cells for an incubation period of 48 hours. Cell viability in the media containing LSMO@SiO_2 particles varied between 50 % and 70 %, whereas Feridex® labelled cells reached 94 % and unlabelled cells 97 %. In the light of our previous results obtained on "poorly" coated particles (viability < 5 %) a substantial improvement of the survival rate due to the coating was evidenced. According to the relaxivity measurements the estimated amount of manganese inside the cells is relatively low (0.24 pg Mn/cell) compared to the amount of iron in the case of Feridex® loaded cells (14.6 pg Fe/cell) but due to high T_2 relaxivity of the LSMO nanoparticles and probably low aggregation of the particles inside the cells, the LSMO labelled cells provide even better contrast than the Feridex® labelled ones. T_2 relaxation rate of the LSMO labelled cells was 18.1 s^{-1}/million of cells compared to Feridex® labeled cells revealing T_2 relaxation rate 17.3 s^{-1}/million of cells.

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Development and properties of Fe-Co ferrite nanoparticles

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Small magnetic particles are of a big interest in medicine [1]. In this work, for low-frequency magnetic hyperthermia [2], were produced Fe-Co ferrite nanoparticles with different Co content using co-precipitation procedures [3, 4], and studied static and dynamic magnetic properties of their solid and liquid suspensions. The co-precipitation is performed in water solution of Co and Fe sulphates by alkali [3, 4]. Particles of $Co_xFe^{II}_{1-x}Fe^{III}_2O_4$ (I) and $CoFe_2O_4$ (II) were obtained with 50-100 and 10-30 nm size respectively. Static and dynamic (in an AC field with frequency of 430 Hz and amplitude of up to 1200 Oe) hysteresis loops were provided. The dynamic response of particles I suspension (Fig. 1) and the energy absorption in one magnetization cycle (Fig.2) are crucial influenced of Co content. Relatively small remanence (Fig. 1) indicates the presence of multidomain particles. The particles II are found to be

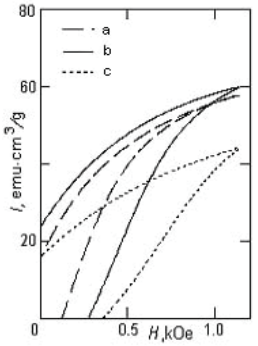

Fig.1. Dynamic magnetization loops of liquid suspensions with Co(II) to Fe(II) ratio 0:40 (a), 1: 40 (b) and 2 : 40 (c)

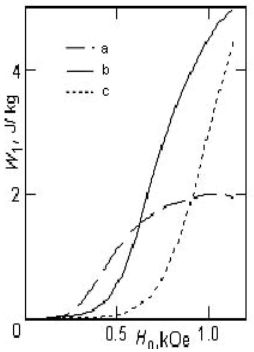

Fig.2. Specific energy absorption in one dynamic magnetization cycle vs. field amplitude for suspension with Co(II) to Fe(II) ratio 0:40 (a), 1: 40 (b) and 2 : 40 (c)

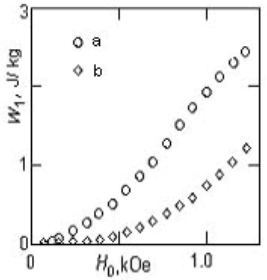

Fig. 3. Specific absorption of energy in liquid (a) and solid (b) Fe-Co ferrites

single-domain with coercivity of about 4000 Oe and to form clusters of 80 nm size. The energy absorption is mainly of viscose nature (Fig.3). It is positioned that the precipitation of silicon dioxide on particles improves stability of magnetic properties of their liquid suspensions.

Support by BRFFR (Projects X08M-140 and X08-257) is acknowledged.

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Synthesis and Characterization of Biocompatible Magnetic Glyconanoparticles

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Due to their variety of applications in the biomedical field, magnetic nanoparticles have generated a great deal of interest in the past few decades. Their magnetic properties and low toxicity make them particularly desirable. Applications in biomedicine include separation and purification of biochemical products and cells, enzyme and protein immobilization, biosensors, diagnosis, contrast agents for magnetic resonance imaging (MRI), targeted drug delivery, hyperthermia treatment of tumours, and tissue repair. Most applications require good chemical stability, narrow size distribution, and uniform morphology. Usually, solubility in aqueous solutions is also required. Carbohydrate coated magnetic nanoparticles were synthesized via the co-precipitation method. Iron (II) chloride and Iron (III) chloride were co-precitpitated out of solution by the addition of ammonium hydroxide in an aqueous solution containing carbohydrate stabilizers such as D-gluconic acid, lactobionic acid and Ficoll® (sucrose polyethers) at 75-80°C. Stable magnetic glyconanoparticles were formed in a simple and direct process. Dynamic light scattering and transmission electron microscopy were used to characterize the surface-coated magnetic nanoparticles. As expected, the polymeric Ficoll® generated much smaller size magnetic glyconanoparticles as compared to the monomeric carbohydrate moieties as stabilizers. In vitro cell viability studies of the magnetic glyconanoparticles using the mouse fibroblast cell lines revealed that biocompatibilities of the glyconanoparticles were similar to those of Ficoll® nanoparticles (see figure 1).

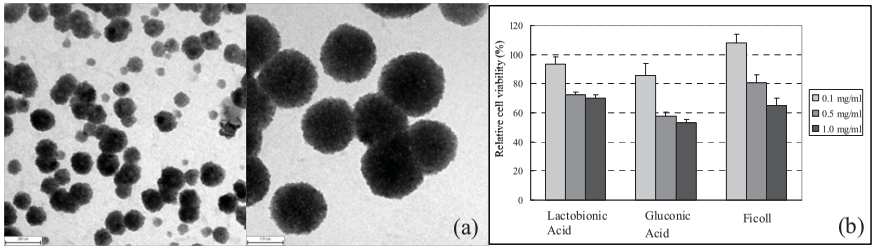

Figure 1. (a) Transmission Electron Micrograph of lactobionic acid stabilized magnetic nanoparticles; (b) Relative cell viability as a function of different concentrations of magnetic glyconanoparticle.

Magnetite Nanoparticles with Active Surface: Synthesis and Surface Chemistry

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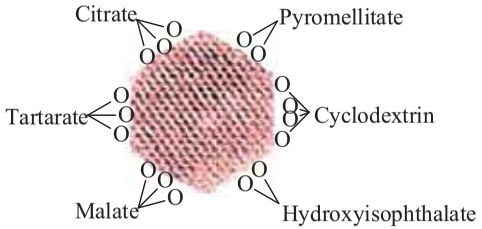
Biomedical applications of magnetic metal oxide nanoparticles require them to form stable aqueous colloids and therefore largely rely on their organic coatings. The function of the coating is to stabilize the particles in colloidal form, prevent them from degradation, to decrease toxicity, and act as a linker for nanoparticles' biofunctionalization; besides, it should be resistant to hydrolysis. The most popular currently used coatings are biocompatible polymers that cloister the nanoparticles, but inevitably bring large diamagnetic contribution and thus decrease the resulting nanocomposites' magnetic response.

In this work we report the synthesis of magnetite nanoparticles with improved morphology and chemically active surface, the studies of their complexation reactions with non-polymeric polyfunctional carboxylic acids, and the studies of their relative aqueous colloidal stability.

Superparamagnetic nanopatricles of magnetite were synthesized by high-temperature solution hydrolysis of iron (II and III) complexes. The injection technique and high temperature provided the conditions for rapid nucleation and consequently, formation of the uniform 5-14 nm nanoparticles. The particles were characterized by powder X-ray diffractometry and transmission electron microscopy. In the next step, the obtained nanoparticles reacted with the multi-functional organic compounds capable of binding to their surface at several sites. The adducts of magnetite with β-cyclodextrin, pyromellitic, 5-hydroxyisophthalic, tartaric and citric acids, were isolated and characterized by IR spectroscopy. Colloid formation and stability studied by Dynamic Light Scattering (DLS) and zeta-potential techniques as a function of pH, showed correlation with the expected binding modes of these compounds. Higher stability was detected for citrate adduct which is due to deprotonation of its coordinated hydroxyl groups, and the lower stability was detected for adducts with cyclodextrin. The unbound OH-groups of hydroxyacids, facing the exterior of the nanoparticles, can be used as sites for adding the protecting shell and the desired functional groups. The nucleophilic properties of these OH-groups are utilized in reactions of new C-O bond formation in new capping ligand synthesis.



Magnetic Iron Oxide Nanopowders Produced by CO_2 Laser Evaporation – 'In Situ' Coating and Particle Embedding in a Ceramic Matrix

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The CO_2 laser evaporation technique is a versatile and capable method for the production of a multiplicity of nanopowders. In this special process, coarse (micrometer…millimeter) starting powders are evaporated in the focus of a CO_2 laser beam (wavelength 10.59 µm, laser power up to 2 kW, focus intensity up to 140 $kWcm^{-2}$) in a continuously flowing condensation gas under atmospheric pressure. Due to the very steep temperature gradient between the hot evaporation zone around the beam focus and the surrounding atmosphere nucleation, condensation and coagulation are proceeding very fast. Thereby ultrafine particles are obtained in a continuously flowing particle aerosol which is separated in a filtering unit. Earlier investigations [1] revealed that this method is well suited for the production of magnetic iron oxide nanoparticles. Two optional methods allow for the conditioning and tailoring of these nanopowders particularly with regard to their magnetic properties:

'In situ' coating: In TEM micrographs of the laser-generated magnetic Fe_xO_y nanopowders, particles ordered in long (several hundreds of nanometers) chain-like structures were frequently observed. In order to stabilize these structures, they can be coated with an organic additive before the powder is separated in the filtering unit. For this, the particle aerosol is led through an oversaturated vapor atmosphere of the coating additive. Here the ultrafine particles act as condensation nuclei for the additive vapor. By heterogeneous condensation, the additive forms a stabilizing layer on the particle chains (Fig. 1) [2,3]. Further investigations are planned on the magnetic alignment of these chains and on the conservation of the alignment. For the alignment, the Fe_xO_y particle aerosol shall be led through the magnetic field of a special designed coil. Subsequently in order to preserve their magnetic alignment, the particle chains shall be coated 'in situ'. It is expected that the stabilized particle chains show a magnetic behavior similar to needle-shaped particles with a high coercivity and remanence due to the high shape anisotropy.

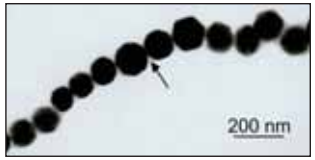

Fig. 1: TEM image of laser-generated Fe_xO_y chains 'in situ' coated with stearic acid; arrow points out the organic layer.

Embedding in a ceramic matrix: First experiments on the co-laser evaporation of hematite and silica were accomplished. Starting from a homogenous mixture of both compounds, the experiments yielded small (diameter about 10 nm) separated Fe_xO_y nanoparticles embedded in bigger spheres (diameter about 40 nm) of amorphous SiO_2 (Fig. 2). The load of the SiO_2 spheres with Fe_xO_y particles can be controlled by the mixing ratio of the coarse starting powders. Further systematic investigations are planned on how the load and the size of the embedded Fe_xO_y nanoparticles influence the magnetic properties of these Fe_xO_y@SiO_2 nanopowders. It is expected that due to the reduced particle interactions caused by the separation of the single cores, the coercivity and the remanence of the magnetic particles will increase markedly. This causes an increase of the specific absorption rate when exposed to an alternating magnetic field. Particles consisting of a SiO_2 matrix with inclusions of separated magnetic single cores are very interesting for the investigation of the magnetic particle-particle interactions.

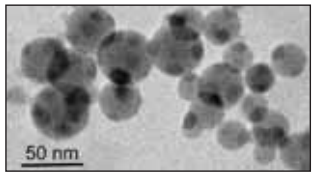

Fig. 2: Fe_xO_y nanoparticles embedded in silica spheres yielded from co-laser evaporation of a hematite/silica mixture.

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Manganese Oxide Nanocrystals as Versatile MRI-T_1 Contrast Agents

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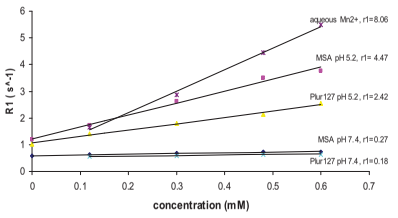
We have developed a systematic route, involving thermal-decomposition of precursors in organic solvents, for the synthesis of manganese oxide nanocrystals. They were subsequently functionalized for specific targeting and their relaxivities evaluated for use as MRI contrast agents. The nanocrystals were monodisperse, and confirmed to be ~ 10nm diameter by TEM, and predominantly MnO by XRD (θ–2θ) measurements. ZFC-FC measurements in a Quantum Design PPMS instrument, with an applied field of 100 Oe showed a transition temperature of 280K. In contrast, similar ZFC-FC measurements of commercial MnO powder showed an antiferromagnetic peak with blocking temperature at 122 K; similarly, a Mn_3O_4 standard showed the expected ferrimagnetic Curie temperature at 42K. Further investigation, including magnetic measurements and high resolution TEM, is in progress to understand the detailed structure of these MnO nanoparticles. In the second step, the MnO nanocrystals were coated with amphiphilic molecules to help disperse them in aqueous solutions for biomedical applications. Mercaptosuccinic acid (MSA), pluronic F127 and silica were utilized as coating materials to successfully transfer nanocrystals to the aqueous phase. In the third step, the Pluronic F127 surface coating was modified with an amino group by esterification for specific binding to small targeting molecules.

We hypothesized that the T_1 contrast enhancement effects of MnO nanocrystals in MRI may result from Mn^{2+} ions released from the surface of nanocrystals. To prove the hypothesis, MSA-, PF127-, and silica-coated nanocrystals were dispersed in variable pH (7.4 and 5.2) aqueous solutions. Small amounts of these solutions were taken and mixed with Mn^{2+} detecting agent (Formaldoxime reagent) and the absorbance of the solution was measured with UV-Vis spectroscopy. The results showed that all three different coated nanocrystals released Mn^{2+} ions dependent on the pH of the solutions. This means that Mn^{2+} can be delivered in nanocrystal form in variable pH for MRI contrast enhancement. To confirm this, MRI relaxivity measurements at 11.7 Tesla, for various concentrations was carried out at pH 7.4 and pH 5.2 (see figure). It showed that MSA coated MnO particles in pH 5.2 had the highest relaxivity (r_1=4.47). This result confirmed that MnO nanocrystals, with the possibility of specific targeting, can be effectively used as T_1 shortening contrast agents. Further work, as a function of time and pH, to monitor the controlled release of Mn^{2+}, with the three different surface coatings, is also in progress.



This work was supported by NSF/DMR #0501421 with partials support for YCL from NIH-NINDS.

Magnetite Nanoparticles for Energy Absorption.

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The synthesis of novel magnetic materials, mainly magnetic nanoparticles (MNPs) for heating purposes in biomedical applications has gained interest along the last years. It has been proved that magnetic intracellular hyperthermia can effectively induce cell apoptosis [1], with the additional benefits related to work in a safer electromagnetic frequency region ($f = 10^2$ - 10^3 kHz) that has no adverse effects on living tissues. However, power delivery from MNPs is still on the low-range (1-10 W per gram of tissue) as compared to traditional microwave or conduction-based hyperthermia methods, which can deliver up to 2-3x10^3 Watts into the target region.[2] Since particles larger than c.a. 20 nm are difficult to stabilize in water, most of the experimental work reported so far has been focused on nanoparticles with sizes < 12-15 nm, thus having single-domain magnetic structure. In order to explore the influence of particle size on power absorption, we have synthesized magnetite-based particles with average sizes 3 < d < 12 nm, using high-temperature decomposition of $Fe(acac)_3$ in the presence of a long-chain alcohol as reported by Sun et al. [3] with a modification developed to control the final size by changing the molar ratio between the metallic precursor and the surfactant [4]. Larger particles (i.e., d > 12 nm) were prepared by a re-growth method using previously synthesized particles as seed for the next ones. The resulting nanoparticles were very stable against agglomeration because of the surfactant molecules attached to the surface of the magnetic cores.

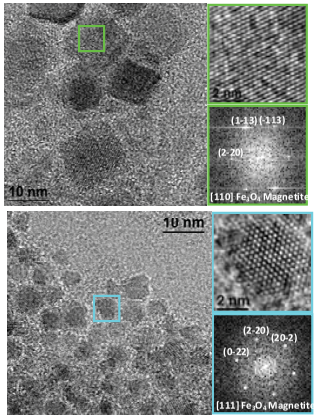

Figure 1. HRTEM images of cubic (top panel) and spherical (bottom panel) Fe_3O_4 particles of average sizes 15 nm and 4.2 nm, respectively.

High-resolution TEM (HRTEM) images and X-ray diffraction (XRD) data confirmed that the average particle size increased with increasing molar precursor/surfactant ratio. A morphology changed from mainly rounded to cubic was observed for particles with d > 15 nm. Magnetization and ac susceptibility measurements showed that the saturation magnetization M_S at room temperature of all samples were lower than the expected 92-98 emu/g of bulk magnetite, but increase as the average particle size increases. Heating experiments were conducted in an adiabatic system at frequency f = 260 kHz and amplitude B = 16 mT. Temperature vs. time curves were taken as a function of particle size revealed a strong increase in the specific power absorption (SPA) values for particles with <d> ~ 25 nm. The largest SPA value obtained was 130 W/g, corresponding to particles with average size values of 25 nm.

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Cell Recognizable Magnetic Nanocarriers for Tumor Targeting, Detection and Hyperthermia Administration: Oligoperoxide Surfactant Based Synthesis

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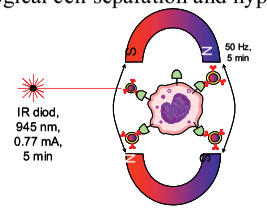
Novel functional water-soluble anionic, cationic and mixed oligoelectrolytes of linear, block, comb-like or branched structures with ditertiary peroxide fragments in side and end substituents or in grafted polymeric blocks and branches were synthesized and studied. They are highly surface-active substances forming micelle-like structures (σ_{CMC} = 20-35 mN/m) of desired size and morphology depending of the length and polarity of oligoelectrolyte structural fragments, media and concentration. Such oligoperoxide surfactants were used for functional magnetite, magnemite and nickel nanoparticle synthesis via technique of homogeneous nucleation as templates and surface modifiers. This not only provides controlling particle size and size distribution but also the availability of predicted amount of functional reactive (including peroxide ones) fragments on nanoparticle surface.

Synthesized nanoparticles comprise of polycrystalline core from a number of small crystals sized 9±2nm (TEM, SAXS measurements, Langevin and Scherrer equations) possessing super paramagnetic properties. Number average particle size is strongly influenced upon functional oligoelectrolyte nature and concentration in the range 70-150nm (SEM measurement).

The values of the adsorption of functional oligoperoxide molecules on nanoparticle surface are 60-160mg/g for linear and block-copolymer oligoelectrolytes and 40-85 mg/g for comb-like ones providing localization of radical forming peroxide groups on the particle surface (6.5 – 26.4)*10^{-5} mole/g. Amphiphilic reactive shell from adsorbed oligoelectrolyte molecules provides both high suspension dispersancy and sedimentation stability in water and polar organic media as well as new functional chain grafting as a result of polymerization initiated from the surface. Experimentally determined activation energy of peroxide group decomposition is 46-86 kJ/mole depending on medium nature. This witnesses the activation of immobilized ditertiary group homolytic dissociation and possibility to form free radicals at comparatively low temperature range (60 - 80C).

Various reactive functional groups (amino, epoxide, aldehyde etc.) included in grafted polymeric spacers at definite distance from the magnetic core were used for binding cell recognizable proteins, antibodies etc. via corresponding polymer analogous reactions.

Modified by this manner biocompatible magnetic and in some cases luminescent also nanoparticles were tested successfully in vitro for targeting, detection and specific binding with pathological cells. There was established the possibility of application of the nanoparticles for pathological cell separation and hyperthermal administration.



Magnetic hyperthermia of apoptotic cells

1) intact cells, 2) cells + nanoparticles 3) cells + nanoparticles + magnetic field 50Hz, 5min, 4) cells + nanoparticles + IR, 945 nm, 5 min

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Size and shape control for water-soluble magnetic cobalt nanoparticles

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In recent years, magnetic nanoparticles (NPs), particularly those fabricated from transition metals, have attracted great interest because of potential biomedical applications such as targeted drug delivery, hyperthermia treatment for solid tumours and magnetic resonance imaging (MRI).[1,2] In order to use magnetic NPs for biomedical applications, they should possess the following properties: stability and solubility in physiological media, biocompatibility, and small size comparable to bio-molecules. More over, they should have fine tuned magnetic properties to be used in certain applications.[2]

A variety of methods exist to synthesize magnetic NPs.[3-6] Thermal decomposition of carbonyl metal compounds in organic solvent with a suitable ligand is preferably used to produce monodisperse particles. However, transferring resulting particles to aqueous solution by ligand exchange has seemingly proved of limited success. The stability and solubility in aqueous medium are still the challenges to prepare magnetic NPs for biomedical applications.

In this work, we report a facile synthesis of monodisperse water-soluble Co NPs by the reduction of cobalt salt in aqueous solution in the presence of alkyl thioether end-functionalized poly(methyacrylic acid) stabilizer with sonication. By varying synthetic conditions such as polymer concentration, polymer molecular weight, speed and order of addition of reactants, the size, shape and stability of the particles can be controlled.

Transmission electron microscope (TEM), dynamic light scattering (DLS) and SQUID magnetometry have been used for characterization of these magnetic nanoparticles. The resulting particles were stable in water for up to two months. It can be seen from the TEM image in figure 1 that the cobalt spherical nanoparticles and nanorods were fairly monodisperse. Currently these nanoparticles were under investigation for use as contrast enhancer in magnetic resonance imaging.

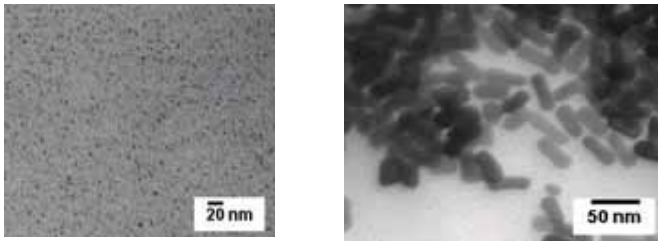

Figure 1: TEM images of 4 nm Co spherical NPs (left panel) and 36 x 15 nm nanorods (right panel).

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Stable water-soluble magnetic hollow CoPt nanoparticles

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Recently, there has been a great of interest in the nanostructure materials, particularly those fabricated from transition metals because of their potential applications in magnetic recording media as well as biomedical applications. Hollow NPs have received a significant attention owing to their high surface area, low density and material saving.

Hollow nanoparticles are often prepared by using templates of silica, polymers.[1-3] This method allows to synthesise hollow NPs with good controlled size and monodispersity. However, the extra step to remove the templates is required, and this sometime complicates the synthetic procedures.

In this work, we present a facile, template-free synthetic method of water-soluble CoPt hollow NPs by simultaneous reduction of cobalt and platinum salts in aqueous solution in the presence of polymer and/or peptide ligands. By varying polymer/peptide ligands as well as cobalt/platinum salt precursors molar ratios and ligands concentration, solid spheres, hollow NPs and nanochains were obtained. Fig.1 shows that the hollow NPs and nanochains were forming from smaller nanoparticles by aggregation and self-assemble, respectively.

Transmission electron microscope (TEM), high resolution transmission electron microscope (HRTEM), dynamic light scattering and SQUID magnetometry were used for characterisation of these magnetic nanoparticles. The resulting hollow nanoparticles and nanochains were superparamagnetic and stable in water for few months. Currently these hollow nanoparticles were under investigation for tracking stem cells using MRI.

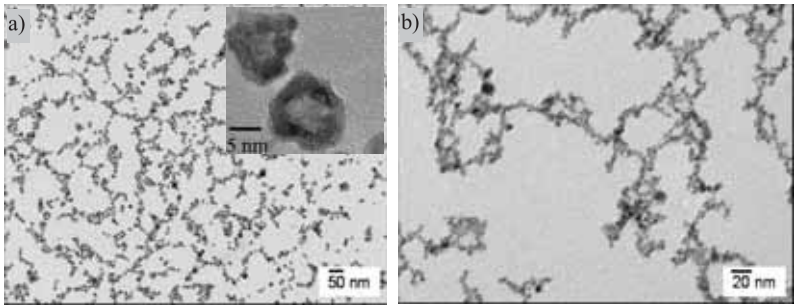

Figure 1: TEM images of water soluble CoPt hollow NPs synthesised by reduction of Co and Pt salts in the presence of mixture of PEG and peptide (a) and PEG (b) ligands under sonication. Inset is HRTEM of CoPt hollow NPs

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Multifunctional, multicompartment polyorganosiloxane nanoparticles for the flow free transport in microfluidic channels

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The synthesis of polyorganosiloxane nanoparticles (POS-NPs) via sol-gel chemistry in aqueous dispersion leads to the formation of narrowly distributed spherical multicompartment nanoparticles in the size range between 10 – 100 nm possessing different architectures, such as homogenous spheres, core-shell spheres and even hollow spheres (Figure 1a,b).[1] The POS-NPs are redispersible in organic solvents and can subsequently be modified to become water soluble. The POS-NPs can easily encapsulate labels such as fluorescent markers or drugs. Additionally, the surface of the particles can be biologically functionalized, which makes the POS-NPs – together with their inherent network-properties – interesting candidates as multifunctional, multicompartment nanoparticles for potential applications including improved drug delivery, sequestering and marking.

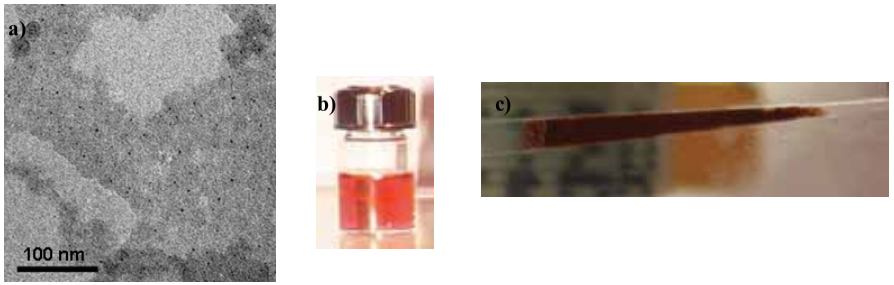

Figure 1: a) TEM of multicompartment POS-NPs (gray) and incorporated metallic NPs (black); b) colloidal solution of modified POS-NPs showing the typical color based on plasmons; c) magnetically based transport of POS-NPs in a microfluidic system.

Additionally, magnetic nanoparticles can be incorporated into the core of the POS-NPs, opening the possibility to magnetically based detection and transport. The basic features of the particles as well as the incorporation in e.g. flow free transport in a microfluidic system are presented (Figure 1c).

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Determination of the Size and Stability of Polydisperse Suspensions of PDMS-Magnetite Nanoparticle Complexes

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The properties and performance of polymer coated magnetic nanoparticles is critically dependent on the size of the polymer -nanoparticle complexes in solution. While the response to electromagnetic stimuli is largely dependent on the size of the core, the colloidal stability, biocompatibility, and functionality, are dependent on the polymer brush. Although it is relative easy to measure the size of the magnetic nanoparticles, the measurement of the entire complex in solution is somewhat more difficult. Techniques such as light scattering, allow one to probe the size of monodisperse systems, but are prone to inaccuracy in polydisperse systems. In this work we present a mathematical approach to determine the polymer brush size based on the polydispersity of the core particle size as determined by TEM. This model is then extended to calculate the colloidal stability of polydisperse polymer-nanoparticle complexes in dilute solutions.

A probability averaging method that incorporates particle size distributions of the magnetite cores derived from TEM is proposed, together with implementation of a polymer brush model for calculating the thickness of the polymer surfactant, for predicting the sizes and size distributions of these complexes in suspension. These calculations of the polymer brush size and density are based on a method originally developed for star polymers. This tool allows us to make close approximation incorporating the effects solvent, molecular weight of the polymer, and the curvature of the particles.

The intensity, volume, and number average size distributions in solution were predicted, and the values were compared to sizes of the complexes measured by DLS. This approach provides a tool for a more precise characterization of the size distributions of polymer-nanoparticle complexes relative to previous methods that utilized only a mean (single) core particle size. The predicted sizes of the complexes in dispersion closely approximate measured values from DLS for particles with narrow size distributions. Agreement between the predicted and measured sizes improves as the particle size distribution becomes narrower.

Having a good understanding of the brush length and the size distribution calculations of the colloidal stability of dispersions of the particles were made. Particle-particle interaction potentials in a theta solvent and in a good solvent for the PDMS were predicted by calculating van der Waals, electrostatic, steric, and magnetic forces as functions of interparticle separation distances. A variety of nanoparticle sizes and size distributions were considered. Calculations of the interparticle potential in dilute suspensions indicated that flocculation was likely for the largest 1% of the population of particles. While a method to combine the interactions of polydisperse particles has yet to be determined, the ability to closely represent the size of polymer-particle complexes and calculate the stability of different slices of the distribution could have a dramatic impact on the design of biocompatible polymer-nanoparticle complexes.

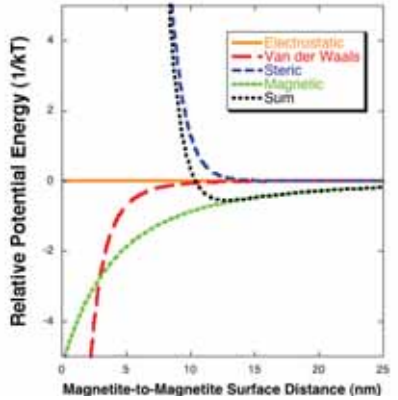

Figure 1: Calculated particle-particle potential energies for PDMS-magnetite complexes that have core radii of 6.3nm and polymer brush length of ~6nm in chloroform.

Control of Phase Composition in Magnetic Iron Oxide Nanopowders Produced by CO_2 Laser Evaporation

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The CO_2 laser evaporation technique is a highly versatile and capable method for the production of a multiplicity of nanopowders. In this special process, coarse (micrometer…millimeter) starting powders are evaporated in the focus of a CO_2 laser beam (wavelength 10.59 µm, laser power up to 2 kW, focus intensity up to 140 kWcm^{-2}) in a continously flowing condensation gas under atmospheric pressure (Fig. 1). Due to the very steep temperature gradient between the hot evaporation zone around the beam focus and the surrounding atmosphere nucleation, condensation and coagulation are proceeding very fast resulting in ultrafine particles. These particles are then transported by the carrier gas (air) to the filter separation unit.

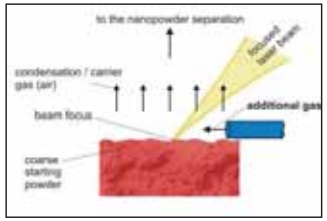

Fig. 1: Principle of the laser evaporation method with the additional gas jet

Basic investigations [1] revealed that this technique is well suited for the production of magnetic iron oxide nanopowders. We found that pure air as condensation gas yielded a mixture of hematite and the magnetic phases maghemite and magnetite, while an additional argon gas jet (Fig. 1) beside the main air flow through the evaporation zone suppressed the hematite phase. In order to clarify the cause of this behavior, laser evaporation experiments in air with various additional gases (Table 1) were accomplished. All experiments started from coarse hematite powder, which was evaporated in the focus of a continuous CO_2 laser beam (power ≈ 1.9 kW) under normal pressure and a total carrier gas flow of 14.5 m^{3}h^{-1} (air plus additional gas jet).

Table 1: Influence of the additional gas on the hematite concentration (XRD measurements) relativ to the total amount of Fe_xO_y; results of BET and VSM measurements

additional gas			XRD	BET	VSM
	gas flow [m^{3}h^{-1}]	heat conduct. [Wm^{-1}K^{-1}]	conc. hematite [vol. %]	particle diam. [nm]	saturation magn. [Am^{2}kg^{-1}]
without			11	42	58.1
O_2	2.0	0.023	20	40	45.3
air	2.0	0.026	10	41	59.9
N_2	2.0	0.020	5	40	67.6
He	5.4	0.144	4	34	69.9
Ar	2.0	0.016	4	43	69.2
Ar	5.4	0.016	5	36	67.1

XRD measurements (Table 1) of the produced powders revealed a strong dependence of the hematite concentration on the additional gas. The results indicate that effects of different heat conductivities, cooling or diluting the laser generated vapour are insignificant. The cause for the suppression of hematite is the lack of O_2 in the zone of evaporation and particle growth. This can be explained by an extremely simple model of the nucleation of Fe_2O_3 particles. Whereas the hematite structure contains only oxygen octahedra around the iron ions, so-called B sites, *i.e.* each Fe ion needs *six* oxygen ions for its complete coordination during the nucleation process, the maghemite structure contains beside octahedra also oxygen tetrahedra, A sites, around Fe^{3+}. Thus, for the first nucleation step already *four* oxygen atoms may suffice if only few oxygen is present.

This consideration may also be applied to other gas phase condensation processes used to produce magnetic Fe_xO_y nanopowders in order to maximize the yield of the magnetic phases.

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Seed crystals added to reaction solution play a critical role in determining the size of highly monodisperse ferrite nanoparticles during synthesis

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Precise size control of ferrite nanoparticles (FNPs) is important for them as high performance magnetic carriers for biomedical applications. We have reported that in the aqueous synthesis of magnetite FNPs, spherical shape and narrower size distribution are attained by adding a disaccharide and seed ferrite crystals, respectively, to the reaction solution.[1] Furthermore, the spherical ferrite particles decrease in average diameter as concentration of seed crystals is increased. Here, we report that the average size of the FNP's is decreased even by improving the dispersibility of seed crystals in reaction solution.

In previous experiments we added coprecipitated seed ferrite crystals, 3-8 nm in size, dispersed in an NH_3 solution to a disaccharide-added reaction solution of $FeCl_2$. In this study we replaced the NH_3 solution by a TMAH (tetramethylammonium hydroxide) solution. This improved the dispersibility (measured by a dynamic light scattering (DLS) method) of the seed crystals in the reaction solution. The figure shows that a doublet spectrum located at ca. 106 nm and 295 nm changed to a singlet centered at ca. 59 nm. The actual diameter calculated from TEM images decreased from 182 ± 19 nm to 85 ± 11 nm. This is because as hydrodynamic size of seed crystals decreased (i.e. dispersibility improved), the effective surface area (where ferrite crystal nucleation occurs) increased as well.

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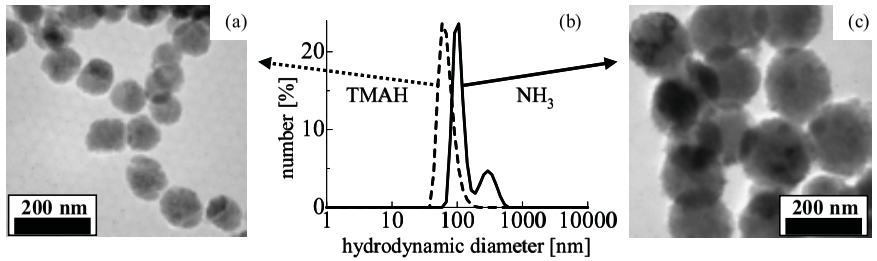

(a) and (c) show TEM images of FNPs prepared from reaction solutions which were added with seed crystals dispersed in TMAH and NH_3 solutions. (b) shows hydrodynamic size of the seed crystals in the reaction solutions used for FNPs shown in (a) and (c).

Thin films of functionalized nanoparticles for magnetic and magneto-transport applications

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Nanoparticles exhibit unique properties mainly due to their high surface to volume ratio and to quantum size effects. Nowadays, they are considered as the building blocks of the future nanotechnological devices and the development of strategies for processing nanoparticles into thin films has become a strategic challenge. Multidimensional assembly of magnetic nanoparticles is used to elaborate many original and significant devices such as filters, magnetic recording head sensors, electronic logic devices, high-density magnetic storage media... Indeed recent studies have demonstrated the interest of the structuration of half metallic magnetic oxide nanoparticles to develop magnetoelectronic devices with enhanced magneto-transport properties. Magnetite, Fe_3O_4, is predicted to be half-metallic at room temperature. It behaves as a metal for one of the spin polarization and as an insulator for the other one and should therefore allow 100% spin polarization. Whereas it has never led to very high magnetoresistance (MR) values in thin films, it has shown extremely interesting MR values of up to 300% when elaborated in films of nanoparticles. [1,2] This demonstrates the interest in developing methodologies towards magnetic nanoparticles structuration. Furthermore the control of the assembling of magnetic nanoparticles will allow building magnetic-force triggered nanodevices.

Magnetite based nanoparticles have been synthesized by co-precipitation or by decomposition of an oleate precursor leading to nanoparticles with sizes of 8, 12 and 40 nm.[3] The grafting of designed organic molecules on these nanoparticles[4] is an interesting way to combine new physical properties and to address their assembling into patterned thin films. Among the techniques for the deposition of thin films of functionalized nanoparticles on solid substrates, we used the Langmuir-Blodgett technique and layer-by –layer deposition which are some of the most promising methods to finely control of the thickness and the homogenous coverage of the substrate in very mild and convenient conditions. The films homogeneity and coverage density appear to depend on the particle size, the type of grafted molecules and the deposition method. Magnetic and resistivity measurements are discussed.

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Multi-technique characterisation of maghemite: determination of the spin canting by NMR and in-field Mössbauer spectrometry

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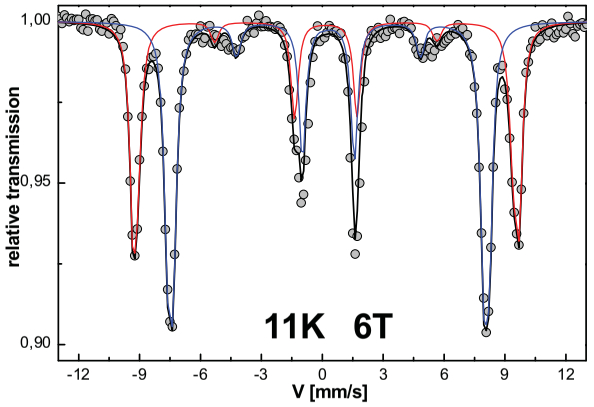
Nanosized powders of magnetic iron oxides are widely used nowadays in biomedical applications, such as hyperthermia, contrast agent for magnetic resonance imaging, cell targeting. Although many works have been devoted in the last ten years to nanosized maghemite, there appears to be a sparcity of data concerning its magnetic structure and particularly the origin of spin canting. Ideally, maghemite is ferrimagnetically ordered, with the magnetic moment of tetrahedral sites oriented antiparallel to the moments of the octahedral sites. However, the saturation magnetization experimentally observed is lower than the theoretical one, due to incomplete alignment of magnetic moments with the magnetic field. The origin of spin canting, generally assigned to finite-size effects, surface effects or disorder of vacancies is still discussed.
We will present a complete characterisation of maghemite nanoparticles, particle sizes, iron oxidation degrees and oxygen stoechiometry, and magnetic structure. Maghemite is obtained by oxidation of magnetite nanoparticles in soft conditions. Its stoechiometry and vacancies order are controlled by TG measurements, X-ray diffraction, Raman and IR spectroscopy. The magnetic structure particularly the canting angle has been determined by in-field ^{57}Fe Mössbauer spectrometry and NMR spectroscopy on particles of 40 nm for which surface to volume effects are negligible. The saturation magnetization is then interpreted in view of these results.



Mössbauer spectrum of 40 nm maghemite recorded at 11K under 6T magnetic field oriented parallel to the γ-radiation: the hyperfine structure suggests a ferrimagnetic canted structure.

Synthesis and Characterization of Core-Shell Monodisperse Fe@Ag Magnetic Nanoparticles and their Bioconjugation

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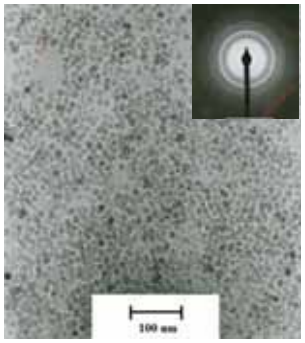
We present a single-pot synthesis of monodisperse core-shell Fe@Ag magnetic nanoparticles via the thermal decomposition of iron pentacarbonyl and reduction of silver acetate by oleylamine, which acts as a reducing and capping agent. The oleylamine ligands can be replaced with 11-mercaptoundecanoic acid (11-MUA), giving particles that can be dispersed in water. The antibodies (GHIgG) were immobilized on 11-MUA modified Fe@Ag nanoparticles by conventional EDC amidation chemistry. The combination of XRD (X-ray powder diffractometry), TEM (Transmission electron microscopy), SAED (Selected area electron diffraction) TGA (Thermogravimetric analysis), EDAX (Energy disperse X-ray analysis), FT-IR (Fourier transform infrared spectroscopy), VSM (Vibrating sample magnetometer), and UV–visible absorption spectroscopy were employed to characterize the structure, morphology, composition, magnetic and optical properties of the core-shell nanoparticles.

XRD was used to confirm the presence of f*cc* silver, as this metal obscures the pattern of *bcc* iron in Fe@Ag nanoparticles. Transmission electron microscopy has shown that particles are well monodispersed with a size of 11 nm (7 nm core of Fe and about 2.5 nm shell of Ag), as well as a narrow size distribution as shown in Fig.1. SAED pattern confirms crystalline nature of pure Fe and Fe@Ag nanoparticles. TGA and EDAX of the samples recorded in air atmosphere have proved that core-shell Fe@Ag nanoparticles are not air-sensitive. All the above characterizations show that in this sample there are no iron oxides inside the particle. The ligand exchange of Fe@Ag nanoparticles with 11-MUA is evident from FTIR. At 295 K, no coercivity (H_c) and remanence (M_r) are observed in the magnetization curve, indicating that particles are superparamagnetic in nature (Fig.2). The absorption band of the Fe@Ag nanoparticles are broadened relative to that of the pure silver nanoparticles. The sharp shifts to a longer wavelength confirms the attachment of antibodies on Fe@Ag nanoparticles.



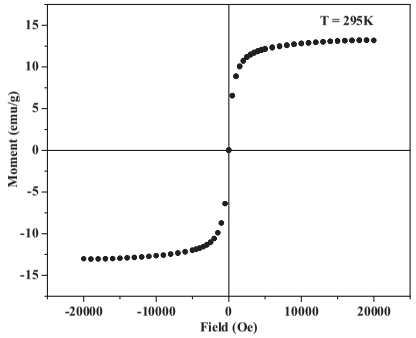


Fig.1. TEM image of Fe@Ag nanoparticles **Fig.2.** Magnetization of Fe@Ag nanoparticles

Novel Core-Shell Magnetic Nanoparticles: Synthesis, Characterization and Biomedical Application*

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Nanoparticles have gained increased attention recently for biomedical and environmental applications. Biocompatible magnetic nanoparticles (MNPs) have been found promising in several biomedical applications for tagging, imaging, drug delivery, sensing and separation in recent years. Most magnetic particles or beads currently used in biomedical applications are based on iron oxides with low specific magnetic moments less than 50 emu/g. In this presentation, we report room-temperature synthesis of novel iron-iron oxide core-shell nanoparticles using a newly developed nanocluster source. Monodispersive MNPs with mean size of diameters from 2 nm to 100 nm are produced in a source chamber and then transmitted into the reaction chamber where a small partial pressure of O_2 is present so that the nanoclusters are coated with uniform iron oxide shell. These shells act as passivation layers preventing further oxidation of the cores upon subsequent or continued exposure to air. The size of the iron-iron oxide core-shell nanoclusters varies with the He:Ar ratio, chamber pressure, and growth distance through the aggregation tube. The MNPs were characterized by XPS, XRD and HRTEM. The bcc-Fe core with magnetite shells was observed by XRD and HRTEM. The core-shell nanoclusters are superparamagnetic at room temperature for sizes less than 15 nm, and then become ferromagnetic when the cluster size increases. The specific magnetic moment of core-shell MNPs is size dependent, and increases rapidly from about 80 emu/g at the cluster size of around 3 nm to over 200 emu/g up to the size of 100 nm (Figure 1). The use of high magnetic moment nanoclusters for biomedical applications dramatically enhanced the contrast for MRI, reduce the concentration of magnetic particle needs for cell separation, or make drug delivery possible with much lower magnetic field gradients. The MNPs were incubated and successfully untaken by lung cancer cells for hyperthermia treatment.

*Research supported by DOE-BES and DOE-EPSCoR.

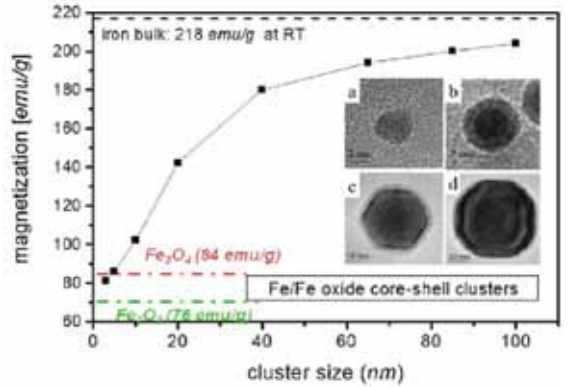

Figure 1: Size dependent specific magnetic moments of core-shell iron-iron oxide MNPs); Inset is the HRTEM micrographs of the MNPs with diameter from around 3 nm to 85 nm prepared on carbon microgrids.

Synthesis of cobalt nanoparticles by laser irradiation in organic solution

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Cobalt and iron oxide magnetic nanoparticles (MNPs) have been synthesised by laser pyrolysis of organometallic precursors in the vapour phase[1-3]. However, to date, no research has been conducted on the use of laser irradiation to produce cobalt NPs in the solution. Here we report, the decomposition of cobalt carbonyl [$Co_2(CO)_8$] in the presence of oleic acid (OA) and trioctylphosphine oxide (TOPO) using laser radiation at a wavelength of 266 nm. The technique offers a unique synthetic method for the production of small (<5 nm) cobalt NPs. After 30 min irradiation, 2-4 nm cobalt nanoparticles were synthesised (figure 1).

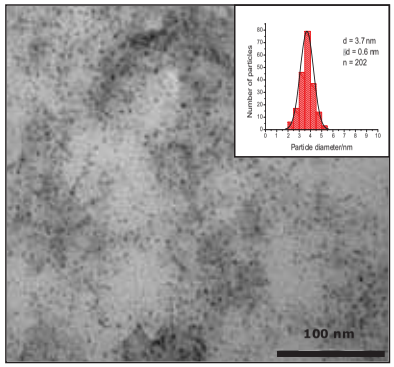

Figure 1. TEM image and size distribution of 3.7 nm cobalt nanoparticles synthesised by laser irradiation at 266 nm.

As both $Co_2(CO)_8$ and the solvent (1,2-diclorobenzne, DCB) can absorb light at 266 nm, it is unclear if the nanoparticles are being formed by chemical-photolysis or by pyrolysis of the precursor resulting from the localised heating of the DCB. The synthesis was repeated at 355 nm (a wavelength where $Co_2(CO)_8$ can absorb light but DCB cannot) and again after 30 min irradiation, 2 - 4 nm nanoparticles were formed. This would indicate that the nanoparticles are formed by photolytic breakdown of cobalt carbonyl, although other factors may contribute e.g. heating of cobalt carbonyl itself.

The effects of the ratio of precursor and ligand were investigated and it was found that decreasing the OA concentration increasing the size of NPs as in a conventional thermal decomposition method.

Transmission electron microscopy was used to observe the morphology of the synthesised nanoparticles and their magnetic properties was characterised using a super conducting quantum interference device (SQUID) magnetometer.

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Acknowledgements

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A versatile approach for producing water-stable magnetic nanoparticles: ligand exchange

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Magnetic nanoparticles (MNPs) have many potential biomedical applications[1]. For most of these applications the NPs should ideally have uniform size and shape[2]. The synthesis of NPs by the thermal decomposition of organometallic precursors in organic solvent is an excellent method for producing MNPs with these desirable properties. This method can also produce MNPs of tuneable size, shape and composition. These NPs are usually stabilised with hydrophobic ligands, however, for use in biomedical applications the NPs need to be water soluble and stable in physiological conditions. There are various *in situ* synthesis methods to make water-soluble MNPs[3,4]; however, different conditions need to be investigated for each of the required systems (e.g. size, shape, composition).

We present a versatile approach to fabricate water soluble MNPs by synthesising them in organic solvent in the presence of hydrophobic ligands which then later be exchanged with hydrophilic ones. As an example, cobalt and γ- Fe_2O_3 NPs were synthesised by thermal decomposition of their respective carbonyl compounds in the presence of oleic acid (OA). Using a process of ligand exchange it was possible to remove the OA and replace it with a hydrophilic synthetic polymer. The polymer coated Co and γ- Fe_2O_3 nanoparticles were water-solube, stable up to 0.2 M and 0.5 M NaCl in phosphate buffer, respectively. All the ligand exchanged nanoparticles were found to stable in solution with pH range from 5.5 to 12.

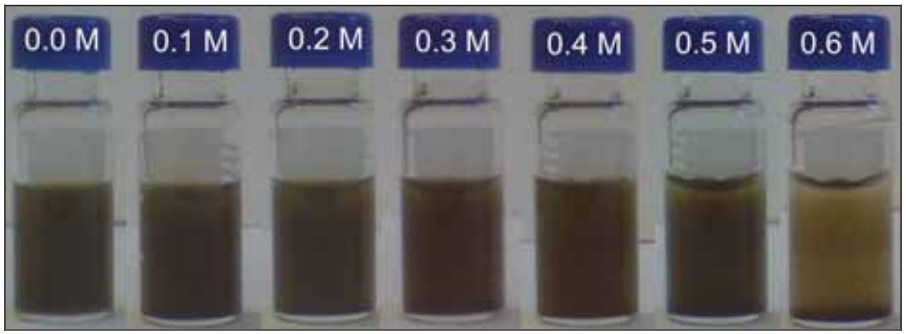

Figure 1. γ- Fe_2O_3 nanoparticles coated with polymer are stable up to 0.5 M NaCl in phosphate buffer.

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One-pot synthesis of monodisperse water soluble 'dual-responsive' magnetic nanoparticles

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The potential use of magnetic nanoparticles in biological applications requires the nanoparticles to be water soluble and stable in aqueous solution[1]. This can be achieved by encapsulating the nanoparticle protective shell. Various substances have been used to form this protective shell such as silica[5], polymer[6], peptide[7, 8].

Here we report the synthesis of monodisperse magnetic nanoparticles using synthetic copolymers as a capping ligand. These copolymers undergo a conformational change in response to changes in temperature that allow the synthesis of monodisperse particles at high temperature, but facilitate the transfer of the synthesised particles into the aqueous phase at room temperature. Furthermore, the fabricated nanoparticles can exhibit 'smart' behaviour such as a non-linear response to an external change, e.g., temperature or pH, which can be used to control drug delivery together with hyperthermia cancer treatment.

The particles were characterised using TEM and the magnetic properties were analysed using a SQUID magnometer. The surface charge of the coated nanoparticles was measured through the ζ-potential over a wide pH range. To investigate their thermal response the synthesised nanoparticles were exposed to a range of temperatures from 5°C to 50°C (fig 1).

These nanoparticles are currently being assessed for their effectiveness in applications such as MRI image enhancement, stem cell tracking, biological separation and a magnetic immunoassay.

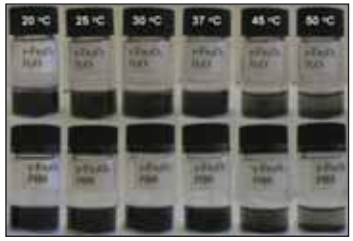

Figure 1. Thermo-responsive polymer coated magnetic nanoparticles dispersed in water or PBS

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Characterization of Amino Surface-modulated Magnetite Nanoparticles

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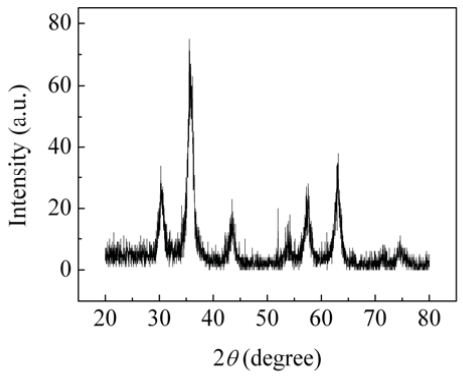
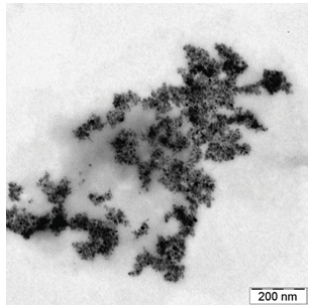
In recent years surface-functionalization of nanosized magnetic particles have received increasing attention with a significant impact on the development of new nanobiocompatible products. This is due to the combination of both superparamagnetism, which allows external manipulation using gradients of magnetic field, with chemotropism associated to the surface molecular coating, allowing biological specificity. One useful strategy is to build a reactive molecular interface (RMI) between the metal-oxide surface provided by the magnetic nanoparticle and the biologically-active molecular coating addressed to a particular application. In searching for RMI candidates the ones providing reactive amino groups facing outside the shell represent an important breakthrough, as far as the nanoparticle surface-functionalization is concerned. In this respect the hydrolysis and condensation of alkoxysilane agents on metal-oxide surfaces is an excellent approach. The organosilanes are represented by the general formula $R_nSiX_{(4-n)}$, where X is a hydrolysable group (typically alkoxy) and R is a non-hydrolysable organic radical, possessing the desired functionality, as for instance (3-aminopropyl)trimethoxisilane, which has the reactive terminal amino group. This reactive amino-termination can be used for chemical conjugation reaction with carboxylic acid from proteins, such as antibodies. In this study we report on successful modulation of the amino surface-grafting of nanosized magnetite particles. In addition, characterization of the surface-functionalized magnetite-based samples using X-ray diffraction (see below figure on left), transmission electron microscopy (see below figure on right), and Fourier transform infrared spectroscopy will be reported in the present study.





Design of Au/Iron-oxide Composite Nanoparticles for Biotechnological Applications

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Takashi Nakagawa[3], Takuya Kinoshita[4] and Takao A. Yamamoto[1]

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Magnetic nanoparticles now offer attractive and versatile applications in the field of biotechnology, such as the separation of specific target, gene transduction, DDS, MRI, etc. We are developing a gold/iron-oxide composite nanoparticle for those in-vitro and in-vivo applications. As the gold part firmly combines with molecules possessing mercapto groups, the composite nanoparticles can be easily modified with molecules appropriate for each application. Here we report on the design of the composite nanoparticles for each application by controlling the support iron-oxide nanoparticles.

The composite nanoparticles were synthesized in aqueous solution systems by radiochemical or sonochemical process. Commercial iron oxide nanoparticles were dispersed in an aqueous solution containing gold ions together with some alcohol and polymer. The solution was irradiated with a high energy electron beam or gamma-rays to reduce the gold ions. After the irradiation, small gold nanoparticles were immobilized on the surface of iron oxide nanoparticles.

For in-vitro applications, magnetic iron-oxides, commercial iron-oxide nanoparticles of NanoTek® with average diameter of 20-100 nm were used as support. The composite nanoparticle showed good dispersibility in aqueous solution system and can be easily collected by a magnet. The composite nanoparticles adsorb thiol-modified probe oligonucleotides. Target oligonucleotides were easily and specifically picked up from an aqueous solution by magnetically attracting these probe-nanoparticle conjugates by a magnet. For the in-vivo applications, commercial MRI agents of Resovist® or Feridex®, which are super paramagnetic iron oxide, were used as support. Figure 1 shows TEM image of the Au/Resovist®. The composite nanoparticles were difficult to collect by a magnet and showed very good dispersibility even in blood serum. These Au/Iron-oxide composite nanoparticles are expected as a new magnetic nanocarrier for various biotechnological applications.

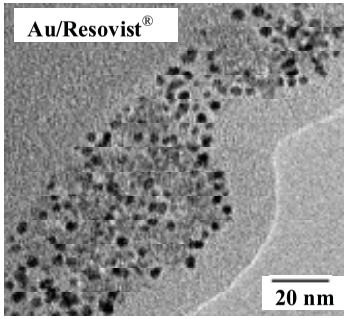

Fig.1 TEM image of the composite nanoparticle for in-vivo applications.

Nanocrystalline Ni-Zn ferrite for bio-medical applications

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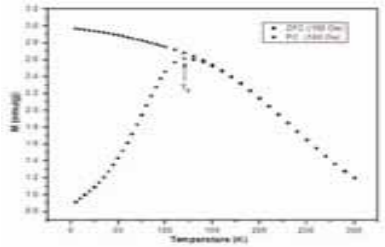
Highly uniform, spherical shape, narrow particle distribution, chemical stable with average grain size of 9 nm behaving superparamagnetic at room temperature, nano ferrite have been synthesized using reverse micelle technique. Blocking temperature of $Ni_{0.58}Zn_{0.42}Fe_2O_4$ nanoparticle is about 147 K and shows negligible hysteresis at low temperature. Also Fe^{2+} ions are resist up to a temperature of 473 K. Superparamagnetic behavior and control of Fe^{2+} ions make this ferrite highly suitable for biomedical application such as drug delivery system as a magnetic fluid carrier.

Fig. 1 shows the Zero field cooled (ZFC) and field cooled (FC) saturation magnetization curves for nano nickel-zinc ferrite at an applied field of 100 Oe. ZFC-FC magnetization curves exhibit the typical blocking process of an assembly of superparamagnetic particles with a distribution of blocking temperatures at $T_B \approx 120$ K. The blocking temperature is a measure of the thermal energy required to overcome the superexchange transition and is defined as the temperature at which the nanoparticles do not relax during the time of measurement; they are blocked [22]. In any real fine particle system, there is always distribution of particle volume f (V) and hence each particle is blocked at different T_B. ZFC and FC curves tend to superimpose at a temperature above T_{SEP} (140 K)



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New Magnetic Polyorganosiloxane Nanoparticles as Biologically Functionalized Targets for Microfluidic Applications

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The need for versatile, multifunctional nanoparticles for e.g. applications in assays as markers or sequestrates is constantly growing. Nanoparticles offer a larger surface to volume ratio as compared to commercially available micron sized particles, making them interesting candidates for improved targeting and detection systems.

We report on the synthesis and characterization of polyorganosiloxane nanoparticles carrying encapsulated magnetic nanoparticles (Mag@Silox-NPs). The nanoparticles are easily functionalized and can be modified to carry different biologically active functionalities (Figure 1a).

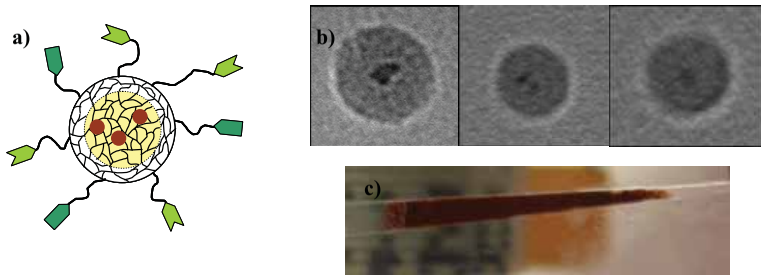

Figure 1: a) Scheme of Mag@Silox-NPs: Siloxane based nano-networks with encapsulated magnetic NPs and biologically modified surface functionalities; b) TEM of Mag@Silox-NPs (gray) and incorporated magnetic NPs (black). Diameter of the Mag@Silox-NPs: 40 nm; c) Mag@Silox-NPs transported in a microfluidic channel.

The synthesis is performed via sol-gel chemistry in aqueous dispersion and leads to the formation of narrowly distributed spherical nanoparticles in the size range between 10 – 100 nm (Figure 1b).[1] The Mag@Silox-NPs are redispersible in aqueous solution. Their inherent network-properties make them interesting candidates for potential applications including drug delivery, sequestering and marking.

The basic features of the particles as well as the incorporation in a microfluidic system (Figure 1c) are presented.

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Imaging and Controlling Magnetic Chain Formation of Magnetic Nanoparticles

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The continuing evolution of magnetic bead techniques for bio-separations has lead to a wide availability of particles with a broad range of magnetic properties which have significant impact on magneto-transport properties. Magnetic field induced chaining is particularly important in that the size and shape of the clusters formed determine the magnetic and viscous drag forces and the diffusion constant.

In this work, we use optical microscopy to study nucleation, growth and diffusion of chains formed from novel metallic nanoparticles, and for some commercial, iron oxide based particles. Individual 100nm metallic magnetic nanoparticles are readily tracked optically, allowing determination of their magnetophoretic mobility and diffusion constants. Application of a suitably strong magnetic field induces magnetic chain formation, wherein particles condense into linear chains which further diffuse and aggregate into large structures, tens of microns in length at sufficient concentrations, best described as composite chains. These chains can be rotated and translated using magnetic fields and gradients, and digital multiple exposures allow deduction and analysis of trajectories of individual chains. Magnetophoresis and diffusion behavior are shown to be strongly affected by chain size and shape. Removing the magnetic field leads to chain disintegration, except in circumstances where the nanoparticles chemically adhere. Finally, we show that one can exploit deliberate variations in nanoparticle magnetism to selectively nucleate aggregation of specific nanoparticle types, or to form composite chains containing distinctive types of nanoparticles.

This work is supported by US National Cancer Institute through Center for Cancer Nanotechnology Excellence focused on Therapeutic Responses (CCNE-TR).

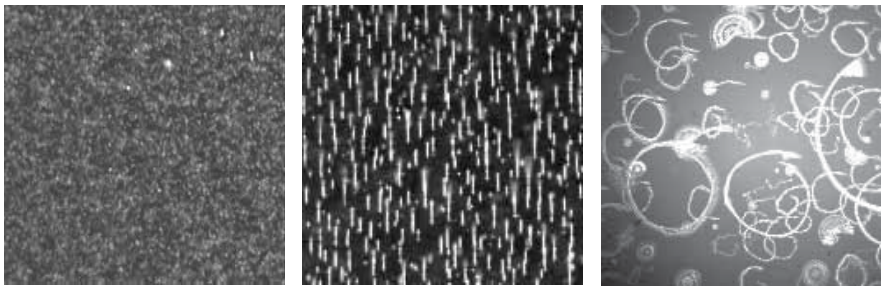

Fig. 1: Optical images of (a) diffusing nanoparticles, (b) chains, and (c) magnetically driven trajectories.

Water Soluble Magnetic Nanoparticles Stabilized With Biologically Active Ligands

Alla Zablotskaya[1], Izolda Segal[1], Edmunds Lukevics[1], Mikhail Maiorov[2], Dmitry Zablotsky[2], Elmars Blums[2], Irina Shestakova[1], Ilona Domracheva[1]

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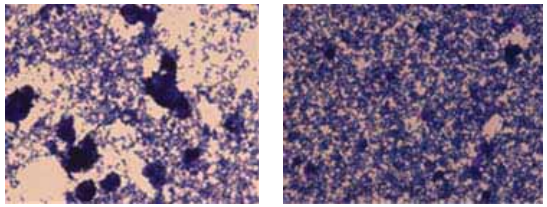
The investigation of nanoparticle based materials has witnessed a period of intense growth over the past 15 years. Iron oxide nanoparticles are promising materials for biomedical applications. At this scale, the grains which exhibit magnetic properties are of great interest in biomedicine. One of the most significant aspects of these studies is the surface modification of nanoparticles. Coating of colloidal particles with a layer of different material in the nanometer scale is an interesting route for modifying the surface properties. Indeed, the nanoparticles can be coated with biological molecules and dispersed in a liquid medium. Due to their liquid properties and sensitivity to an applied magnetic field, such materials can be made, for example, to deliver an anticancer drug to a targeted region of the body such as a tumor.

An important stage for study of iron oxide particles is to devise synthetic routes which allow the control of the particles morphological characteristics. In this context, we have been interested on the synthesis of aqueous iron oxide colloids bearing biologically active organic ligands. Earlier following this strategy we have obtained double layer water based colloids of iron oxide nanoparticles covered with oleic acid (first cover) and different surfactants of the series of biologically active silyl modified choline derivatives (second cover), which revealed cytotoxic properties.

In this work, we present the results of the interaction of naked iron oxide nanoparticles with different surfactants, namely, oleic acid, n-decyldimethyl(β-dimethylaminoethoxy)silane methiodide and n-hexadecyldimethyl(β-dimethylamino-ethoxy)silane in toluene. Different behavior is observed for the interaction of silylated alkanolamines with naked nanoparticles to that with surface modified by oleic acid nanoparticles. We have obtained water soluble iron oxide nanoparticles covered with oleic acid and evaluate the influence of the latter content on physico-chemical and anti-tumor properties. The nanoparticles were prepared by co-precipitation and followed by the double-layer-surfactant process to entail their hydrophilic property. Physico-chemical and structural characterization (magnetic properties, particle size, magnetite concentration) of the nanoparticles synthesized was carried out by the method of magnetogranulometry and transmission electron microscopy.

Magnetic colloids prepared were screened for *in vitro* cytotoxicity on monolayer HT-1080 (human fibrosarcoma), MG-22A (mouse hepatoma) tumor cell lines and normal mouse fibroblasts (NIH 3T3).



View of human fibrosarcoma (left) and mouse hepatoma (right) cells with magnetic nanoparticles visualized with crystal violet (300x).

Preparation and Characterization of Multiple-functional $CoFe_2O_4$@ZnO Nanocomposites

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Abstract

The multiple-functional nanocomposites can be formed by the following method: $CoFe_2O_4$ ferrite possessing large magnetocrystalline anisotropy and reasonable magnetization can be coated with microcrystal ZnO with properties of photoluminescence and photocatalysis.

In this work, multiple-functional $CoFe_2O_4$@ZnO nanocomposites with multiple function of magnetism, photoluminescence and photocatalysis are successfully synthesized by sol-gel method. Preliminary $CoFe_2O_4$ nanoparticles are prepared with the method of coprecipitation. And the $CoFe_2O_4$@ZnO nanocomposites are characterized by transmission electron microscopy (TEM), X-ray diffractometer (XRD), magnetic hysteresis loop (MHL), differential thermal analysis (DTA) and, photoluminescence (PL), respectively. The results show that the average diameter of $CoFe_2O_4$@ZnO nanocomposites prepared is 50±5 nm, and $CoFe_2O_4$ with face center cubic structure are coated by microcrystal ZnO. The saturation magnetization of $CoFe_2O_4$@ZnO nanocomposites is 15.18meu/g. An endothermal peak was observed at 131□ in DTA. The fluorescence emission peak lie at 443 nm when excitated at 350 nm. And methyl blue can be depigmented by $CoFe_2O_4$@ZnO nanocomposites under ultraviolet radiation.

Acknowledgements

This work supported by the National Natural Science Foundation of China (10405034).

Key words: nanocomposites; multiple-function; $CoFe_2O_4$; ZnO; sol-gel

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Preparation of Fe_3O_4 / P(AA-MMA-GMA)Magnetic Composite Microspheres by Controlled Radical Polymerization

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In this paper, Fe_3O_4 organic magnetic nanopaticles have been made by chemical co-precipitation firstly. Then, an functional magnetic composite microsphere with epoxy groups was synthesized by controlled radical polymerization with 1,1-Diphenylethylene(DPE) as a control agent of radical polymerization. The particles are characterized by electron microscopy , thermogravitometry analysis and vibricated sample magnetic.

The transmission electron microscopy (TEM) micrographs of magnetic composite microspheres, which was shown in Figure 1, indicated that the magnetic composite microspheres are nearly sphere with an average particle size in the range of 200–400*n*m. The magnetite content of magnetic composite microspheres measured from TG is about 40%, and saturation magnetization is 14.24emu/g by vibrating sample magntometer.

The content of epoxy groups were larger than 1mmol/g. Fe_3O_4/P(AA-MMA-GMA) magnetic composite microspheres, prepared by DPE controlled radical polymerization method show a good medium-resistance in salt , acid and alkali solution.

Figure1 TEM micrograph of Fe_3O_4/P(AA-MMA-GMA)

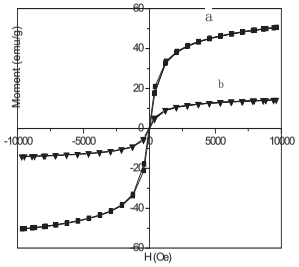

Figure 2 Hysteresis loop of magnetite particles Fe_3O_4 (a) and Fe_3O_4/P(AA-MMA-GMA) magnetic composite microspheres (b)

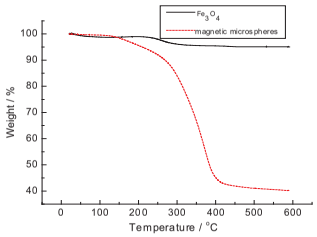

Figure 3 TGA curves of magnetite particles

Easy to use and rapid detection of viral nucleic acid by using of paramagnetic particles modified by streptavidin

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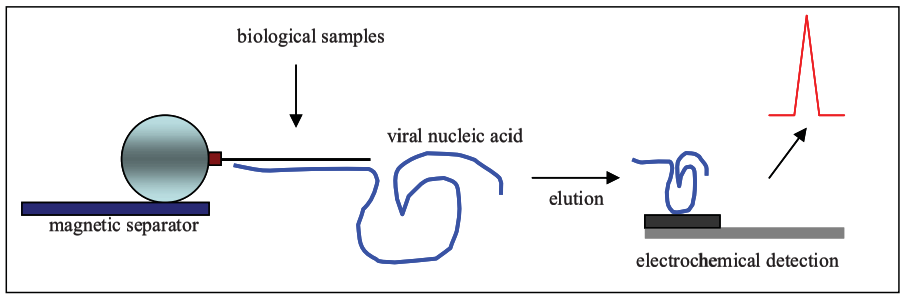
Easy to use, rapid and low-cost detection of viral nucleic acid in a biological sample represents the essential tool in targeted therapy. Human population is globally threatened by expansive pandemics caused by viruses such as Human Immunodeficiency Virus (HIV), hepatitis (HVB, HVC) and influenza's viruses. In addition in tropical and subtropical areas the very dangerous epidemics evoked by different viral species causing hemorrhagic fevers occur. All these viral diseases can be distinguished by the rapid spreading in population, considerable morbidity and also mortality. Diagnostic possibilities are relatively limited. Nowadays they are focused on the direct cultural proof of identity or they result from the detection of viral antigen by using of antibodies. Nevertheless the procedures utilizing molecular-biological methods, especially polymerase chain reaction, are also implemented. Real time Polymerase chain reaction coupled with capillary electrophoresis on chip represents very important step in viral sequence detection. In our work we report on the suggestion of new method in detection of viral nucleic acid based on magnetic microparticles. We utilized microparticles modified by streptavidin with high affinity to biotin for detection of viruses (HIV, VHB and influenza H5N1), which have been labelled by biotin. The interaction between biotin and streptavidin is very rapid and strong, dissociation constant of this bound is 10^{-15} M. Primarily we aimed on optimization of experimental conditions such as temperature and composition of the buffer, where interaction proceed, and time of the interaction itself. Viral nucleic acids (0, 1, 10, 50 or 100 mg/ml) labelled by biotin were added to solution with paramagnetic particles. Due to biotin-streptavidin affinity the labelled nucleic acids were bounded. Bounded nucleic acids were isolated, released using heat treatment and detected. For this purpose the miniaturised carbon electrodes as well as carbon electrodes modified by carbon nanoparticles were utilised. Under the optimized experimental conditions it was possible to detect 75-80 % of added viral nucleic acid. Limits of detection of nucleic acids were estimated down to nanomolar concentrations. In addition the whole proposed process was tested in fully automated arrangement.



Acknowledgement: The financial support from the grant KAN 208130801 is highly acknowledged.

"Hairy" magnetic micro and nano particles for diagnostic applications

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[3]Max Planck Institut, Munich, Germany *

Magnetic particles are widely used as carriers for biomolecules in diagnostics. Thus, surface functionalization of beads is now of a great interest to enhance biomolecules immobilization.
In this study, we report a new polymeric coating based on Acrylamide (AM) polymers and leading to thick polymer brushes at the surface of the magnetic particles. These brushes include reactive functions for the attachments of biomolecules of interest, such as antibodies, enzymes or nucleic acids.
"Hairy beads", thus obtained were extensively characterized with several techniques: zeta potential, size measurements (Dynamic Light Scattering), Transmission Electron Microscopy (TEM) (Fig 1) and Cryo-TEM (Fig 2). Reactive functional groups were quantified by a colorimetric assay. The molecular weight and composition of the polymer brushes were also evaluated, applying Size Exclusion Chromatography (SEC) and H-NMR to polymers present in the supernatant during synthesis.
This new coating provides easy protein grafting, reduced non-specific interactions, and increased colloidal stability, in particular in the presence of salts.
The prepared particles were first tested for their ability to support nucleic acid assays. Aminated oligonucleotides were covalently linked to polymeric magnetic beads and complementary oligonucleotides labelled with an FITC-probe were hybridized. Efficiency of oligonucleotides capture was determined by UV-visible spectroscopy, fluorescence spectroscopy and fluorescence microscopy and compared to magnetic particles activated by conventional carbo-diimide coupling.

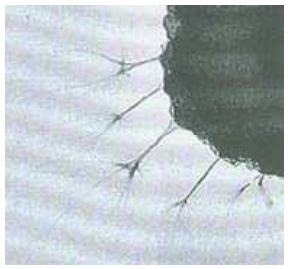

Fig 1 TEM images of polymer brushes from 1µm magnetic particles

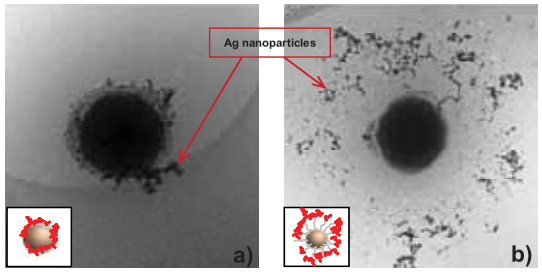

Fig 2 Cryo-TEM images of silver nanoparticles prepared **a)** on 500nm magnetic particles without polymer and **b)** on polymer coated magnetic particles (17500 magnification)

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Magnetic Fluids for Glioblastoma Cancer Treatment

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Despite the success of magnetic hyperthermia, up to date magnetic nanoparticles are rarely used to treat specific cancers which are buried deep in the body or show unusual cell mobility. Among those cancers are glioblastoma. The main problem of these tumours is that they diffusely invade the brain of a patient and therefore cannot be completely resected neurosurgically. A completely new therapeutical approach is to exploit the exceptional migratory propensity of glioma cells. The aim is to load the tumour cells with magnetic nanoparticles and induce directed migration by applying a gradient magnetic field. If tumor cells can thus be directed to a defined location, it is conceivable that this would increase their amenability to surgical resection or hyperthermia.

In a first step, the proper source of magnetic nanoparticles was searched among magnetic fluids with sterical stabilization based on double coating of magnetite with different surfactants in water [1] including citric (CA+CA), oleic (OA+OA), myristic (MA+MA) and lauric (LA+LA) acids. Main aspects of the given investigation were the structural characterization of magnetic fluids, their stability in the cancer cell culture, toxicity and absorption by the cells. Structure analysis comprising magnetization, transmission electron microscopy and small-angle neutron scattering showed that a part of magnetite particles in the fluids (size ~7 nm, polydispersity 40%) form stable aggregates with the mean size up to 40 nm depending on the type of the surfactant layer. The magnetic fluids (volume fraction of dispersed magnetite within 1-3 %) were added to the culture medium (Dulbecco modified Eagle medium Glutamax supplemented with 10 % fetal calf serum), where brain cancer cell lines G44, G55-T2, G87, G112 (UMC Hamburg Eppendorf) were incubated for three days at 37°C. The incorporation of nanoparticles into cells was determined via magnetic cell separation, atomic absorption spectroscopy, fluorimetric measurements, as well as Berliner Blue staining. The cytotoxicity varied with the stabilization layers. A clear preference of LA+LA coated nanoparticles was found for all cancer cell lines. The difference in the nanoparticle absorption by the cells was related to structural features of the initial magnetic fluids. The penetration ability of magnetite into the cells strongly depends on the aggregation rate in the fluids. This explains high absorption of LA+LA stabilized nanoparticles and might also be the reason of their lowest cytotoxicity for the cells.

(*a*) (*b*)

Fig.1. Absorption of magnetite nanoparticles by glioblastoma cancer cells G55-T2 incubated in the culture medium with 0.5 µg/mL content of magnetic fluids
(*a*) MA+MA and (*b*) LA+LA.

The work is done in the frame of the project Helmholtz-Russian Joint Research Group (RFBR-Helmholtz - HRJRG-016). It has been also partially supported by the National Research&Development Program II of the Romanian Ministry of Education and Research, through the project nr. 71-068/2007 NANOMAGPOLI.

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THE FIRST STEPS ON WAYS TO OPENING THE MECHANISMS CELL REGULATION BY NANOTECHNOLOGY PREPARATIONS

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The synthesis and study of nanotechnology preparations in XXI century is the great extent actuality.

At now officially medicines following products nanotechnologies are applied in Ukraine: "Micromage-B" by way of biologically active additive and magnet-controlled sorbent (MCS-B) for extracorporal detoxication of biological liquids. A basis of the given preparations makes of nanoparticle magnetites (Fe_3O_4) size from 6 till 12 nm. 300-400 kA/m is value of magnetic field which inducing by nanoparticle magnetites.

In this scientific work for the first time are make an attempt investigate mechanisms action of nanoparticles magnetite on cell regulation and metabolism as a whole.

Material: the nanoparticles of magnet-controlled sorbent (MCS-B).

Object of research: erythrocytes and leucocytes the patients of blood.

Is established, that action point of nanoparticle magnetites are proteins superficial cells membrane.

As a result of research is established: nanoparticle magnetites (MCS-B) had sorption activity about to proteins superficial cells membrane spectrine on 7±1 %, ancirine on 3±0.5 %.

After processing blood by nanoparticle magnetites (MCS-B) was discovered restoration regulator mechanisms metabolism of cell at the patients with syndrome intoxication. Normalization of a level POL and parameters antioxydation system (AOS) was evidenced of blockade oxidizing stress. On the contrary after processing blood by magnetite particles, restoration of metabolism cell was not discovered at the patients terminal. It is possible this effect explainable of high level intoxication, damage metabolism apparatus of cell, substratum metabolism depletion (tab. 1).

Probably, the influence by nanoparticle magnetites was caused, on the one hand, the properties nanoparticle magnetites sorption selectively of molecule proteins from a surface membranes cell; with another, probably, change of conformation structure proteins of the superficial membranes as a result influence of magnetic field which induced by nanoparticle magnetites.

Table 1. Parameters of metabolism processes in leucocytes in various persons (M±m)

The metabolism products of leucocytes	Health persons (n=12)			Patients with syndrome intoxication and insufficiency of polyorganic (n=10)			The terminal patients (n=6)		
	Initial date	After to process by MCS-B	P	Initial date	After to process by MCS-B	P	Initial date	After to process by MCS-B	P
Glycogen, mg/10^{10} cell	51.4±0.5	32.4±0.3	<0.001	32.3±0.4	21.4±0.5	<0.001	27.6±0.4	23.0±0.3	<0.001
Phospholipines, mgx10^6 cell	2.5±0.2	3.4 ±0.1	>0.05	2.1±0.2	3.9±0.1	<0.05	1.8±0.1	1.9±0.2	>0.05
Total protein, mcgx10^{10} cell	36.2± 0.4	44.2±0.5	<0.001	32.3±0.3	47.6±0.5	<0.001	28.6±0.3	29.3±0.4	>0.05
Total lipids, mcgx10^6 cell	98.1±0.6	92.0±0.5	<0.001	89.8±0.6	74.2±0.5	<0.001	84.6±0.8	83.1±0.7	>0.05
Creatinine, mgx10^9 cell	1.01±0.2	2.3±0.1	>0.05	3.1±0.2	3.8±0.1	>0.05	3.4±0.1	3.0±0.2	>0.05
Glutathione (restored), mgx10^{10} cell	3.2±0.2	4.2±0.1	<0.001	2.7±0.1	4.7±0.2	<0.001	1.0±0.1	1.0±0.2	>0.05
Hexocynase, mmol/1hx10^{11} cell	9.5±0.2	11.4±0.2	<0.001	7.7±0.1	10.8±0.2	<0.001	6.3±0.1	6.8±0.2	>0.05
Lactate DG, mmol/1hx10^{11} cell	542±10.5	611±11.4	<0.001	467±10.6	591±11.1	<0.001	348±12.1	330±10.9	>0.05
Gl-6PH-DG, mmol/1hx10^{11} cell	500±10.6	302.4±11.2	<0.001	378±10.8	187.3±12.0	<0.001	278.6±10.1	211.5±10.3	<0.001
Succinate DG, mmol/1hx10^{11} cell	3.8±0.1	6.9±0.2	<0.001	2.7±0.1	6.7±0.2	<0.001	2.0±0.2	1.6±0.1	>0.05
MDA, nmol/mg	22.9±0.5	30.6±0.7	<0.001	32.5±0.5	34.4±0.7	<0.05	36.8±0.5	37.1±0.6	>0.05
DC, nmol/mg	190±10.3	256.8±10.5	<0.001	197±11.2	261.4±12.1	<0.001	205.7±10.3	200.1±10.7	>0.05
SOD, IUx10^6 cell/h	0.08±0.02	0.18±0.04	>0.05	0.12±0.03	0.28±0.02	>0.05	0.22±0.03	0.22±0.02	>0.05

Magnetizable 3D matrix biomaterial for cell therapy.

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The aim of this work is to elaborate functionalized cardiovascular biomaterials which are magnetically-assisted for tissue engineering and cell therapy. Magnetic labelling of cells makes them responsive to a magnetic force generated by a magnetic field gradient. We propose to use this magnetic force to enhance cell seeding and cell migration efficiency in a biocompatible 3D matrix.

This 3D matrix is a hydrogel prepared from polysaccharides (pullulan/dextran), the cross-linking process uses sodium trimetaphosphate in aqueous solution. We synthesize alginate beads containing magnetic nanoparticles and biopolymer extracted from algae, sodium alginate. In first, the synthesis used for the preparation of magnetic alginate microbeads is a micro-emulsion process (diameter ranging from 15 µm to 200 µm) in order to reduce the size of the beads. Second, we develop the preparation of 3D matrix with these magnetic microbeads. The quantity of magnetic nanoparticles could be adjusted in function of the targeted application until 10% in volume fraction. The microbeads should be dispersed directly in the matrix before polymerization so as to obtain a homogeneous distribution of these magnetic 3D matrix attractors.

Under a homogeneous magnetic field, each magnetized structure could induce local forces on surrounding cells, enhancing the efficiency of cell attachment. The distribution of magnetic attractors should in turn modulate the distribution of cells within the matrix. One major advantage of these magnetizable devices is that cell seeding, assisted by magnetic force, could be performed in vivo (instead of in vitro) after injection of magnetically labelled therapeutic cells.

Interaction of Magnetic Nickel Nanowires with THP-1 Cells and High Content Analysis of their Cytotoxic Effects.

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The interaction between nanowires and biological specimens has been attracting much interest due to the magnetic properties of nanowires and their ability to be functionalised with various antibodies and fluorescently tagged [1]. It is thus important to evaluate the interaction between magnetic materials and biological entities for future scientific and clinical applications.

Magnetic nickel nanowires, with a diameter of 200 nm and 20 μm in length, were synthesised by chemical electrodeposition into alumina templates. The experimental set-up involved an electrolyte bath containing nickel ions and a three-electrode cell with a platinum electrode, a working electrode and a Ag/AgCl (saturated KCl) reference electrode. Once deposition was complete, the membrane was dissolved in 1 M NaOH to obtain the free-standing nickel nanowires. Complete characterisation on these nanowires was achieved using SQUID, X-Ray Diffraction and Scanning Electron Microscopy. The nanowires have a face centered cubic (FCC) structure with a lattice parameter a_0 of 3.53 x 10^{-10} m. The saturation magnetisation was also found to be 40 Am^2/kg.

A time course study was successfully carried out using High Content Analysis (HCA) (Cellomics, KineticScan Reader, Thermo Fisher Scientific, USA) to evaluate the possible cytotoxic effects of nickel nanowires on differentiated THP-1 cell line-derived macrophages. The time points selected were 0, 3, 6, 24 and 72 hours with matching negative and positive controls. The nanowire to cell ratios used were 1:1, 10:1, 100:1 and 500:1.

A multiparameter analysis was effectively implemented over a series of experiments to evaluate and identify the critical time points and concentrations of nickel nanowires on THP-1 cellular response. The full content analysis involved examining cell viability, nuclear size, cell membrane permeability, cell lysosomal mass and pH.

From the results it can be seen that there is an inhibition of the cell growth response due to the increase in phagocytic activity of the THP-1 cells induced by prolonged exposure to the nanowires. Nickel nanowires appeared to have no substantial effect on THP-1 cellular response after incubation times of 3 and 6 hours regardless of nanowire concentration. However, there was a decrease in cell viability after 24 hours for high nanowire concentrations. The lethal dose time occurred at 72 hours when there was a 50 % loss in cell viability for 100 nanowires plated to every cell.

This emphasises the necessity for more rigorous health and safety regulations with regard to the future scientific and clinical application of these novel magnetic nanocarriers.

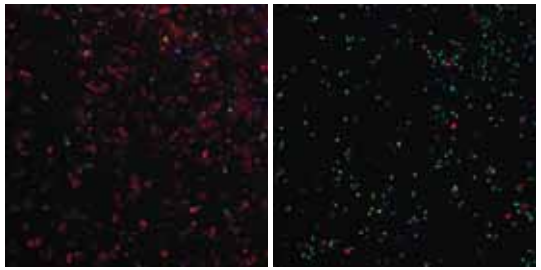

Figure 1: (a) HCA image of control well at 48 hour time point indicating healthy THP-1 cells due to large number of lysosomes present (red stain). (b) HCA image of cells plated with high concentration of nanowires at 48 hour time point. High intensity of cell permeability (green stain) and low intensity of lysosome (red stain) indicates cell death has occured.

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The Use of Static Magnetic Fields in The Prevention of The Conception: The Design and The Biological Assessment of a New Intrauterine Device

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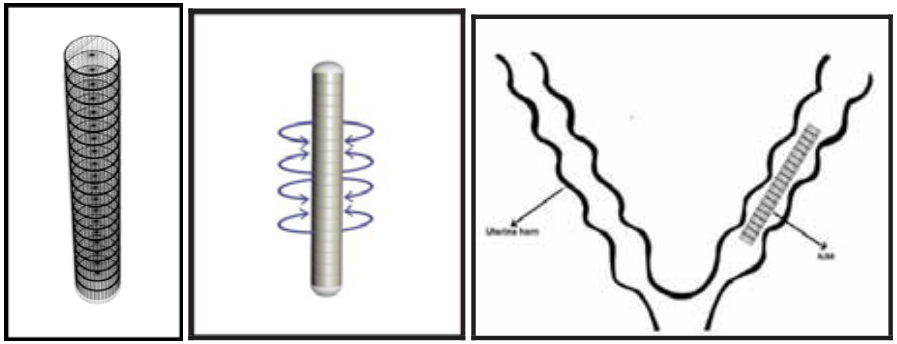
Many studies have reported various effects of magnetic fields on biological molecules and cells and some significant results have been reported in this direction. We evaluated in vitro effects of a magnet producing static magnetic field (SMF) on ejeculated human spermatozoa and also in vivo effects on endometrial histology of rats.

In vivo experiment included 20 adult female Wistar albino rats. They were divided into five groups. Group 1: sham-operated rats, Group 2: intrauterine copper implanted rats, Group 3: intrauterine uncovered magnetic rod implanted rats, Group 4: intrauterine silicone-coated magnetic rod implanted rats, Group 5: subcutaneous magnetic rod implanted rats. Magnetic orientation of ejeculated human spermatozoa and endometrial histology of rats were evaluated. In Group 2, endometrial stroma contained small amount of eosinophil and neutrophil leukocytes. In Groups 3 and 4, endometrial stroma exhibited more leukocyte infiltration than Group 2. The sperm were found partially being oriented perpendicular to the magnetic field direction.

We have demonstrated that intrauterine magnet to cause for more inflammatory cells in stroma and endometrial cavity than copper. Presence of more intense leukocytic infiltration with magnet may mean much valuable spermatozoaicidal effect and thus, more effective prevention of intrauterine and extra-uterine pregnancies.



Schematic representation of the intrauterine magnet consisted of NdFeB magnets had a diameter of 2 mm and a height of 1mm and a magnetic field intensity of 450-500 mT and the application of magnet in rat uterine horn.

CHARACTERIZATION OF A COMPACT HIGH GRADIENT MAGNETIC SEPARATOR FOR BLOOD DETOXIFICATION

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The increasing use of medicated or functionalized magnetic spheres in biomedical applications necessitates the development of technologies for selective and rapid blood removal of such spheres, i.e., to terminate toxic side effects. Applying our theoretical simulations results, we designed a compact magnetic separator device (Figure) consisting of alternating arrays of stainless steel 430 wires and biocompatible tubing which are immerged in an externally applied magnetic field. The local magnetic field gradients generated at the wire-tube intersections allow the sequestration of magnetic spheres from the freely circulating blood within the tubes. Device performance was validated *in vitro* employing blood replacement fluid (glycol-water solution) and whole blood as well as various field strengths and flow rates. We delineated that the design of our compact (i.e., portable, zero-power) separator device allowed rapid, selective, and highly efficient magnetic sphere removal (>90% single pass capture) at field strengths of <0.5T while maintaining substantial flow rates of up to ~30 ml/min. Further, prolonged (>2 hrs) blood circulation through the device did not lead to flow obstruction or cell lysis. Based on these results, we concluded that the designed compact magnetic separator device allows highly efficient, on-demand clearance of magnetic spheres from circulating blood and that subsequent *in vivo* animal studies are warranted.

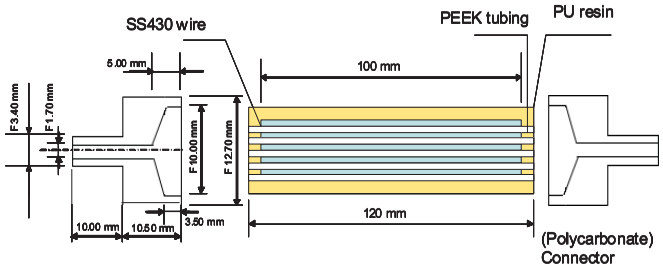

Figure. Diagram of the designed, compact separator device.

Magnetic albumin polymers as drug carriers

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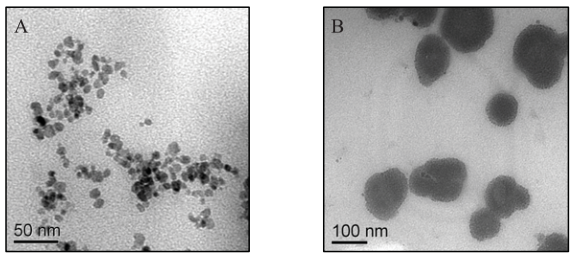
Magnetic carriers have been the focus of intense research in the last decade. As a rule, drug delivery systems may minimize the collateral effects of very toxic drugs, such as chemotherapics for cancer treatment. The use of magnetic nanoparticles in these systems may add more advantages, as for instance, reaching the target more efficiently, improving the diagnosis while permiting other biomedical applications, such as hyperthermia. The aim of the present research was to evaluate the biocompatibility and biodistribution aspects of a new sample called magnetic albumin polymers (MAPs) developed with maghemite nanoparticles encapsulated in albumin polymers (73 nm diameter) and lyophilized, with biomedical purposes.

To perform the investigation, MAPs containing 1.23×10^{13} particles were intraperitoneally injected in Swiss female mice. Cytometry analysis, viability test of peritoneal leukocytes, and histological analysis of the liver, lungs, and spleen were done from 30 minutes until 30 days after the administration of lyophilized MAPs dilluted in bovine serum albumin (BSA). Evaluation of MAPs genotoxicity and cytotoxicity in bone marrow erythrocytes were carried out from 24 hours until 30 days after the sample application. It was verified that MAPs cause light and temporary alterations in both leukocyte populations and viability of peritoneal cells, as expected for foreign materials. Interestingly, the studied sample did not present any genotoxicity or cytotoxicity effects in bone marrow erythrocytes. Further, it was not detected the presence of nanoparticle clusters in the investigated organs through light microscopy analysis. These results (absence of both DNA damage and nanoparticle clusters) were rarely observed in the *in vivo* investigations of other magnetic samples. It was not verified any histological alteration in the liver and spleen, but all animal lungs presented alveolar septs thickening and inflammatory infiltration. This adverse effect was probably caused by the diluent BSA once it was also found in BSA treated control animals.

The observed PAMs biocompatibility is probably due to the presence of albumin, the main serum protein. The results sugest that the MAPs presents high potential to the treatment of cancer. Cancer cells overexpress some receptors to albumin and may catch more effectively the MAPs. MAPs are now under investigation to be used as a drug delivery system.



A – Maghemite nanoparticles of ionic magnetic fluid; B – MAPs

Keywords: Biocompatibility, magnetic nanoparticles, albumin.

Engineered ferrofluid for pathogen detection

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Ferrofluids have significant potential in a variety of biological applications, including pathogen sensing and cellular manipulation. One of the main challenges that still remains in this context is the difficulty to render a ferrofluid sample biofunctional without labor-intensive and lengthy chemical procedures and drastic changes in its physical properties.
Here we present a novel, rapid and labor-free biofunctionalization scheme for ferrofluids based on silica covered magnetic nanoparticles. The procedure is as easy as mixing two solutions. On the one hand, the ferrofluid comprised of cobalt-ferrite nanoparticles have been synthesized via a co-precipitation method and covered with a thin silica shell [1]. The resulting ferrofluid is then mixed with genetically engineered peptides for inorganics [2] (GEPI's); such GEPI's selected for specific and strong binding to the silica surface of the nanoparticles not only increase colloidal stability by acting as a thin surfactant, but they also enable an efficient route for rendering the ferrofluid biofunctional and biocompatible.
We have demonstrated the utility of this simple biofunctionalization process in the context of pathogen sensing via ferrofluids (figure 1 top). In experiments, avidin-coated microbeads of 3.6 µm diameter are used as pathogen stimulants, and biotinylated, silica-binding GEPI's are used to functionalize the water-based ferrofluid comprised of cobalt-ferrite/silica nanoparticles. The increase in the hydrodynamic size of magnetic nanoparticles is detected via AC-susceptibility measurements (figure 1 bottom). Only samples functionalized with biotinylated GEPI's respond to the presence of the microbeads (while controls with no GEPI's or with non-biotinylated GEPI's result in no change in signal), demonstrating the specificity of the detection mechanism. This biofunctionalization approach could be applied to other GEPI's selected for specific adhesion to different inorganic surfaces (including magnetite or cobalt-ferrite), leading to the possibility of using a wide variety of ferrofluids with different compositions in biomedical applications.

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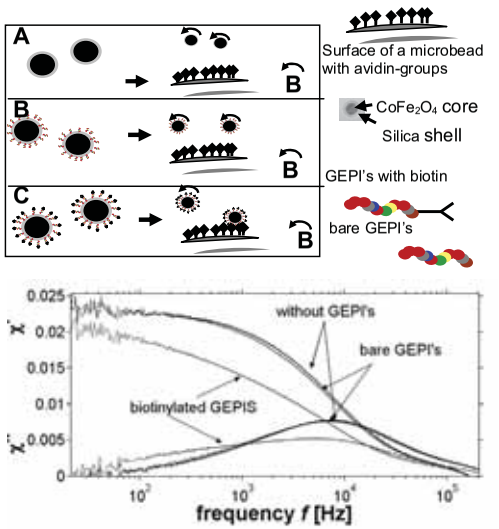

Figure 1 Top: Schematic overview for pathogen detection: The sample with no GEPI's (Sample A) and with bare GEPI's (Sample B) show no response to the avidin covered microbeads, whereas the sample with biotinylated GEPI's (Sample C) can attach to the avidin-coated microbeads. If an alternating magnetic field is then applied, the binding event can be monitored by measuring the relaxation of the particles. Binding of a magnetic nanoparticle to the surface of the large bead stops the free rotation of that magnetic particle. Binding events can then be monitored via AC susceptibility measurements (Bottom).

Magnetic nanoparticles for protein purification and water treatment

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Drinking water is a scarce world resource and the treatment of water to render it fit for human consumption has become a significant problem, both in developing and developed countries. An efficient and easy way to purify water is to use natural coagulant proteins, for example from *Moringa Oleifera*, a plant growing in semi-arid tropical and subtropical areas.
Our work is focused on developing an efficient water purification system based on superparamagnetic nanoparticles with coagulant protein on the surface.

A stable colloidal suspension of magnetic nanoparticles with narrow size distribution was prepared through co-precipitation method in water. In order to allow protein binding on the surface of the nanoparticles different surface modification routes were studied, namely silica, carboxylic and amino groups. Several characterization techniques were used to fully characterize the nanoparticles' morphology, size, composition and surface charges.
The surface modified nanoparticles were then used for the purification of the coagulant protein from crude extract of Moringa protein by magnetic separation. After a few steps, the elution procedure was undertaken in order to collect the purified protein.

In order to prove the possibility of using proteins attached to magnetic nanoparticles for water purification, we performed some studies to understand the binding of protein *Moringa Oleifera* on nanoparticles with different surface groups. Nanoparticles with carboxylic surface groups presented the best binding capacity towards *Moringa Oleifera* protein. These nanoparticle-protein assemblies were then used for activity studies and water purification experiments were performed to remove kaolin clay particles from dirty water.

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Keywords: Nanoparticles, Moringa Oleifera, Water purification

Bi-functionalization of $\gamma Fe_2O_3@SiO_2$ core-shell nanoparticles for enzyme conjugation

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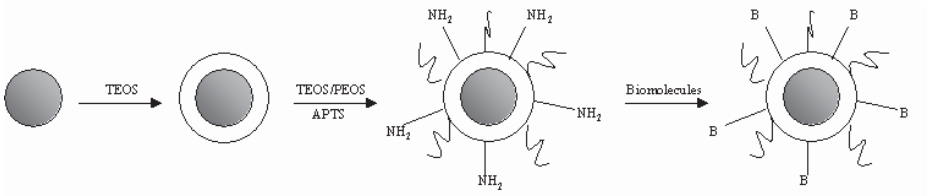
Many research groups have demonstrated their interest for immobilization of bio-molecules onto magnetic nanoparticles (MNP). These hybrid materials are supposed to provide a great improvement of bio-molecules transport.

We describe here the synthesis of γ-$Fe_2O_3@SiO_2$ core-shell nanoparticles functionalized at the same time by primary amino groups and PEG-chains. These functionalized MNP particles are slightly polydisperse, with a mean diameter around 40nm. They stay dispersed as individual objects in aqueous solutions between pH 4 and pH 8. The PEG chains ensure at the same time colloidal stability and *in vivo* furtivity, while the presence of amino groups onto the particles surface allows the covalent anchoring of biomolecules. Condensation of silane derivated compounds have been carry out in a two steps procedure without any surfactant and with relatively high nanoparticles concentration.

After a first amination step with glutaraldehyde, enzyme conjugation is performed in the presence of a soft reducing agent like $NaBH_3CN$. The enzyme functionalized MNP are finally dispersed in aqueous solutions buffered by the MOPS buffer (100mM, pH 7.4). Enzymatic activity after bio-conjugation has been studied by UV-spectroscopy and the activity of the MNP conjugated enzyme was compared to free enzyme activity. Enzymatic works were based on the Michaelis-Menten model. Preliminary analyses show the preservation of the catalytic properties of the enzyme after conjugation to MNP indicating that the covalent anchoring does not alter neither enzyme conformation nor the active site.



MNPs MNP@SiO_2 MNP@SiO_2-PEG/NH_2 Biomolecules conjugation

The bioavailability of magnetic nanoparticle: in vitro and in vivo study

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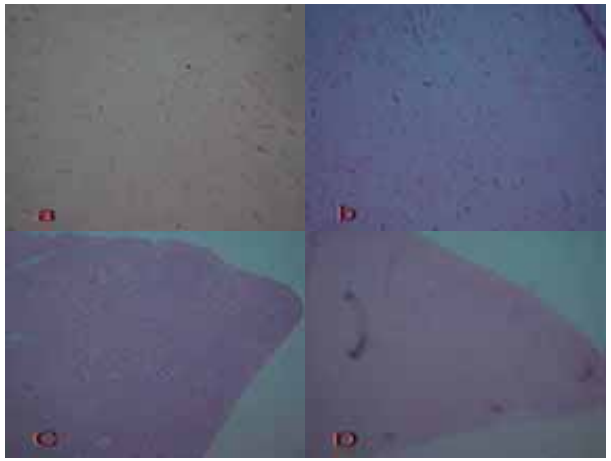
Abstract:

This study investigates the bioavailability of carboxymethyldextran-coated magnetic nanoparticles (CMD-MNP). In vitro study, we analyzed the cytotoxicity of CMD-MNP in murine fibroblast using MTT assay as well as agar diffusion method. The nanoparticles demonstrated good cell viability even in high concentration (10^5nM). In vivo study, we designed the external magnet on rat brain and investigated the magnetic effect on biodistribution. The CMD-MNP was injected via external jugular vein and the organs were taken 2 or 24hrs later. Prussian blue stain revealed the nanoparticles increased aggregation and quantity in rat brain and reduced retained quantity in liver, spleen, lung and kidney after application of external magnet on brain. The magnetic effect was time dependent.



a: brain without magnetic effect 24hrs later. b: brain with magnetic effect 24hrs later. c: liver without magnetic effect on brain 24hrs later. d: liver with magnetic effect on brain 24hrs later.

Magnetic Microspheres as versatile Tool for simple and quick removal of Endotoxines and bacterial DNA

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The removal of bacterial endotoxines is an important factor in producing recombinant proteins. Endotoxines consisting of lipopolysaccharides (LPS) and also bacterial DNA can cause pathophysiological reactions *in vivo* and in cell cultures. For the preparation of proteins, an almost complete removal of these contaminants is necessary.

The modification of superparamagnetic microspheres with polycationic ligands (Endotoxin Removal Beads) allows very strong interactions with polyanionic substances like LPS and DNA. In combination with the magnetophoretic mobility and the good kinetic properties, these particles offer a good approach to eliminate contaminants and retain a high yield of the target protein.
Due to a bead size of about 1.5 µm and a very large surface area, the capacity of 70,000 Endotoxin Units (EU) or 7,000 ng DNA per mg of Endotoxin Removal Beads is very high. The yield of the retained protein is depending on the net charge of the target protein and the pH value of the used buffer. Therefore, in a model assay with BSA as protein and a contamination level of 10,000 EU and 5,000 ng DNA per mg protein, a depletion of 99.0 % of Endotoxin and 96.4 % of DNA could be achieved while more than 90 % of BSA was retained.

The combination of the particles with the MACSiMAG™ Separator allows a quick and simple processing of sample volumes of up to 80 mL in about 60 minutes.

Fig.1: Separation of Endotoxin Removal Beads in MACSiMAG™ Separator

Automated isolation of total mRNA from plant cells affected by cadmium(II) ions using paramagnetic particles

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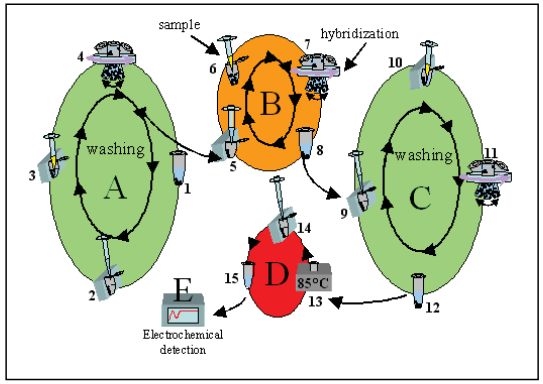
Easy, efficient and low demanding separation of mRNA from biological material is needed to study gene expression and to use in chip technologies. To rapid and selective isolation of mRNA new approaches using modified surface of paramagnetic beads have been developed. Each mRNA molecule contains sequence of 25 adenines. This feature can be used for binding of mRNA on the surface of the beads coated by thymine chains. Based on the recently published findings it can be assumed that the expression of genes responsible for stress proteins biosynthesis corresponds to exposition of organism to stress, abiotic as well as biotic. Thus total mRNA (transcriptome) analysis could be suitable indicator of stress response. In this work we are focused on total mRNA isolation by using of paramagnetic beads oligo(dT)25 (DBT). Total mRNA was isolated from the cells of early somatic embryos of Norway spruce (*Picea abies* (L.) Karst.), clone 2/32. These embryos were cultivated in cell suspension culture supplemented by cadmium(II) ions 0, 50, 100, 250 and 500 µM for 11 days. The isolated mRNA was further determined by cyclic voltammetry. Primarily it was necessary to optimise as well as automate the process of mRNA isolation by DBT. The figure shows the optimized procedure of isolation of total mRNA, which minimized the interference caused by human operator during mRNA isolation from a biological sample. Under well controlled shaking, centrifugation and temperature of the hybridization the effectiveness of mRNA isolation increased from 10-15 % at the beginning optimization up to 60-75 % at the end of this process. The optimized procedure using DBT enabled us to analyse 30 biological samples within 6 hours. We determined that the exposure of the embryonic suspension cell cultures of spruce to cadmium(II) ions enhanced the total mRNA about 10 % at the second day of the treatment. Then the level of total mRNA decreased. The amount of isolated mRNA was within the range from 30 to 300 µg/ml. Paramagnetic particles coupled with electrochemical detection represents suitable tool to quantify the total mRNA in biological samples rapidly and effectively.



Isolation of total mRNA from a sample by using of paramagnetic beads oligo(dT)25.

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Development and Characterization of CD34-Conjugated Nanoparticles and a Magnetic Bone Marrow Biopsy for Evaluating Acute Leukemia

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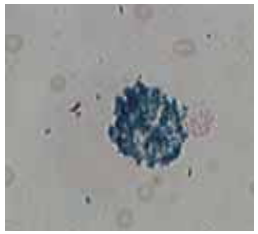
Acute leukemia is a malignant cancer of the blood and bone marrow and a disease in which early detection is key to improving survival. The current standard for diagnosis and reevaluation of leukemia is a bone marrow biopsy. The absolute and relative detection sensitivities of a standard bone marrow biopsy are approximately $1x10^5$ blast cells and 1% blast cells, respectively. This limitation is due to nontargeted sampling. The use of antibody-conjugated superparamagnetic iron oxide (SPIO) nanoparticles against acute leukemia antigens coupled with a "magnetic needle" presents a powerful option for targeted sampling. Both high and minimal CD34 expressing cell lines were used in this study with anti-CD34-conjugated SPIO nanoparticles (SiMAG-TCL, chemicell, Germany). Various timecourse conditions were used to ascertain optimal binding parameters. Microscopy, Superconducting Quantum Interference Device (SQUID) magnetometry, and *in vitro* magnetic needle extraction were used to assess nanoparticle-cell binding.

The CD34-conjugated nanoparticles were shown to preferentially bind high CD34 expressing cell lines as identified with Prussian blue aided microscopy and SQUID measurements. The newly developed magnetic needle exhibited the capacity to isolate CD34 positive cell lines from non-malignant cells from peripheral blood *in vitro*. Furthermore, the magnetic needle collected blast cells bearing magnetic nanoparticles at relative levels of below $5x10^4$ cells and absolute levels of below 0.7% which, at the minimum, meet levels of conventional bone marrow biopsy. These data suggest the potential to increase the sensitivity of bone marrow biopsy using antigen-targeted magnetic nanoparticles and a magnetic needle for the evaluation and diagnosis of acute leukemia.



Photomicrograph of CD34-conjugated NP's bound to a human leukemia cell collected with the magnetic needle. The slide is stained with Prussian blue for the presence of iron-oxide nanoparticles, which appear blue.

Senior Scientific, LLC, acknowledges the support of the National Institutes of Health. This work was performed, in part, at the Center for Integrated Nanotechnologies, a U.S. Department of Energy, Office of Basic Energy Sciences user facility. Sandia National Laboratories is a multi-program laboratory operated by Sandia Corporation, a Lockheed-Martin Company, for the U. S. Department of Energy under Contract No. DE-AC04-94AL85000.

Immunomagnetic carriers for microfluidic analytical device to capture and concentrate of A• peptides or Tau proteins, the most valid biomarkers of Alzheimer disease

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The project aims to prepare the highly effective immunosorbent based on superparamagnetic micro/nanospheres for magnetically active microfluidic device. This system derives benefit from self-organized micro/nanoparticles in compact microcolumn with auto-calibrated micron-sized pores [1]. It combines a high loading capacity and fast affinity reaction, thanks to a high surface to volume ratio and extremely thin diffusion layer. The goal in the construction of an efficient immunosorbent was to immobilize the antibody to the solid-phase support without adversely affecting the antibody's function to capture antigen. The high steric accessibility of the binding sites, high operational and storage stability of binding activity and low non-specific sorption of protein were the criteria for validation of carrier. Carboxylated maghemite silica non-porous microparticles (SiMAG-Carboxyl, Chemicell, Germany, 1µm) and hydrazide maghemite silica non-porous microparticles (SiMAG-Hydrazide, Chemicell, Germany, 1µm) were carriers utilized for rabbit polyclonal IgG to β-amyloid (1-14 AA) or rabbit polyclonal IgG to Tau protein (S396) immobilization. Binding efficiency was approximately estimated by difference of absorbances before and after immobilization ([1.55*A(280 nm)]-[0.77*A(260 nm)] and by SDS-PAGE. About 15 % of total amount of IgG were orientedly immobilized. The binding activity and specificity of prepared immunosorbents were validated by of β-amyloid antigen 1-42, synthetic peptide (Appronex, Czech Republic) or Tau protein (S369), (Abcam, UK). The problem with tendency of β-amyloid antigen 1-42 to aggregate was solved. The 1-3 M Urea disrupts undesired precipitation of antigen; this low level of chaotropic compounds in equilibration buffer does not disturb the immunocomplex. The original samples of β-Amyloid and Tau protein, elution fractions were analyzed by improved electrophoretic separation Tris-Tricin-SDS-PAGE-urea [2] and MALDI-TOF-MS. The immunosorbents with anti-Aβ or anti-Tau IgG molecules immobilized orientedly to the magnetic particles were prepared and provided for microchip application.

Acknowledgements
This work was supported in part by the Ministry of Education of Czech Republic (MSMT 0021627502) and by the E.C. project "NEUROTAS" No. 037953.

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Detection of DNA Hybridization Using a New Type of Electrochemical Sensor

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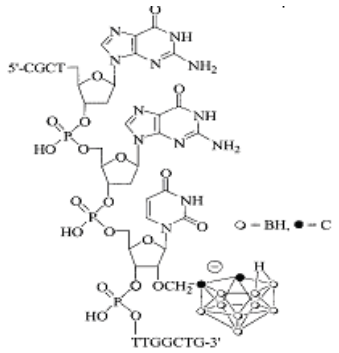
We present electrochemical detection of DNA hybridization connected with commercial paramagnetic beads and a carbon paste electrode. Our electrochemical analysis is based on the adsorptive transfer stripping technique, which is combined with other electrochemical methods to increase sensitivity and achieve lower detection limits in different electrochemical sensors (1). We have found that carborane cluster bound to oligonucleotide chain (example shown on the scheme) (2,3) can be used as a suitable electrochemical redox label of DNA hybridization.



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An Improved Method of Phage DNA Isolation Using Magnetic Microspheres

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Staphylococcus aureus is a major human bacterial pathogen capable of causing a wide range of infections. The invasive potential of *Staphylococcus aureus* strains depends on the presence of virulence factors. Several bacterial virulence factors are carried on genetic elements such as phages. Molecular biology-based methods have been developed for *Staphylococcus aureus* identification. The aim of this contribution was focused on the isolation of PCR-ready DNA from small volumes of phage lysates. High-titre (10^9 particles per ml) and low-titre (10^3 particles per ml) phage lysates of bacteriophages f77 and f53 were used for sample preparation and DNA isolation. The release of phage DNA from phage heads was performed using proteinase K. The conditions of sample preparation and proteinase K treatment (Ca^{2+} concentration, incubation time) were optimised. PCR-ready phage DNAs were purified by magnetic particles containing carboxyl groups on their surface. The adsorbed DNA was eluted by TE buffer and used as matrix in multiplex PCR for phage DNA identification. The DNA extracted using magnetic microspheres was amplified in all cases.

Enhanced Detection of Gametocytes by Magnetic Deposition Microscopy Predicts Higher Potential for Malaria Transmission

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Background – Hemozoin crystals are naturally occurring, biosynthetic magnetic particles in malaria infected red blood cells and can be utilized for malaria diagnosis by magnetic separation. Aggregated hemozoin crystals within malaria-infected erythrocytes confer susceptibility of parasitized cells to a magnetic field. Exploiting this property we have demonstrated that trophozoites, schizonts and gametocytes, and all four human malaria species infecting nonhuman primate hosts were concentrated on microscope slides by magnetic field capture. Here we assess the utility of this method for diagnosis of human malaria in an endemic region of Papua New Guinea (PNG).

Methods and Findings – Individuals observed with *Plasmodium falciparum* malaria symptoms (n=55) at local health centers provided samples for malaria blood smear diagnosis and magnetic deposition microscopy (MDM). Blood samples were applied to transparent polymer slides exposed to a high gradient magnetic field (Bmax=1.8 Tesla) under constant flow (8.3 µL/minute). Standard Giemsa staining and light microscopy was performed to evaluate parasites. *P.falciparum* parasitemia observed on MDM slides was significantly higher than parasitemia observed by conventional blood smear (CBS). Within individual blood samples MDM detected consistently elevated ring (CBS = 2.6 vs. MDM = 3.4%; t-test P-value = 0.13), trophozoite (CBS = 0.5 vs. MDM = 1.6%; t-test P-value = 0.01), schizont (CBS = 0.003 vs. MDM = 0.1%; t-test P-value = 0.08) and gametocyte (CBS = 0.001 vs. MDM = 0.4%; t-test P-value = 0.0002) parasitemias. Among the 55 study participants, gametocyte prevalence determined by CBS compared to MDM increased from 7.3 % to 45 %, respectively.

Conclusions – MDM increased detection sensitivity of *P. falciparum*-infected, hemozoin-containing erythrocytes from infected humans while maintaining detection of ring-stage parasites. Gametocyte prevalence 5-fold higher than observed by CBS suggests a higher malaria transmission potential in PNG endemic sites compared to previous estimates.

New Approach for Applications of Asynchronous Rotation of Magnetic Microspheres

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Asynchronous rotation of magnetic particles has many potential applications, including measurement of local magnetic fields, magnetic particle characterization, viscosity, and pathogen detection -- all of which can be done in real time (Applied Physics Letters **91**, 224105 (2007)).

One of our technological and research goals is to develop an accurate, easy to use, low power, and portable device that utilizes a magnetic field to asynchronously rotate magnetic particles. This device could be used in many different applications with only minor modifications. This poster will discuss progress toward this device as well as the applications of asynchronous rotation.

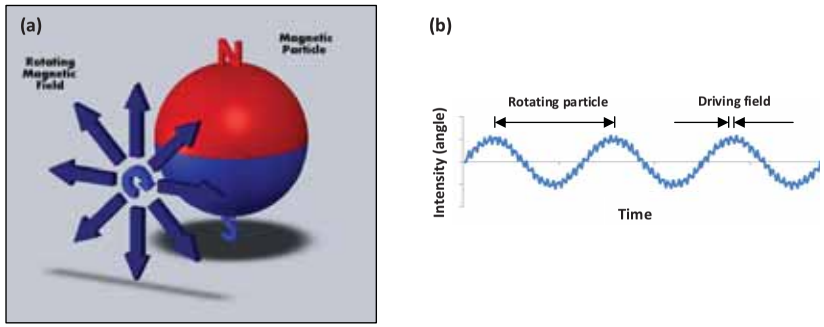

Figure 1: (a) Schematic of a magnetic particle in a rotating magnetic field. (b) Schematic plot of a magnetic particle slowly rotating and rocking back and forth in faster rotating driving magnetic field.

Hemin Immobilization on Iron-Based Composites, its Desorbtion and Oxidant Activity

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It is known that hemin has shown a cytotoxic action on tumor cells, which is explained, evidently, by its oxidation activity. That is why it was interesting to carry out hemin immobilization on magnetic microparticles and to study its desorption and oxidation activity.

We have worked out immobilization methods and suggested hemin immobilization mechanisms on microparticles of iron-based composites of different chemical content: iron-carbon, magnetite, restored-iron. Bovine hemin immobilization on iron-carbon composites was carried out by physical absorption in alkaline solution (pH 10.5) with further coating of the particles by gelatin or albumin; on magnetite and restored iron - by conjugation with these proteins. Some of the hemin immobilization experiments on particles were carried out in acidified hemolizates of bovine blood. The highest sorption capacity of 121.4 mg/g for hemin was reached by the iron-carbon composite. Hemin desorption in the alkaline and alcohol-alkaline solutions reached 65% and 95%.

The dynamics of immobilized hemin desorption in a model biological liquid (0.6% albumin in physiological solution, pH 7.2) was studied during 4 days. It was shown that the highest hemin desorption immobilized on the iron-carbon particles by absorption with further coating by gelatin was 38.0% and was reached after 2 days. Hemin desorption immobilized on the iron particles by gelatin conjugating during 2 days didn't exceed 4.2%. For elucidating the conjugating mechanism of hemin with gelatin, we conducted the reaction of conjugation in the solution and have got a new compound hemigelatin. IK-spectrum analysis of hemigelatin has demonstrated the covalent coupling hemin with gelatin.

Hemin influence on the kinetic of limonene fluid phase oxidation was studied. Hemin on a dose-dependent manner in 2 and 4 times correspondingly exceeded the speed of limonene oxidation, i.e. manifested oxidation activity.

Switching Characteristics of Magnetic Spin-Valve Traps for Magnetic Bead Manipulation in Microfluidics

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Magneto-optical Kerr effect (MOKE) was used to measure the switching properties of spin-valve arrays as a function of size, array spacing, and composition. The device structures consisted of Ta (5 nm)-NiFe(15 nm)-CoFe(5 nm)-Cu(10 nm)-CoFe(5 nm)-NiFe(15 nm)-IrMn(10 nm)-Ta(5 nm) multilayers sputtered on nitride coated Si substrates. The films were deposited in field to obtain an easy axis along the long dimension of the spin-valves and to set the pinning layer. The wafers were patterned with the following spin-valves sizes: 0.5 μm x 2 μm, 0.5 μm x 3 μm, 1 μm x 3 μm, 1 μm x 4 μm, 2 μm x 4 μm, 2 μm x 5 μm, and 3 μm x 6 μm. In addition to multiple spin-valve sizes, the spacing between spin-valves was varied to determine the minimum required spacing to prevent dipole interactions amongst the arrayed spin-valves. The high field and low field switching fields were determined using the MOKE data as shown in the graph below.

Recently, addressable arrays of magnetic spin-valve traps in combination with paramagnetic beads have been proposed as a potential tool for nanometer-scale biomolecule manipulation.[1] Our data is being used to optimize the spin-valve array layout for micro paramagnetic bead manipulation on a microfluidic platform where each spin-valve device acts as a bistable magnet that can be switched on or off. Future plans include scaling down the platform to control nanometer beads. Nanometer-scale manipulation, using torsion and linear extension, is crucial to the study of the mechanics and dynamics of single biomolecules as well as inter-molecular interactions between multiple molecules.

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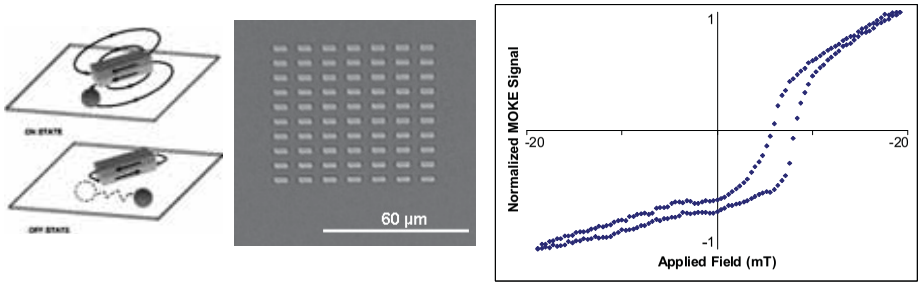

Left: Concept of spin-valve trap for magnetic bead control; **Middle:** Array of 2 μm x 6 μm spin-valves and schematic of spin-valve on/off states; **Right**: MOKE easy-axis, high-field hysteresis curve for a 7 x 10 array of 2 μm x 6 μm spin-valves. Data is averaged over 100 cycles.

A rapid and trace quantitive immunoassay of *Salmonella dubolin* by the magnetosome conjugated antibody

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In this study, we aimed to capture the salmonella cells in food samples based on conjugation of BMPs (bacterial magnetic particles) and anti-salmonella antibodies, and detect the compound of BMPs-Ab-salmonella by real-time fluorescence quantitative PCR. The anti-*Salmonella* antibodies and BMPs were conjugated by heterobifunctional reagent N-succinimdyl-3-(2-pyridyldithio) propionate (SPDP). In addition, Bis (Sulfosuccinimidyl) suberate (BS3) and biotin-avidin were also used as cross-linking reagents to compare the conjugation efficiency with SPDP. The conjugation conditions including the ratio of BMP and antibody, the quantity of the linker, the deoxidization of DL-Dithiothreitol (DTT) and the reaction time were optimized respectively. It was found that the best linker is SPDP and the superior conjugation efficiency reached 682 μg/mg (antibody/BMPs).

The compound of BMPs-antibodies were added to suspension of a small quantity of *Salmonella dubolin* and tried to capture the cells. The BMP-Ab-salmonella complexes were then assayed by real-time fluorescence quantitative PCR, using primers and probes specifically targeting the gene *fimY* of *Salmonella* genus. The results showed that approximately 50% of cells in the suspension were captured by the compound, and the detection limit was 4×10^2 cells ml^{-1}. To investigate the speciality of the capture, equal quantity of *Vibrio parahaemolyicus* was added to the salmonella suspension as disturbing cells. No cross-reactivity between the compound and *Vibrio parahaemolyicus* was detected, while the capture efficiency was decreased to 29%, and the detection limit was 4.5×10^3 cells ml^{-1} for the mixed suspension.

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Comparative study of toxicity between cell line J774-A1 and a cell line B16-F10 using a cationic magneticliposome: a potential drug delivery system for cancer treatment

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Advances in nanotechnology have stimulated the development of innovative systems for the delivery of many drugs or new diagnosis agents. Among the different systems available, liposomes continue to be of great interest, mainly due to their biocompatibility and vesicular structure which can include a wide range of substances, either hydrophilic within the internal aqueous compartments or hydrophobic inside the lipid bilayer shell. In the last few years hybrid systems formed by encapsulating biocompatible magnetic fluids within liposomes, known as magnetoliposomes, have been investigated as new tools for hyperthermia. Photodynamic therapy (PDT) is a clinically approved therapeutic modality used for the management of several types of tumors as well as non-malignant diseases. Most of the effects of this treatment result from direct action of singlet oxygen and others reactive oxygen species. However, accumulating evidence indicates that antitumor effects are also mediated by indirect stimulation of inflammatory and immune responses. These responses include rapid local infiltration of tumors by neutrophils and macrophages accompanied by systemic release of inflammatory mediators. Therefore macrophages play an important role in immune system, every alteration in their nuclear material can alter all the biological responses and they constitute like this an adequate model for in vivo studies. In this work, we realized a comparative study the toxicity of cationic magneticliposomes (CMLs) in mouse macrophage carcinoma cells J774-A1 and in melaoma murine cells B16-F10. Maghemite nanoparticles are surface-coated with phosphate (PPT) and Chlorine e_6 ($Chle_6$) is used as a PS drug. The concentration of the PPT used was $1.5x10^{13}$ particle/ml and for the $Chle_6$ the concentration was 5μM. The CMLs was prepared by injection techniques. This drug delivery system leading to an expected enhancement of the tumor damage after minimum doses of heat dissipation and/or visible light photosensitization. The methodology used to investigate the toxicity of CMLs (CML-PPT and CML-PPT.Chl_{e6}) was the classical MTT assay. Statistical analysis was performed on the data in order to compare the differences among groups using the Newman-Keuls t-test and ANOVA. In agreement with the toxicity in the dark results found for the investigated CMLs samples it could be verified that there is no significant statistical difference between the cellular viability of the control and samples. Nevertheless the phototoxicity observed in the cell line J774-A1 is low than observed it in the cell line B16-F10 (see table 1). Therefore the macrophages showed a larger resistance that melanoma cells. Probably the cell line J774-A1 exhibited would provide some protection against phototoxicity.

Table 1: Viability cellular of J774-A1 and B16-F10

J774-A1 (Viability cellular (%))			B16-F10 (Viability cellular (%))		
CML	In the dark	With light	CML	In the dark	With light
CML-PPT	93±2	91±2	CML-PPT	93±4	97±3
CML- PPT.Chl_{e6}	92±7	28±4	CML- PPT.Chl_{e6}	89±2	5±2

Single Bacterial Cell Detection and Analysis with Nonlinear Rotational Frequency Shifts of Driven Magnetic Microspheres

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The nonlinear rotation response of a magnetic particle, suspended in a viscous fluid, occurs when a driving magnetic field, used to rotate the magnetic particle, exceeds a critical frequency. Above this critical frequency, the particle is asynchronous with the external field. This type of rotational dynamic depends on physical parameters, such as the particle's magnetic moment, the external magnetic field strength, and the rotational drag that the particle experiences.

Shifts in the nonlinear rotational frequency of magnetic microspheres offer a dynamic approach for the detection of single bacterial cells and measurement of their growth. We demonstrate this detection capability by measuring frequency shifts when an Escherichia coli attaches to the surface of a 2.0 micron magnetic microsphere, thereby affecting the drag of the system. From this change in drag, the nonlinear rotation rate was reduced, on average, by a factor of 3.8. Sequential bacterial attachments and bacterial growth were also monitored using this approach (see Applied Physics Letters **91**, 224105 (2007)). Development of a stand-alone prototype that utilizes this effect will also be discussed.

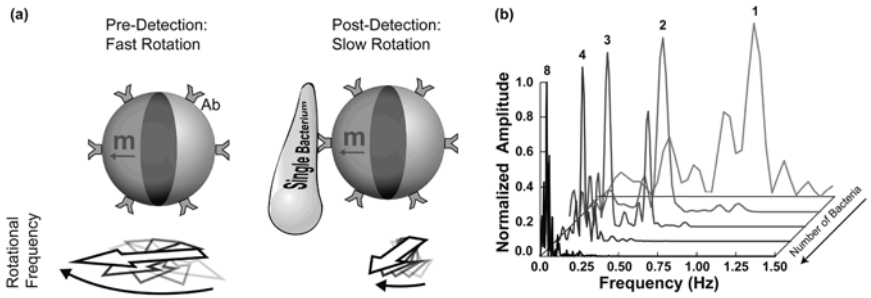

FIGURE 1: (a) Schematic of the asynchronous (nonlinear) rotation changes that a magnetic microsphere undergoes when bound to a bacterium (i.e. the rotational frequency is reduced). The magnetic microspheres are functionalized with an antibody that specifically binds to the bacterial strain of interest. (b) Power spectral density of a rotating magnetic microsphere dimer driven at 3.75 Hz, where bacterial cells were sequentially attached with total cell counts of 1, 2, 3, 4, and 8.

Morphological analysis of monkey (*Cebus apella*) liver, spleen, and lymph nodes, by optical and transmission electron microscopy following single intravenous injection of maghemite-based magnetic fluid stabilized with DMSA coating.

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To our knowledge, the present work represents the first study to research the effects of a type of magnetic fluid (MF) in an experimental model other than culture cells or *in vivo* rodent models. The aim of this study was to assess any morphological alterations in liver, spleen, and lymph nodes after single intravenous injection with maghemite (γFe_2O_4) - based MF stabilized with DMSA (2,3-dimercaptosuccinic acid) in a non-human primate model, the capuchin monkey (*Cebus apella*), bringing nanobiotechnology applications closer to an experimental clinical stage.

Samples of spleen, liver and lymph node from control animal (CA), 12 h post injection animal (EA12h) and 90 days experimental subject (EA90d) were observed by light microscopy in order to assess any possible morphological and ultra-structural changes, and by transmission electron microscopy to locate MNP in cellular structures. Samples of spleen and lymph nodes 12 h and 90 days after DMSA-MF administration showed no morphological changes. Nevertheless, for EA90d liver samples, morphological changes in the tissue structure and Disse space were detected. In all analyses performed, the DMSA-MF was internalized in cytoplasmic vesicles; however, for EA90d liver samples, secondary lysosomes were also observed.

Morphometrical tests of hepatic mitochondria were performed and analyzed for all animals. Our study suggests that there exists a clear positive correlation between mitochondrial area and administration time with DMSA-MF. For EA90d, the organelle tripled its size when compared with mitochondria from CA. This suggests a metabolic alteration in the liver.

Apparently, DMSA-MF was not proven to be completely harmless, due to the fact that hepatic alterations were attributed to the MF presence in the tissue. On the other hand, spleen and lymph nodes did not show any kind of toxicity effects to their cells and no pathological process was observed, demonstrating the biocompatibility of DMSA-MF. With this, the present study demonstrated the possibility of using maghemite-based magnetic fluid stabilized with DMSA coating as a promissory tool for diagnostic and therapeutic ends in primates, including human beings.

Magnetic Protein Microspheres as Dynamic Contrast Agents for Targeting Integrin Receptors

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Optical coherence tomography (OCT) is an emerging biomedical optical imaging modality that has been developed over the last 15 years in a number of clinical applications. Recently, OCT has been used for the intraoperative imaging of tumor margins and axillary lymph nodes in breast cancer providing a real time in-vivo assessment of the tissue morphology for the staging of breast cancer. OCT has been extensively studied in the field of cardiology. In comparison to intravascular ultrasound, OCT provides much higher resolution by at least an order of magnitude. The strengths of structural OCT as an imaging modality lie in its high-resolution (μm and sub-μm) capabilities, deep depth penetrations in highly-scattering tissue (2-3 mm), and its high acquisition rate.

As diagnostic medicine continues to push for earlier detection, the development of functional imaging modalities to probe molecular information in-vivo continues to increase. This information can be combined with the morphological information (observed under OCT) allowing for a real-time microscopic analysis of the tissue specimen. A novel functional modality of OCT called magnetomotive-OCT (MM-OCT) uses a magnetic field that is modulated at half the axial scan frequency. This modulates the OCT image between an "on" and "off" state generating an MM-OCT image that is overlaid on top of the structural OCT image. The contrast agent itself does not necessarily need to exhibit significant scattering or absorbing properties (the contrast mechanism needed for OCT) in their optical profiles in order to be detected in MM-OCT, but simply need to be able to displace nearby tissue scatterers adding dimensionality to the contrast mechanisms needed for OCT.

The contrast agent in this study designed to work with MM-OCT is a protein microsphere with an oil core and a BSA protein shell functionalized with RGD peptide sequences for targeting integrin receptors. Magnetic nanoparticles (Fe3O4) and Nile Red fluorescent dye have been encapsulated into its oil core. These microspheres have been functionalized with RGD using an LBL electrostatic adhesion process. Results show that these magnetic microspheres (2.0-5.0μm), are readily detectable under MM-OCT when embedded in a different number of environments including 5% agarose gel, a 3-D scaffold of macrophage cells previously incubated with the microspheres, and when injected in-vivo into a tumor from an NMU-carcinogen rat animal model. Current studies also confirm the increased binding affinity and specificity of these microspheres to the integrin receptors in cancer cell lines. Initial *ex-vivo* studies in an atherosclerotic rat model have confirmed their binding affinity to atherosclerotic lesions found in the aortas using histology. Ongoing studies are underway to further study the sensitivity and specificity of the RGD targeting to the α(v)β(3) receptor.

The Utilization of Magnetic Nanoparticles to Target the Pulmonary Vasculature

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A defining feature of Acute Lung Injury (ALI) is inflammation-induced disruption of the endothelial cell (EC) barrier that lines the pulmonary vasculature, resulting in leakage of fluid, protein, and cells into the airspaces of the lung. Therefore, the ability to target the lung endothelium with barrier restorative and/or enhancing agents has important therapeutic implications. We examined the use of nanoparticles composed of polylactic glycolic acids (PLGA), polyethylene glycol (PEG) with FITC, and magnetite (iron oxide, 5-20 nm) to target the pulmonary vasculature using various delivery methods (retro-orbital and intratracheal administration). The leaky blood vessels provide a unique morphologic feature to concentrate nanoparticles less than 400 nm combined with the potential of magnetic guidance to the lung region. The Zeta potential of the magnetic nanoparticles was -5.4mV, a magnetic susceptibility of 0.44 emu/g, and an effective size distribution of 300 nm. Nanoparticles containing LacZ DNA as payload were also used to confirm biofunctionality.

Fluorescent histochemical analysis of murine lungs (see Figure 1) indicates that retro-ocular administration of nanoparticles targets the pulmonary vasculature. In contrast, intratracheal administration of nanoparticles targets multiple regions of the lung. Therefore, retro-orbital administration of nanoparticles can potentially be used to target barrier enhancing agents to the pulmonary vasculature to treat diseases including ALI. A series of nanoparticle systems containing siRNA payloads and monoclonal antibody will be used to explore retro-orbital administration for selective in vivo ALI treatment.

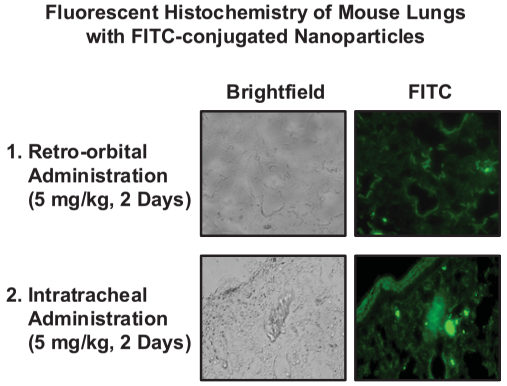

Figure 1 – Male C57BL/6J mice (8-10 weeks, Jackson Laboratories, Bar Harbor, ME) were anesthetized with intraperitoneal ketamine (150 mg/kg) and acetylpromazine (15 mg/kg) and injected with nanoparticles (5 mg/kg) retro-orbitally (1) or intratracheally (2). The animals were allowed to recover for 48 hours. The mice were then sacrificed, lungs extracted, formalin fixed, 5 micron paraffin sections were obtained and analyzed with either brightfield or fluorescent microscopy.

Sterically stabilized superparamagnetic liposomes for MR imaging and cancer therapy: pharmacokinetics, biodistribution and *in-vitro* activity on MCF-7 cell line

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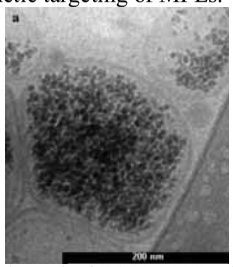
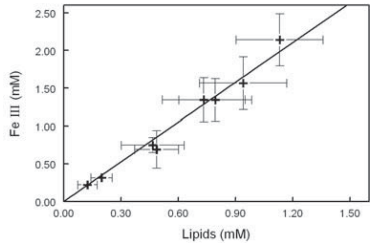
Tumor-specific drug delivery is of actual importance in cancer therapy. We have recently shown that sterically stabilized liposomes loaded with superparamagnetic nanocrystals of maghemite, referred to as magnetic-fluid-loaded liposomes (MFLs), can be successfully exploited to target different tissues *in vivo*, especially solid tumors and brain, by applying an external magnetic field gradient to the region of interest [1-4]. Now, we aim at using MFLs for targeting of a pure anti-estrogen (RU 58668) towards breast cancer tumors. RU 58668 was already shown to be efficient *in vivo* to arrest the growth of estrogen-dependent tumors when passively carried by PEG-ylated liposomes empty of magnetic fluid [5].

Pharmacokinetics and biodistribution of MFLs after intravenous administration to mice were studied by double tracking of lipid bilayer and magnetic fluid. MFLs mainly exhibited long circulation behaviour over a 24-hour period (12.6 h half-time) as intact vesicle structures that conserved their content[6]. RU loading of MFLs (3.6 mol% RU:lipids) was characterized by DSC and UV-visible absorption spectrophotometry. Three-month stability was confirmed by quasi-elastic light scattering. First evaluation of RU-loaded MFLs activity on breast cancer cells was performed *in vitro*. Toxicity and anti-estrogenic potential of free RU, non-loaded MFLs and RU-loaded MFLs towards MCF-7 cell line were compared. Our results as a whole were very promising and encouraged further *in-vivo* investigations by combining RU loading and magnetic targeting of MFLs.



Cryo-TEM micrographs of EPC:DSPE-PEG2000 (95:5 mol %) MFL with 1.8 mol of ferrofluid loaded per mol of lipids. Total lipid concentration is equal to 1 mM [from ref 1].



Variation of iron oxide concentrations versus total lipid concentrations in blood samples taken from mice at different time intervals after iv injection of magnetic-fluid-loaded liposomes [from ref 6].

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Magnetic fluids impact on vegetal tissues

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In the last decades, the impact of nanotechnology and the development of new nano-sized materials opened up the potential novel applications in biotechnology. A special interest is presented by the plant behavior in the biocompatible magnetic fluids presence. The iron oxides from magnetic fluid composition could be a source of colloidal iron for the plant development on a magnetic fluid supplemented culture medium. The biosynthesis of siderophores was assumed to be stimulated by the ferrous and ferric iron from magnetic fluid ferrophase.

Lobreaux et al., 1992 have reported that iron treatment of *Zea mays* induced ferritin protein accumulation in roots and leaves over a period of 3 days. Gonzalez et al.2008, in yours study showed that the biocompatible magnetic fluids can be uptaken into whole living plants and further can move inside using the vascular system being concentrated in specific areas by application of magnetic gradients.

The present experimental investigation was focused on the study of length plants, assimilatory pigments and nucleic acid levels in young plants, intended for agricultural use *(Zea mays*), in presence of water based magnetic fluid in the culture medium. *Zea mays* plants were cultivated ***in vivo*** conditions being treated with Fe_3O_4 nanoparticles coated with different biocompatible compounds. To study the magnetic fluids impact on plants growing, we used three magnetic fluid samples synthesized in our laboratory coated with different biocompatible compounds (β-cyclodextrin ($C_{42}H_{70}O_{35}$), citric acid ($C_6H_8O_7$) and respectively tartaric acid ($C_4H_6O_6$)) and dispersed in water. The average magnetic particle diameter was range from 8.94nm to 12.3nm.

After seeds germination, daily supply with 15ml magnetic fluid aqueous suspension per dish of sample was carried out for 12 days, plant growth being conducted in controlled conditions of temperature (22.0 ± 0.5^{o}C), illumination (dark/light cycle: 14h/10h) and 70% humidity into a climate room. Water based magnetic fluid was added daily in various volume fractions (10 – 50 – 100 – 150 – 200 – 250 µl/) in the culture medium of *Zea mays* plants during their early ontogenetic stages.

After 12 days of plant growth assimilatory pigments and average nucleic acids level in the green tissues of all experimental variants as well as in the control plants were assayed by spectrophotometric methods. The assay of the assimilatory pigments extracts in acetone 80% was performed following the Lichtenthaler & Welburn's method (Lichtenthaler et al. 1983), while the assay of nucleic acid level accordingly to modified Spirin's method (Spirin 1958, Struchkov et al. 2002) was carried out. During the experiment, we have noticed that toxicity symptoms led to brown spots covering the leaf surface for the highest magnetic fluid volume fractions, which show an oxidative stress in leaf cells as generated by the iron excess addition for all tested ferrofluids. In this case, photosynthesis may be greatly affected leading to decrease of the process rate. Magnetic fluid samples used in the experiment have disruptive effects such as the ratio chlorophyll**a**/chlorophyll**b**, ratio value decreasing to the increase of the magnetic particle diameter.

We found that for increasing volume fraction of magnetic fluid solution, the nucleic acid biosynthesis is inhibited in comparison to the control sample. Based on the presumption of magnetic fluid interference with the nucleic acid biosynthesis, the ferrophase could penetrate the nuclear membrane but the existence of extra-nuclear DNA and RNA need to be also taken into account. In this frame, the DNA from the chloroplasts is the most probable target of magnetic fluid effect in this experiment.

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Plant culture medium polluted with magnetic fluid induced oxidative stress

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The interest in the utilization of the magnetic fluids in plant biotechnology seems to increase in the latest decades. Relatively small number of studies is dedicated to the influence of magnetic fluids on the enzymes activity (Pintilei et al. 2005).

This experimental project was suggested by the assumption that iron, though is the fourth most abundant element in the Earth crust, in aerobic conditions and neutral pH, it is unavailable to living organisms, being in the form of ferric hydroxides highly insoluble. Nevertheless, iron is highly involved in the cell metabolism of either plants or animals and microorganisms, since various living species are able of iron internalization in the form of chemically specific iron ligands (iron chelates). More, the iron ions are present within the molecules of various enzymes that are involved in the energy conversion via photosynthesis or the peroxide substrate processing.

In the following study the authors report their results regarding the oxidative stress induced by a biocompatible magnetic fluid prepared from magnetite colloidal particles suspended in water.

The plant sensitivity to the magnetic fluid addition was revealed by means of the changes in catalase and peroxidase activity. Water based magnetic fluid, stabilized with citric acid (Racuciu et al. 2005) was added in various volume fractions (10 – 50 – 100 – 150 – 200 – 250 µl/l) in the culture medium of pumpkin (*Cucurbita pepo*) plantlets in their early ontogenetic stages. Saturation magnetization was of 23kA/m; the ferrophase volume fraction was 5 % while the average particle diameter was 7.9nm.

The standard spectrophotometrical assay techniques have been utilized to reveal the activity of some peroxidase like enzymes in the plant green tissues. The catalase activity was assayed by the spectrophotometric method accordingly to Beers and Sizer (Beers et al. 1952) while the peroxidase activity was measured accordingly to (Artenie et al. 1997). For catalase activity the assay method is based on the dynamics of the hydrogen peroxide consuming (spectrophotometrical measurement at 240 nm wavelength). Peroxidase activity was measured by assaying the amount of orto-dianisidine, which is oxidized by the H_2O_2 under peroxidase action (spectrophotometrical measurement at 540 nm wavelength). Soluble protein was assayed by applying Bradford method (Bradford, 1976). The average values and standard deviations for the enzyme activity of catalase and peroxidase have been graphically represented for magnetic fluid supply samples and control.

The values obtained for the enzyme activity, provided indirect evidences on the levels of hydrogen peroxide and other peroxide substrates in the young plant leaves. The catalase activity was found diminished while the peroxidase activity was found enhanced following magnetic fluid supply. Further experiments are planned to get additional information on the oxidative stress induced by magnetic fluid in vegetal tissues.

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Application of Two-phase system and Hydrophilic Magnetic Microspheres for Isolation of Bacterial PCR-ready DNA from Dairy Products

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PCR identification of bacteria in real dairy samples involves two steps: preparation of PCR-ready DNA and identification of bacterial cells by PCR. False-negative results can occur due to the presence of extracellular PCR inhibitors in the samples tested. The problem can be solved by adsorption of genomic DNA on the surface of silica or magnetic particles. An aqueous two-phase system for removal of PCR inhibitors from real samples prior to PCR was also used. The aim of this work was the application of both procedures for the isolation of DNA from dairy products. Magnetic hydrophilic poly(2-hydroxyethyl methacrylate-*co*-ethylene dimethylmethacrylate) P(HEMA-co-EDMA) microspheres employing carboxyl groups on their surface were used for the isolation of PCR-ready DNA in the presence of PEG 600 and/or PEG 6000 and 2 M NaCl. The method developed was applied for isolation of PCR-ready DNA from different fermented dairy products (yoghurt and fermented dairy drinks and hard cheeses supplemented with *Bifidobacterium* strains). The influence of the PEG molecular mass on the sensitivity of PCR amplification is discussed. PCR was performed with primers specific to the *Lactobacillus* or *Bifidobacterium* genera.

Comparison of PCR-ready DNA Isolation Methods from Lactic Acid Bacteria used in Dairy Industry

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The importance of lactic acid bacteria (LAB) for dairy industry is well known. Therefore it is important to know true taxonomic classification and relatedness among LABs used in dairy products. Increasing interest has been focused on some LAB strains that are proposed to be probiotic. Polymerase chain reaction (PCR)-based methods are specific to and sensitive for identification of microorganisms. Genome analysis involves DNA purification, amplification, and detection. The occurrence of false-negative results is a great problem in the analysis of real samples. Thus, DNA purification is an essential step. DNA was isolated from bacterial cells (*Bifidobacterium* and *Lactobacillus*) using the phenol extraction method, salting out using 6 M NaCl, and by adsorption on magnetic nonporous poly(2-hydroxyethyl methacrylate-co-glycidyl methacrylate) (HEMA-co-GMA) microspheres containing carboxyl groups. The advantage of hydrophilic microspheres consists in low non-specific adsorption of biologically active compounds. It was shown that DNA eluted from magnetic particles into TE buffer can be successfully used for PCR amplification and identification of *Lactobacillus* and *Bifidobacterium* cells in cheeses and fermented dairy products. DNA fingerprinting methods (RAPD, rep-PCR) have been frequently employed for the characterisation, discrimination, and identification of LAB strains. The reproducibility of these methods depends on DNA purity and therefore on the DNA isolation method used. The influence of the DNA isolation procedures tested on the reproducibility of RAPD and rep-PCR was also studied for this reason. The reproducibility of amplification of DNA isolated by the salting-out method and adsorption on magnetic nonporous microspheres was comparable with the phenol extraction method.

Biocompatibility of various ferrite nanoparticles evaluated by in vitro cytotoxicity using HeLa cells

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Magnetic nanoparticles have attracted attention as drug delivery systems and heat source for hyperthermia because of their reaction to a magnetic force. The aim of this study is to evaluate the biocompatibility of the nanoparticles in order to use inside of the body.

We investigated in vitro cytotoxicity of ferrite nanoparticles composed of several materials (iron, zinc and nickel) by colony efficiency and cell count. The samples were Fe_3O_4 (20~30 nm), $ZnFe_2O_4$ (15~30 nm), $NiFe_2O_4$ (20~30nm), and human cervical carcinoma cells HeLa was used for cell culture study.

In the cell adhesion study, HeLa cultured in petri dish have exposed to magnetic nanoparticles at a concentration of 0.01 mg and 0.1 mg. for 7 days. This study shows that Fe_3O_4 and $ZnFe_2O_4$ don't have much effect on cultured cells while $NiFe_2O_4$ gives serious damage to the number of cell colonies.

In the cellular viability study, HeLa was cultured in 24 well plates with ferrite particles at a concentration of 0.01 mg and 0.1 mg for 24 hours and 48 hours. This study shows that the cells cultured with nanoparticles at a concentration of 0.01 mg has high viability. However, $ZnFe_2O_4$ and $NiFe_2O_4$ at higher concentration have much effect on the number of cells.

These studies indicate that the material of magnetic nanoparticles has much effect on in vitro biocompatibility.

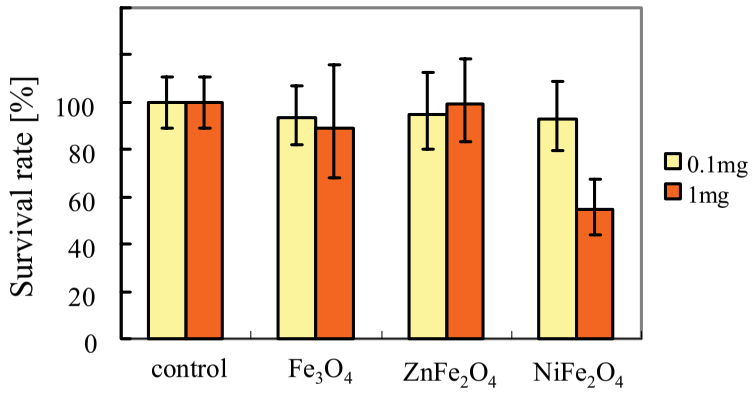

Fig. 1 Colony efficiency of cultured cells with ferrite nanoparticles.

Magnetophoretic Behaviour of Ferrofluid Droplets Suspended in Diamagnetic Viscous Media in Non-Uniform Magnetic Fields

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Biocompatible ferrofluids, comprised of magnetic nanoparticles coated with surfactants, are increasingly being investigated for use in biomedicine and biotechnology. One potential biomedical application of ferrofluids is their use in the treatment of retinal detachment [1]. To optimise the properties of ferrofluids for such applications, it is important to understand how droplets will behave in a fluid in response to an applied magnetic field.

We have developed an instrument for studying the motion and shape of ferrofluid droplets suspended in diamagnetic viscous media in the presence of a controlled applied magnetic field and field gradient. A schematic diagram of the instrument is shown in Fig. 1. The instrument consists of two main coil sets and pole pieces to generate a large static magnetic field of up to 8 x 10^5 A m^{-1}. Additionally, a pair of counter-wound coils enable the generation of magnetic field gradients of up to 7.3 x 10^5 A m^{-2}. The field gradients can be varied as a function of time with a waveform generator enabling droplets to be driven in an oscillatory fashion. Droplets of ferrofluid can be placed in viscous media in a cuvette at the centre of the coil system. A digital video camera is used to record shape and position changes of the droplet as a function of field, field gradient, and time.

The motion of a ferrofluid droplet through a viscous medium can be quantified as its "magnetophoretic mobility", a characteristic property of the droplet relating its velocity to the magnetic field and field gradient inducing the motion [2]. The magnetophoretic mobility of a droplet is dependent upon both its magnetic properties and its size and shape. The low field magnetic properties are in turn determined by the size of the magnetic nanoparticles.

By measuring the magnetophoretic mobility of ferrofluid droplets in well-defined magnetic field environments, properties such as magnetic susceptibility and size can be optimised for particular ferrofluid applications.

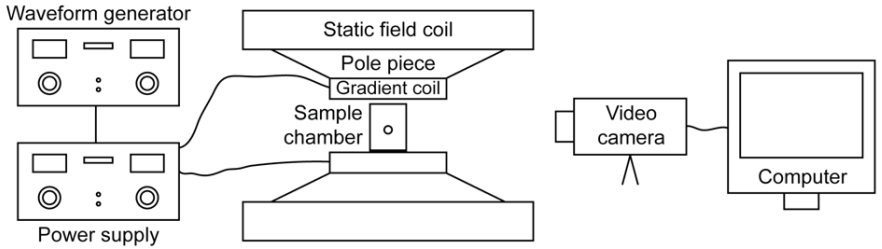

Figure 1. Schematic diagram of the instrument to observe magnetophoretic behaviour.

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Magnetic Protein Cages Characterized by Electron Magnetic Resonance (EMR) Spectroscopy

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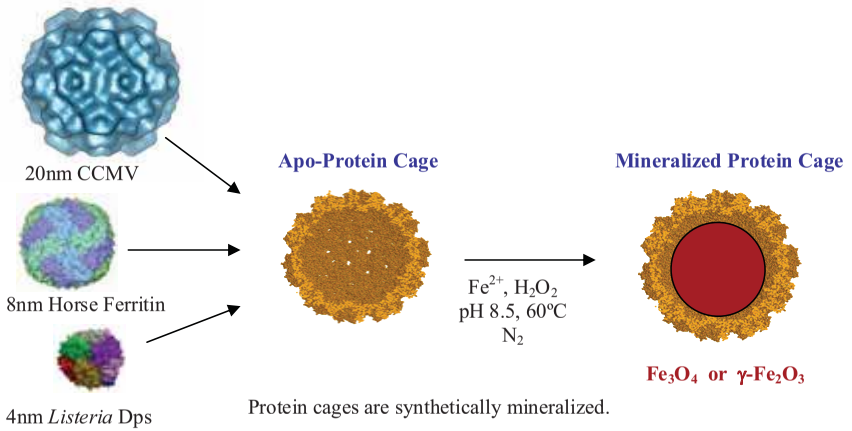
Magnetic nanoparticles have recently attracted substantial interest for applications in nanoscience and will form the basis for advances in technologies such as biological diagnostics, chemical sensing, enhanced magnetic resonance imaging (MRI), drug delivery, and cellular tracking.

Magnetic properties of nanostructures are highly dependent on the size, shape, composition, and topology. The controlled synthesis of nanostructures with a narrow size distribution and controlled shape thus remains a central goal. Bio-mimetic mineralization within protein cages is an attractive approach since maximum particle sizes are limited by the cage inner diameter. The protein shell surrounding the nanoparticles presents a uniform spatial array of amino acid side-chains for further synthetic processing such as covalently attaching relevant functional groups for cellular targeting.

Variable temperature electron magnetic resonance (EMR) spectroscopy was used to determine size distributions and magnetic properties of three different sized protein cages: Cowpea chlorotic mottle virus (CCMV), horse spleen ferritin, and *Listeria* Dps with inner diameters of 20, 8, and 5 nm, respectively.

The EMR lineshapes show characteristic changes as a function of particles size. These results demonstrate EMR as a diagnostic for nanoparticle physical properties. The protein cages provide a vantage point for preparing different metal compositions having unique magnetic characteristics that can be utilized in nanobiotechnology.



MagNA Pure LC 2.0: Modern design and improved performance and handling combined with a proven, magnetic particle based, nucleic acid isolation technology

Thomas Walter[1], Michael Kirchgesser[1], Christoph von Felten[2], Christian Kälin[2], Dan Liao[2], Manuel Herzog[2], Claudia Kappelsberger[1] and Peter Wenzig[1]
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The fast, flexible and reliable isolation of pure nucleic acids from various sample materials is crucial for sensitive molecular analysis by e.g. PCR, RT-PCR, sequencing, arrays etc. In addition, automation of this process is becoming increasingly important.

The MagNA Pure LC system from Roche Applied Sciences consists of ferromagnetic glass particles combined with an optimized chemistry for isolation of genomic DNA or total RNA from biological sample materials as well as a pipetting robot capable of magnetic separation and processing up to 32 samples at a time. Dedicated disposables and optimized pre-programmed protocols complete the MagNA Pure LC system which was launched in 1999.

We have now updated MagNA Pure LC system to version 2.0 with respect to hardware design, software, LIMS compatibility and operating system. The improvements are:

Hardware:
- New modern housing
- Front Door with improved opening mechanism (more robust, less height)
- Integrated PC with touch screen
- New UV lamp design for decontamination
- Stage easier to clean, less corners and crevices
- New, solid waste container instead of the current plastic bag

Software:
- New Software based on Windows XP (currently Windows 2000)
- Roche GUI
- LIMS connectivity
- Improved message handling and display (e.g. for service and errors)
- Instrument Overview (status) implemented
- Result database

The performance of the new system was evaluated in direct comparison with the current instrument. The MagNA Pure LC 2.0 proved to be equal regarding performance in the isolation procedures and superior regarding handling, programming, error management and data processing and management. We will present representative application data of our new MagNA Pure LC 2.0 system.

Multifunctional iron oxide nanocrystals for biomedical applications

Y. Andrew Wang[1], Sarah Geng[1], John Dixon[1], Tieshan Jiang[1], Xiaohu Gao[2], Lily Yang[3], Hui Mao[4], Hongwei Duan[5], Shuming Nie[5]

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Iron oxide magnetic nanocrystals have been investigated for use in the biomedical applications for many years. The recent advances in molecular and cell biology, biotechnologies as well as the nanotechnologies in the preparation of iron oxide nanocrystals further open a wealth of possibilities. Many biomedical applications have been developed or under development such as magnetic cell separations, cell purifications, drug delivery, biological detection and contrast enhancement for magnetic resonance imaging (MRI). Ocean NanoTech have commercialized new generation of the size tunable and monodisperse iron oxide nanoparticles with monolayer polymer coating. The features of our iron oxide nanocrystals are: 1) bioconjugation ready. Iron oxide surface is modified with the most widely used linkable functional groups including carboxylic acid, amine, biotin, streptavidin, which make the nanocrystals easy to conjugate most biomolecules. 2) size controllable. The core size of iron oxide nanocrystals can be controlled within the range of 5-50 nm. 3) high mass magnetization value. It originates from the high crystalline of iron oxide nanocrystals and thin organic layer coating. 4) controllable magnetic properties. Iron oxide nanoparticles can be magnetically manipulated and chemically change their magnetic properties according to size. In this presentation, we will summary our progress in the preparation and applications in target specific MRI, magnetic separation, drug delivery, and hyperthermia.

Modification of Magnetic Microsphere with Antibody-Antigen complex for the Diagnosing of Tumor Markers

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Traditional diagnostic methods, such as ELISA usually are not sensitive enough for the detection of early stage cancer markers. A novel technology based on the combination of a magnetic-immune-microsphere (MIMS) and an oligonucleotide magnifying probe has been proposed. The system is mainly composed of a magnetic microsphere (MMS) carrying an antibody against specific cancer marker; and a non-magnetic nanosphere loaded with a detection antibody in lower number and a kind of oligonucleotide in greatly higher number as magnifying probe. By detecting the oligonucleotide as end point, the signal of the corresponding cancer marker collected by the MIMS would be greatly magnified. This abstract reported the preparation of MIMS and its immunological availability was evaluated.

Methods: The MMS of 3μm with amine or carboxyl functional groups and with different length (C_3 and C_7) of space arms were purchased from the Base Line ChromTech (Tianjin, China). A mouse anti-human IgG was used as the model antibody (Ab). The Ab and a human IgG (the antigen, Ag) were labeled with red fluorescence and green fluorescence respectively. The Ab was covalently bound on the MMS-NH_2 or MMS-COOH by using SPDP or EDC/NHS as cross-linkers. The Ab-immobilized MIMS (Ab-MIMS) was collected over a magnetic field and was further put into the Ag solution of 100ng/ml to test immune activity and sensitivity of the MIMS. The complexes of MIMS-Ab-Ag, after separation and rinse, were examined under a laser scanning confocal microscope (LSCM,Germany Leica, SPE) to evaluate the binding of Ab and Ag. **Results:** The LSCM images (Figure 1) showed strong green and red fluorescence circles around the surface of the amine-functionalized MMS when use either SPDP or EDC/NHS as cross linkers. The results indicated that the Ab was effective bound on the MMS-NH_2, and the Ab-MIMS was highly immunological active for the conjugation of corresponding Ag. The influence of spacer length was unclear from this experiment. Further study will quantity the amount of Ag conjugated on the Ab-MIMS by radioimmunoassay (RIA) to optimize the Ab immobilization process.

Fig.1 LSCM image of the MMS-Ab-Ag complexes: The color circles on top shows the Ab (right with red fluorescence) and Ag (left with green fluorescence) bound to the surface of the MMS respectively. The orange circle in the bottom is the overlay of the two pictures on the top.

a SPDP/ C_3-MMS-NH_2 **b** SPDP/ C_7-MMS-NH_2 **c** EDC/NHS / C_3-MMS-NH_2
d EDC/NHS/C_7-MMS-NH_2 **e** EDC/NHS/ C_7-MMS-COOH

Nickel/Polyaniline Nano-cmpositesas a Target for Thrombolysis

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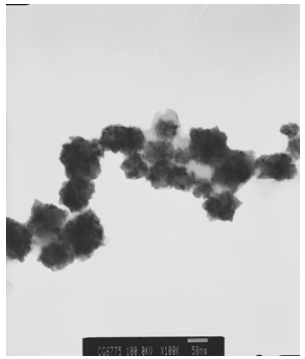
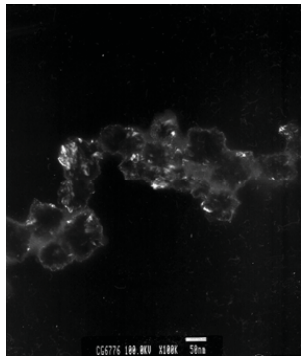
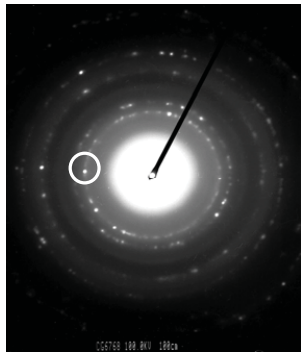
The purpose of this study is to synthesize magnetic Ni/PAn nano-composites by addition of polyaniline (PAn) with nickel. The nickel was prepared from to the nickel ion by chemical reduction in the mixed aqueous solution of hydrazine, ethylene glycol, and polyvinyl alcohol. Their structure, physical properties and applications to dissolute the formed thrombus were also investigated. These composites were successfully prepared as analyzed by ultraviolet-visible near IR spectroscopy (UV-Vis near IR), infrared spectroscopy (IR), X-ray diffractometer (XRD) and transmission electron microscope (TEM). In addition, no oxidation reaction of Ni was found for Ni/PAn composites as evidenced from isothermal experiment of thermo-gravimetric analyzer (TGA), implying that nickel particles were uniform covered with polyaniline. The magnetic properties of the composites were contributed from Ni particles as measured by super-conduction quantum interference device magnetometer (SQUID). It's found that the surface area, pore volume and size of the nano-composites were increased from 14.7 m^3/g, 0.05 cc/g, and 132.7 Å to 35.6 m^3/g, 0.14 cc/g, and 156.6 Å, respectively, as analyzed by Brunauer-Emmett-Teller (BET) analysis, indicating that PAn can isolate the inter-attraction of Ni particles. After doping with 0.1 N $FeCl_3$/$CHCl_3$ solution for 4 hrs, the molar ratio of Ni/C of Ni/PAn was decreased from 0.69 to 0.21 as measured by energy dispersive X-ray spectrometer (EDS). Meanwhile, the morphologies of Ni/PAn were changed from spherical-like to laminar-like particles and the saturated magnetization decreased from 17.6 to 1.7 emu/g. For activity assay with D-lle-Pro-Arg *p*-nitroanilide dihydrochloride, the rt-PA (recombinant tissue plasminogen activator) was combined with Ni/PAn by covalent-bond still had 81.2% reactive specific activity, which can use as a target for thrombolysis.







Kinetics of Nanoparticles Uptake and Retention by Liver and Spleen: A MRI study

Kuo-Chen Wei[1],Yeu-Jhy Chang[2], Yunn-Hwa Ma[3], Mei-Jie Jou[3], Mu-Yi Hua[4], Jyh-ping Chen[4], Peter Wu Hung[2], and Tony Wu[2]*

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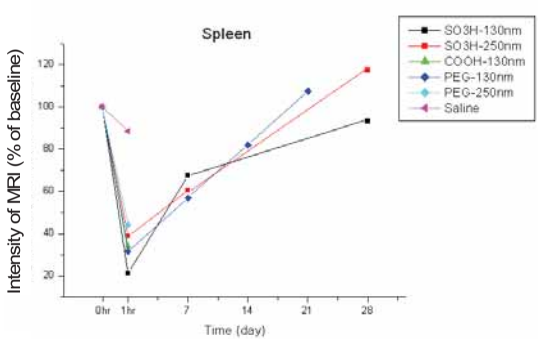
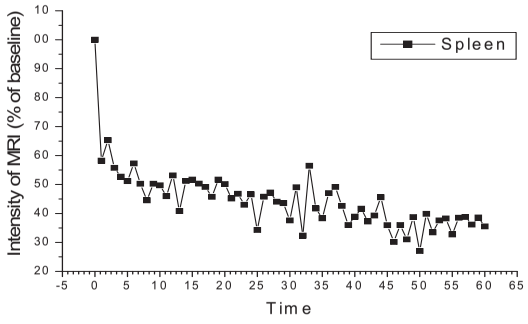
Previous histological study demonstrated magnetic nanoparticles (MNP) were rapidly retained in the liver and spleen, and the externally applied magnetic field modified its biodistribution pattern. For the application of MNP as a drug carrier administered via a systemic injection and guided to target site, a sensitive and dynamic method of monitoring biodistribution is needed. The present study examined the time dependent MNP retention in liver and spleen. MRI was performed using a 3.0-T Trio A Tim System, Siemens. Animals were imaged before and both acutely and over the long term after injection of MNP via tail vein. The T1WI and T2WI are acquired with turbo spin-echo pulse sequence, and an imaging block lasted for about 3 minutes. Images were analyzed with Syngo software (Siemens Inc). To determine temporal changes of NP in various tissues, ellipsoid regions of interest were placed in the liver and spleen region by visual inspection. To minimize partial volume effects, care was taken not to include the anatomical borders of the organs. Retention of MNP had low attenuation on T2 weighted MRI, the results of MNP retention in spleen was demonstrated in the figures.





Novel Magnetic Nanowires for Cell Labelling

Aine M. Whelan,[1]* Joseph E. McCarthy[2], Fiona Byrne[1], Frank Dowd[1], Adriele Prina Mello[1], J. M. D. Coey[1], Yuri Volkov[1] and Yurii Gunko[1]

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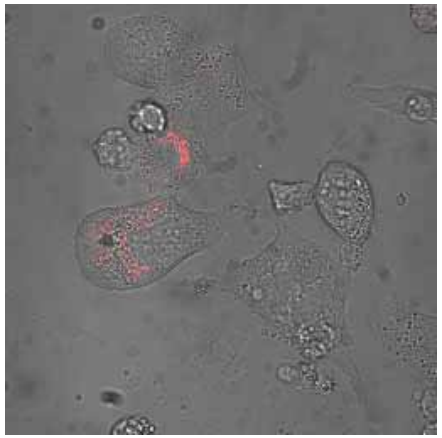
In recent years, there has been intense effort devoted to the application of nanoparticles and microparticles in a variety of biomedical technologies, for example, cell separation, biosensing and cell imaging. While much of this work has focussed on utilising the unique optical features of semiconductor nanoparticles, there has also been great interest in exploiting the properties of magnetic micro- and nanoparticles.

Herein, we report the synthesis of novel magnetic silica and polystyrene nanowires. The nanowires have been characterised by electron microscopy, vibrating sample magnetometry (VSM) and Superconducting Quantum Interference Device measurements (SQUID). The nanowires have been further functionalised with fluorescently labelled antibodies and then characterised by fluorescence microscopy and flow cytometry. The interaction of these wires with THP 1 monocytes and Hut78 cells has been studied, so that their potential as cell labels can be evaluated.



Fluorescence microscope image showing antibody labelled silica nanowires internalised by THP 1 cells

Magnetic nanoparticle – Dendritic Cells interaction: influence of surface charge and size

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It is well-known that surface characteristics of nanoparticles play a key role in the interaction with cells [1] but how determining are their molecular pattern (size, surface charge and functionality) in these processes is still largely unknown. Iron-based magnetic nanoparticles (MNP) interact with polyelectrolytes (such as polypeptides and polysaccharides) which are present in all living tissues. We exploit these strong interactions to impart tailored properties to the MNP via surface modification.

The main focus of this work is the preparation of polymeric shells around MNP so that the first contact with cells and/or subcellular structures is no longer the MNP core itself but the surrounding shell [1-3]. MNP size represents an important property for interaction with, and uptake into, cells [1, 2, 4]. MNP used here are superparamagnetic in nature. Upon addition of the polymeric shell on the MNP the resulting core-shell structures retain their superparamagnetic property [3]. We employ the sequential adsorption of polyelectrolyte multilayers using the layer-by-layer (LbL) assembly [5] on MNP to tailor and tune the (i) effect of surface charge, (ii) shell thickness and (iii) surface chemistry, and study their interaction with dendritic cells in vitro and in vivo.

We report the synthesis, LbL modification and characterizations of MNP, and their interactions with cells (cytotoxicity, cell adhesion or uptake, uptake mechanism and internalization).

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Interaction of tumor cells and peripheral blood cells with magnetic nanoparticles coated with tailored polysaccharide-based shells

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It is known that most of the cancer patients do not die from the primary tumor but from distant metastases. Once the primary tumor is formed, cells may begin to dissociate from the tumor and spread to other parts of the body via the circulatory or lymph system. These disseminated tumor cells are able to form metastases. To prevent a spreading of tumor cells to other parts of the body and forming metastasis, it is necessary to eliminate the tumor cells out of the blood quantitatively.
In hematology and oncology magnetic nanoparticles are regularly used for labeling and detection of cells. In addition to antibodies, the diversity of the shell surface is a promising opportunity to get a pure fraction. It has been shown that magnetic nanoparticles with a polysaccharide-based shell interact with living cells in a cell type specific manner. Pure dextran (D) and carboxy dextran (CD) as well as carboxymethyl derivatives of dextran (CMD), pullulan (CMP), cellulose (CMC), curdlan (CMCd) and starch (CMS) were studied as coating materials. In case of CMC and CMCd coated particles no uptake was observed. In contrast the CMD shell is most efficient in the separation of tumor cells from leukocytes. Tumor cells showed an intense interaction with CMD coated particles whereas the leukocytes exhibited a lower tendency to interact. To achieve a more pronounced discrimination between cancer cells (MCF-7 cells) and leukocytes, the influence of the structure of the CMD coating material was investigated. Neither variation of the degree of carboxymethylation nor the molecular masses of the dextran did yield coating materials with an increased difference in the interaction with the two cell types.
Thus, the pattern of functionalization of the CMD was modified. Using different synthesis paths for the carboxymethylation of dextran, it is possible to obtain CMD with a statistic pattern of carboxymethyl groups along the dextran chain or a non-statistic distribution of the substituents. Interestingly, first experiments show that a non-statistical distribution of carboxymethyl groups intensifies the difference of interaction with the cells. MCF-7 cells are only slightly affected in their interaction with CMD coated nanoparticles whereas the leukocytes exhibited a significant decrease of interaction.

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Plasmid DNA purification with silica-coated magnetic nanoparticles

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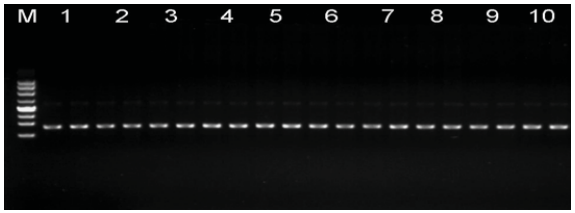
Abstract The work developed a simple and reliable method for the rapid isolation of plasmid DNA from crude cell lysates with the silica-coated magnetic nanoparticles. The silica-coated magnetic nanoparticles with average particle size of 20 nm were synthesized. PUC19 plasmid has been successfully concentrated and purified from the *E. coli* DH5α under the magnetic fields, it took 20 min to yield approximately 15μg DNA from 1ml bacterial culture and OD260/OD280 ration was 1.82, indicating that the DNA was of a high purity with negligible protein contamination. This method has shown excellent reproducibility, Fig. shows the plasmid DNA extracted from the same volume bacterial cultures by using 10 different batches of silica-coated magnetic particles, demonstrated that the method is very stable. The plasmid obtained by this approach retains biological activity that can be suitable for restriction enzyme digestion and cells transformation.



Keywords: silica-coated magnetic nanoparticles; plasmid DNA; purification

Detection of toxA genes DNA Hybridized with coloured microspheres using a Planar Hall sensor

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This paper describes the promising technology for detection of toxA genes DNA with high resolution screening using Planar Hall sensors (Ta/NiFe/NiFe/Cu/NiFe/IrMn/Ta) of size 50 x 50 μm² [1].We are also showing the impact of surface chemistry for removal of non-specific binding of magnetic beads. In this work we are using M-280 Dynabeads and 300nm coloured beads .The PHR profiles of sensors when DNA immobilzed with magnetic bead has smaller amplitude as compare to without DNA immobilised magnetic beads. The coloured beads has 450-500 molecules binding capacity per particle as well as exhibits high resolution for screening,Which makes our sensor a good candidate for real sensor applications

Keywords toxA gene,coloured magnetc beads,surface chemistry

Reference

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A Magnetic Biosensor System Based on Brownian Relaxation Measurements

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An ideal biosensor should detect target molecules directly without using labeled ligands or washing steps. We have overcome these obstacles by designing a sensor system based on magnetic nanoparticles with a surface modified to bind any specific molecule we want to detect. Upon binding of target molecules (i.e. antibodies) to the particles, the hydrodynamic particle volume increases which can be detected using dynamic magnetic measurements.

Nanoparticles in suspension undergo Brownian rotation that is a stochastic motion due to collisions with water molecules. The frequency of Brownian rotation is dependent on the size of the particles, the rotation of smaller particles occurs at higher frequencies than larger particles. For thermally blocked magnetic particles in colloidal suspension, one can monitor Brownian rotational motion by measuring the frequency dependent magnetic susceptibility of the particle system. Since the Brownian frequency can be detected in this way, the particle hydrodynamic size be determined and most important, a change in particle size can be observed upon binding of target molecules.

We have designed an instrument to measure the frequency dependent magnetic susceptibility of nanoparticle systems and find experimentally for neutravidin particles a good correlation between biotinylated DNA strand length and the observed change in hydrodynamic volume of the particles. We also present a few other biological applications using magnetic nanoparticles functionalized with different biomolecules.

The technique can be used for a wide variety of analytes with biological applications. Detection of disease markers in patient samples constitute one area, other areas can be analysis of environmental samples or to follow chemical reactions or synthesis in (bio-) chemical industry.

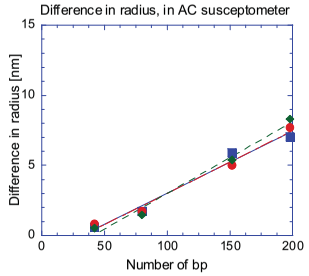

Figure 1: The difference in hydrodynamic particle radius is plotted versus the number of bp (base pair) added. Biotinylated PCR product of different length but of the same concentration (a concentration which saturates the magnetic nanoparticles) was added to magnetic nanoparticles conjugated with neutravidin.

DETECTION OF MAGNETICALLY LABELED DNA MICROARRAYS USING A SCANNING MAGNETIC TUNNEL JUNCTION SENSOR

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Microarray technology has become one of the principal platform technologies for the high throughput analysis of DNA, proteins and cells. A principal focus of research in DNA microarray technology lies in the development of a low cost, portable and sensitive tool to detect the hybridization event. Through integrating the state of the art magnetic tunnel junction (MTJ) sensor widely used in conventional magnetic recording technology with magnetic particles as the detection labels for the DNA microarrays, we are developing the fundamental technology for a low-cost disk-drive based bioassay system for the detection of magnetically-labeled DNA microarrays.

Using standard DNA microarray protocols, single stranded DNA (ss-DNA) probe spots are printed on a silanized glass substrate. A test sample containing an unknown quantity of biotinylated target ss-DNA is introduced and hybridization is allowed to occur. The hybridized DNA is labeled with streptavidin-functionalized paramagnetic beads, as shown in Fig 1a. Finally, the magnetic labels are detected using a magnetic tunnel junction (MTJ) sensor that scans across the microarray and measures the magnetic signature of the particles, as illustrated in Fig 1b. Scanning the MTJ sensor across the array allows both a large scanning area (> 1 cm^2) and high spatial resolution (~ 1 μm)

The MTJ sensor noise level is ~300nT in a 1Hz bandwidth, and the theoretical noise-limited resolution corresponds to a single 2.8 μm magnetic bead at sensor-bead gap of 5μm. Current sensor-bead spacing of ~20μm results in a resolution of ~130 magnetic particles within a single 100μm diameter DNA spot, as shown in Fig 1c. Efforts to reduce the gap to 10 μm through focused ion beam (FIB) machining of the sensor are currently underway and are expected to improve the resolution by approximately an order of magnitude.

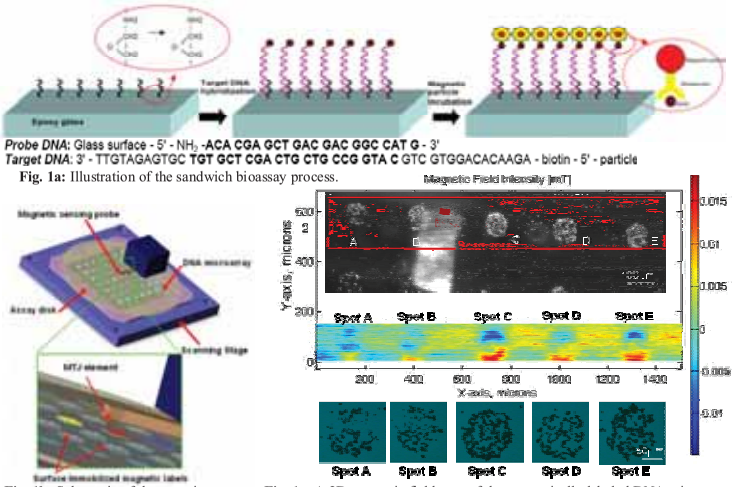

Fig. 1a: Illustration of the sandwich bioassay process.

Fig. 1b: Schematic of the scanning setup.

Fig. 1c: A 2D magnetic field map of the magnetically labeled DNA microarrays.

Binding assays with streptavidin-functionalized superparamagnetic nanoparticles and biotinylated analytes by fluxgate magnetorelaxometry

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Magnetorelaxometry (MRX) has been introduced using superconducting quantum interference devices (SQUIDs) [1]. In our measurement system we use a differential fluxgate arrangement to measure the magnetic stray field of a sample at room temperature [2]. MRX was proposed to perform magnetic relaxation immunoassays [3].

In this work we present binding measurements of streptavidin-functionalized superparamagnetic nanoparticles (MNP-StAv) and two types of biotinylated analytes which essentially differ in size. To quantify the different assay results we discuss different analysis methods. In our MRX experiment the sample is magnetized by a magnetic field in the order of 2 mT for about 2 seconds. After switching off the magnetizing field, the stray field of the MNPs relaxes either via the Brownian or the Néel relaxation mechanism.

In one experiment 9 samples were prepared each with 19 pmol of MNP-StAv and an increasing amount of biotinylated BSA protein ranging from 2.1 nmol to 10.7 nmol. Here the BSA protein acts as a linker that cross-links the MNP-StAv. The formation of clusters of different sizes takes place during the cross-linking process. For a simple analysis the area under the relaxation curves was calculated and related to the area of the immobilized reference sample. The size of the area is a measure for the cluster size and thus for the cross-linked MNP-StAv. Fig. 1 shows the evolution of the cross-linking state. By increasing the amount of BSA linkers the cross-linking has a maximum at 4.3-5.3 nmol of biotinylated BSA. By further increasing the BSA linker concentrations the cross-linking decreases again. This is due to the occupation of all binding sites of the MNP-StAv by the BSA linker.

In a second experiment 19 pmol MNP-StAv was bound to biotinylated agarose beads (~5µm diameter) ranging from 40 to 140 µL. It is found that all MNPs are immobilized for biotinylated agarose bead contents above about 120 µL.

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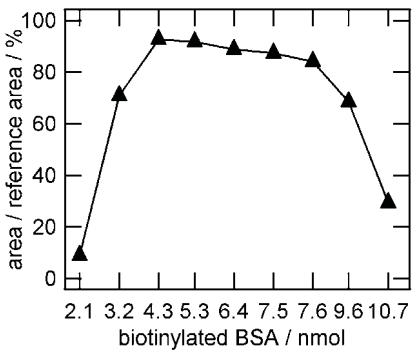

Fig. 1: Evolution of the cross-linking state of 9 samples with 19 pmol streptavidin-functionalized MNPs and increasing amount of biotinylated BSA protein.

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Probing Temperature-Sensitive Behavior of PNIPAAm Coated Iron Oxide Nanoparticles Using Frequency Dependent Magnetic Measurements

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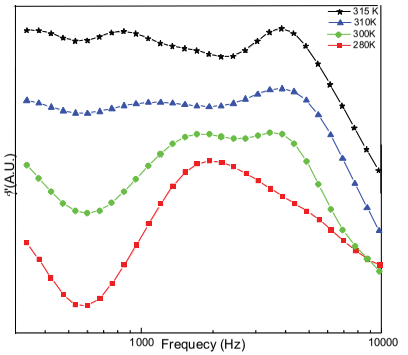
The relaxation of magnetic nanoparticles in an AC magnetic field can be used for diagnostic purposes [1]. Ferrofluid of blocked particles relax via Brownian relaxation, with a relaxation frequency given by $f_B = k_B T / 3\eta V_H$, where η is the viscosity of the ferrofluid and V_H is the hydrodynamic volume of the particle. Brownian relaxation is dependent on the ability of the particles to rotate in their matrix, thus changes in the viscosity or hydrodynamic radius can be detected by measuring the shift in frequency at which there is a peak in the imaginary component of the susceptibility. In this sense the nanoparticles can be used as a biosensor to detect binding events of biomolecules of interest on the surface of the functionalized particles [2].

In this regard ferromagnetic iron oxide nanoparticles (size ~33 nm) were prepared by high temperature pyrolysis of ferric oleate in a coordinating solvent and in the presence of oleic acid as a surfactant [3]. Later these particles were individually coated with PNIPAAm by surfactant exchange method and dialysed to get aqueous dispersion of PNIPAAm coated iron oxide nanoparticles (size ~ 80 nm) [4]. These particles have lower critical solution temperature (LCST) between 305-310 K. Their temperature dependent physical behavior could be monitored by measuring the imaginary component of the magnetic susceptibility, χ". Hence, AC magnetic measurements of these particles at different temperatures, above and below LCST (i.e. at 280K, 300K, 310K and 315K), were carried out. The clear trend in the peak in χ" (see figure) is correlated with swelling and deswelling of PNIPAAm. Potential use of this behavior as a field-dependent triggering mechanism is envisioned [5].



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A MICROFLUIDIC DEVICE FOR THE DETECTION OF MAGNETORELAXATION IN BIOLOGICALLY-FUNCTIONALIZED FERROFLUIDS

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Ferrofluids composed of aqueous suspensions of biologically-functionalized magnetic nanoparticles (MNPs) have promising applications in microfluidic lab-on-a-chip devices. One such application is in miniaturized immunoassays based on the detection of changes in the magnetorelaxation signature of antibody-functionalized MNPs. The principal of these magnetorelaxation assays is that nanometer-scale antigen molecules bind to the MNPs, increasing the hydrodynamic diameter and therefore increasing their Brownian relaxation time.

Here we describe a microfluidic device for magnetorelaxation-based assays. In this device, magnetorelaxation is measured using optical birefringence, a technique that is capable of detecting extremely small concentrations of magnetic material and readily performed using low-cost, polymer microfluidic substrates. The prototype device consists of a 14 mm x 1 mm x 0.5 mm microchannel formed in a polydimethylsiloxane (PDMS) layer using micromolding (Fig. 1a). The fabrication method produces optically-smooth sidewalls, allowing low-loss transmission of the detection laser through the microchannel. The microchannel volume is 7 µl and may be reduced to ~0.1 µl without reduction in optical pathlength.

Preliminary measurements have been performed using a 2 mW laser that is collimated to a ~200 µm diameter. The magnitude of the birefringence frequency response for a ferrofluid containing 0.2 mg/ml Fe_2O_3 (40 ppm by volume) was measured using a small coil producing a 60 Oe AC field (Fig. 1b). The measurement shows an 11x improvement in SNR relative to measurements performed in a 1 mm glass cuvette, and confirm that measurements at sub-ppm concentrations are possible in this device. The measured response has a 3 dB frequency of 2079 Hz, corresponding to a hydrodynamic diameter of 84.6 nm, in good agreement with diameter measurements performed using dynamic light scattering (DLS).

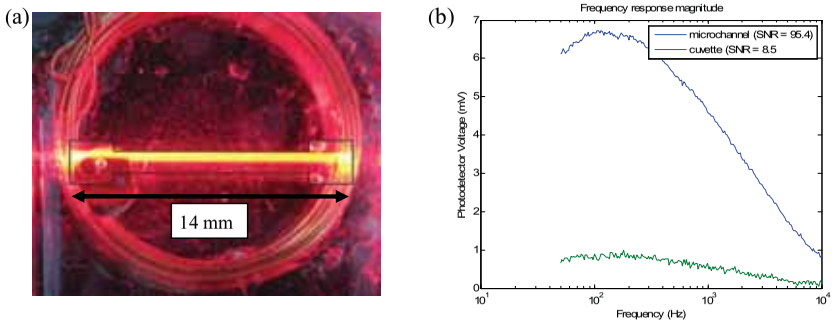

Fig. 1. (a) Top view of molded PDMS microchannel mounted above excitation coil. The detection laser beam is visible due to scattering from the magnetic nanoparticles within the ferrofluid.
(b) Magnetorelaxation frequency response magnitude measured using an AC field of 60 Oe with a ferrofluid containing 0.2 mg/ml Fe_3O_4.

Use of self-assembled magnetic particles for lab-on-chip Prion protein analysis

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At present, there is no accurate pre-mortem diagnosis for Prion diseases, and post-mortem analyses have many drawbacks: They are rather time consuming and have a low sensitivity. The advantages of miniaturized systems, such as small consumption of reagents, short reaction times, potential for parallelization and integration of many analytical steps, make them very interesting for the development of new Prion diagnosis.

To this purpose, we present a microfluidic device for organizing into a porous microcolumn magnetic beads grafted with proteinase K (PK) or antibodies, as a matrix for protein reaction, namely digestion and immunocapture.
The PolyDiMethylSiloxane device integrates strong magnets to create a magnetic field parallel to the flow, with a strong gradient pointing through the centre of the channel (Fig. 1a and 1b).When exposed to a uniform external field, the magnetic beads self-organize into a supraparticle structure consisting of a columnar clustering in the direction of the field ("labyrinth" phase). This microcolumn has an anisotropic porosity parallel to the flow, a uniform pore size, and self-healing properties. It can also be replaced easily by a strong hydrodynamic "flush", and reassembled with new beads ([1, 2]). It was used here for protein reaction (digestion) and immunocapture. Another advantage with regards e.g. functionalized microchannels or "monolith" phases is that the biofunctionalization can be performed ex situ in large quantities, allowing for reduced cost, better reproducibility and quality control.

Proteins (enzymes and antibodies) were covalently grafted on the –COOH functional groups of magnetic particles (0.5 and 1 µm) using EDC (water soluble carbodiimide) and S-NHS (hydroxysulfosuccinimide). A plug of these functionalized magnetic particles was then immobilized between the magnets in a microchannel for flow-through reaction with off-chip analysis. Results of digestion studies showed accelerated kinetics in microchip compared to batch reactions, by a factor of 100 (Fig. 1c) due to reduced diffusion distance. A PK reactor dedicated to the digestion of the Prion Protein was developed and controlled proteolysis could be obtained while varying the on-chip flow rate [2]. On-chip capture of protein was also demonstrated.

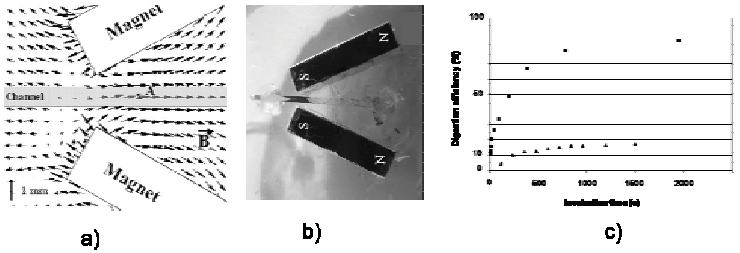

Figure 1.a) Simulation of the magnetic field. b) View of the plug of magnetic beads immobilized between two magnets in the microchannel. c) Comparison of the efficiency of PK substrate digestion between batch-wise (▲) and on-chip (■) experiments for different incubation times.

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Towards understanding anisotropic bead variance in a magnetic tweezers instrument

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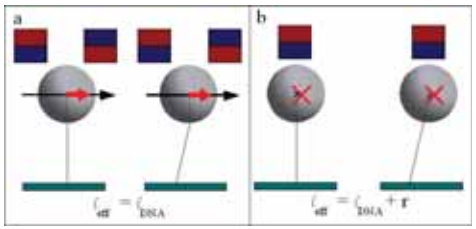
In recent years, magnetic tweezers have emerged as an excellent tool for applying forces and torques to single molecules, probing systems such as supercoiled DNA and the mechanism of DNA-modifying enzymes. In this technique, an external magnetic field interacts with a superparamagnetic bead to exert sub-pN to pN forces and torque on a tethered molecule.

The force on the bead, **F**, can be calculated and experimentally determined. Theoretically, **F** is given by $\mathbf{F} = \nabla(\mathbf{m} \cdot \mathbf{B})$, where **m** is the induced magnetic moment of the bead and **B** is the external magnetic field generated by a pair of rare-earth magnets. In the most common set-up (see figure), **F** acts in the vertical (z) direction, applying a stretching force to the tethered molecule. Experimentally, this force is evaluated from the variance of the measured position of the bead parallel to the surface, σ^2, and the extension of the molecule, ℓ: $F_z = k_B T \ell \sigma^{-2}$. Calculations of the magnetic fields and forces acting on a spherical superparamagnetic bead provide estimates of the expected position variance of a bead tethered to a substrate by DNA. These can be compared with experimentally measured variances to assess the validity of the measurements and improve experimental factors such as system alignment and number of DNA molecules attached to the bead.

Our measurements exhibit anisotropy in the position variance of the bead between the directions parallel and perpendicular to the magnetic field direction: $\sigma^2_{\perp} > \sigma^2_{||}$. A possible explanation for this difference is that shape anisotropy in the bead gives rise to a preferred orientation with respect to the field axis. In this case, the effective tether length used in force calculations, ℓ_{eff}, would differ between parallel and perpendicular directions: $\sigma^2_{||} / \sigma^2_{\perp} = \ell_{DNA} / (\ell_{DNA} + r)$. In order to assess this quantitatively, we experimentally determine the ratio $\sigma^2_{||} / \sigma^2_{\perp}$ for different lengths of DNA. We also compare the resultant forces with those expected for our experimental configuration and for a single molecule of DNA, and identify important experimental factors to consider in these types of measurements.



Asymmetric Brownian motion of the bead (a) parallel or (b) perpendicular to the field direction is a possible explanation for the difference between $\sigma^2_{\perp}$ and $\sigma^2_{||}$.

Magnetic biosensor for screening small molecular ligands against protein targets

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Over the past few years, the detection of the superparamagnetic particles binding with biomolecules using giantmagnetoresistive (GMR) sensors, has gained a lot of interest as a candidate for the realization of highly sensitive bio-detection. In this paper, we demonstrate nanoparticle based GMR sensor system to screen various small molecular ligands against different protein targets. The idea of nanoparticle based GMR sensor system can be potentially extended to pathogen detection and even to cellular level.

The giant magnetoresistive sensors consist of a multilayer structure of $Ir_{0.2}Mn_{0.8}$(10nm)/$Co_{0.9}Fe_{0.1}$(2.5nm)/Cu(3.3nm)/$Co_{0.9}Fe_{0.1}$(1nm)/$Ni_{0.82}Fe_{0.12}$(2nm). The multilayer films were deposited on the substrate by Shamrock sputtering system. The GMR multilayer was patterned into rectangular shape with different size using photolithography and ion milling. To prove the detection, CoFe magnetic nanoparticles were first deposited directly on the surface of the GMR sensors. The GMR sensors were tested by a four-probe magnetic station. An external magnetic field was applied to polarize the nanoparticles so that stray field could be generated by the nanoparticles. The stray field, which generated by the nanoparticles has opposite direction to the applied magnetic field. Therefore, by comparing the magnetoresistance loop of the GMR sensors before and after their deposition, the magnetic nanoparitcles can be detected. The sensitivity of the GMR is 0.24 ohm/Oe. The MR values are in a range of 2.5% to 3.0% depending on the GMR size and aspect ratio. A 0.5 ohm net resistance change was obtained. It is suggested that the net resistance change from one particle is 0.6 micro ohm. The nanoparticle detection using GMR sensor was confirmed.

Subsequently, we modified the GMR sensor surface covalently with amino groups using 3-aminopropyltriethoxysilane (APTES) and then with biotin. APTES modified CoFe nanoparticles were covalently immobilized with dye labeled streptavidin which specifically binds to biotin. We also developed protocols for quantifying loading level at each stage of modification. The magnetic nanoparticles modified by streptavidin were immobilized to biotin modified GMR sensor surface as a model study. The nanoparticles binding to the GMR surface resulted in a 0.25 ohm net resistance change.

In conclusion, the detection of magnetic nanoparticle on GMR sensor has been shown. More importantly, we successfully demonstrated a streptavidin-biotin binding biosensing system, which has potential application for biomolecular detection.

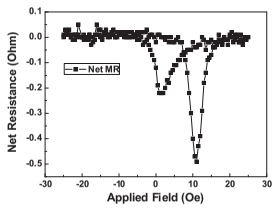

Fig. 1. Net MR change with and without CoFe nanoparticles

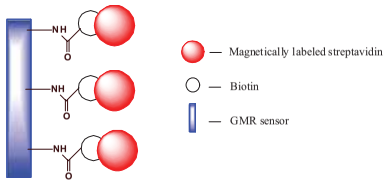

Fig. 2. Schematic showing of magnetically labeled streptavidin immobilization to biotin modified GMR sensor surface

Magnetic Encoding for High Throughput Suspension-based Biochemical Assays

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Microarrays and suspension (or bead)-based technologies have attracted significant interest in the last few years for their broad applications in high throughput molecular biology. However, due to fundamental limitations in the operating principle, the throughput of microarrays will always be limited by the array density and the slow diffusion of molecules to their binding sites. Suspension -based technologies, in which all the reactions take place directly on the surface of microcarriers functionalised with probes, promise a conceptually different approach. Such technologies could offer true multiplexing due to the possibility of extending their detection capability by a straightforward expansion of the size of the chemical library of probes. To fully exploit the potential of suspension based methods, the microcarriers must be encoded, but the number of distinct codes available from spectrometric/graphical/physical encoding methods is currently fairly limited.

We are developing a novel digital magnetic encoding method based on magnetic tags that can be remotely encoded and decoded as they flow in microfluidic channels, by means of an external magnetic read/write head. The planar tags consist of a substrate and magnetic microbars that are individually addressable by a magnetic sensor and can be functionalized with various chemical or biological probes. The main elements that are necessary to show the feasibility of our technology, namely, the design and fabrication of releasable multibit magnetic tags, static measurements showing remote reading and writing and the manipulation and functionalisation of the tags, have been demonstrated and will be discussed in this paper.

We present experimental data demonstrating the reading of individual magnetic microbars from samples comprising 50x20 µm Ni elements, as well as micromagnetic simulations that show the feasibility of stray field detection with a large element-sensor spacing when a microfluxgate sensor is used. A gold capping layer provides the chemical base for the functionalisation of the tags; alternatively the tags can be encapsulated in SU8, the epoxy groups of which can also be used for functionalisation. The magnetic properties of the planar tags are analysed and a comparison between magnetic microbeads and tags for efficient encoding of biomolecules is also presented.

In addition, we present data showing the fabrication of multicoercivity planar tags comprising thin permalloy films and encoding/decoding of the tags using Kerr microscopy and remotely applied global magnetic fields. Furthermore, we demonstrate the successful release of the tags in suspension and their manipulation in microfluidic SU8 channels using hydrodynamic focusing. The design principles of a novel magnetic lab-on-a-chip device, integrated with microfluidics, that encodes/decodes the suspended magnetic tags in flow are also presented in this paper (Figure 1). The magnetic lab-on-a-chip could be used for labelling chemical moieties that are then utilised as probes for various applications, such as genome sequencing, immunoassays, proteomics and clinical diagnostics.

We envisage that the digital magnetic encoding technology will be implemented for the synthesis of vast *in situ* encoded chemical libraries prepared by combinatorial chemistry methods, benefiting drug discovery and screening and high throughput multiplexed biological analysis.

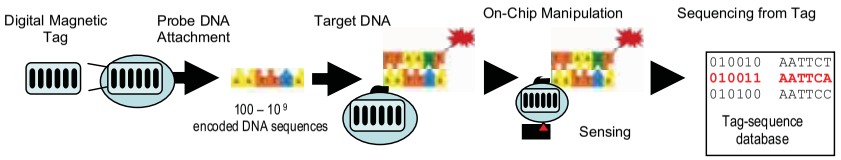

Figure 1: Schematic diagram showing the concept of digital magnetic encoding for DNA screening. A variety of other biochemical moieties can also be attached to the tags, such as antibodies or proteins.

Active superparamagnetic bead manipulation on-chip

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We report an innovative and highly integrated approach for the mixing of superparamagnetic beads in a PDMS microchannel. The end goal of our project is to improve the analyte capture efficiency on functionalized beads, in particular for the HIV-p24 protein [1]. For this purpose a plug of magnetic beads is retained in the sample solution flow by means of two soft magnetic poles (laser cut foils with 100 x 100 µm^2 tips) that focus a magnetic field generated by an electromagnet across the microchannel (Figure 1a). Bead chain rotation in an AC magnetic field using high-coercivity beads has been reported previously as a possible mixing mechanism [2]. However, this type of beads is not suitable for the present application where the beads are required to have a very low coercivity. Indeed, once the external magnetic field is removed, beads should be released from the plug for subsequent analysis. Low-coercivity bead chain rotation using a rotating magnetic field was also reported [3]. However, actual approaches are based on more complex systems making microfluidic integration more difficult.

In order to increase the effective plug cross-section, i.e. the antigen capture probability, bead motion across the channel is induced by applying an AC field in between the magnetic tips. Two additional permanent magnets on top of the microchannel serve to create a weak permanent bead magnetization and an asymmetric field distribution in the system. The resulting alternating magnetic field maxima attract beads subsequently to each side of the microchannel (Figure 1 b and c).

Figure 2a shows a plug of about 2x10^4 1 µm strepatavidin-coated beads (Dynabeads MyOne C1) moving at 20 Hz. Beads are moving from one side of the channel to the other forming alternately a dense plug on each side wall. By increasing the frequency of the magnetic field up to 70 Hz, beads are in perpetual motion in the center of the channel (see Figure 2b). The mixing system was designed to minimize the amount of beads while keeping a good protein capture probability.

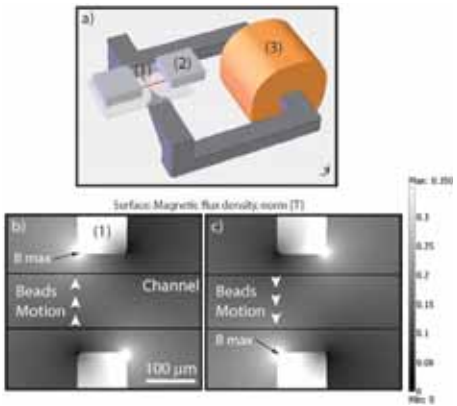

Figure 1: a) Magnetic actuation system with soft magnetic poles (1), permanent magnets (2) and the coil (3). Simulation of the magnetic flux density B with (b) positive and (c) negative current in the coil. The maxima of the magnetic field moves from one side of the channel to the other.

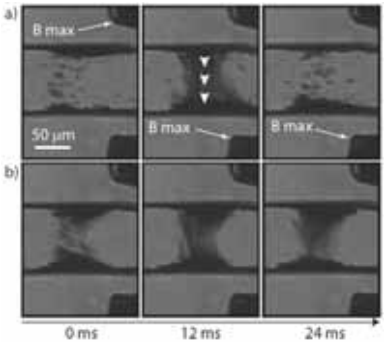

Figure 2: a) Beads moving from one side of the microchannel to the other at 20 Hz induced by the displacement of the magnetic field maxima. b) Beads moving at 70 Hz across the microchannel, resulting in a more uniform plug distribution.

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[2] A. Rida and M. A. M. Gijs, "Dynamics of magnetically retained supraparticle structures in a liquid flow," Applied Physics Letters, vol. 85, pp. 4986-4988, 2004.
[3] I. Petousis, E. Homburg, R. Derks, and A. Dietzel, "Transient behaviour of magnetic micro-bead chains rotating in a fluid by external fields," Lab on a Chip - Miniaturisation for Chemistry and Biology, vol. 7, pp. 1746-1751, 2007.

Multicomponent Bioassays Based on Magnetic Nanolabels

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Application of superparamagnetic particles or magnetic beads (MB) as labels in biosensing has become very popular. Quantification of such labels can be done for various Magnetic Immunoassay (MIAtek™) formats by non-linear magnetization of MB at two frequencies f_1 and f_2 and recording response at combinatorial frequencies $f = mf_1 + nf_2$, where m and n are integers (one of them can be zero, and they may be varied to get the best signal-to-noise ratio), e.g. at $f = f_1 \pm 2f_2$ [1-3].

The present paper is devoted to extension of this technology for detection of several biological agents simultaneously. Different types of readers have been developed to count MB in several recognition zones with various capture antibodies and tested for multicomponent bioassays.

The first version of the device family is designated to readout planar biochips or micro fluidic cartridges by a number of small coils with radius R = 0.5 - 1 mm (Fig. 1a). The experiments demonstrated that such coils can be successfully used to count magnetic nanoparticles inside a semisphere of the same radius. This fact allows separation of the main electronic units from biochips or microfluidic cartridges, which can be interrogated from outside through 0.1 mm thick glass or plastic bottom. Such magnetic biochip can be a very affordable consumable for medical diagnostic as compared with single-used magnetoresistive biochips that incorporate arrays of multi-layer magnetic sensors. The tested MIA chip can analyze small sample volumes < 100 µL. The next developed approach is designated for multi-component analysis of big volume samples ~ 0.1-1 L by pumping the sample though a flow channel with several sequentially connected recognition zones based on filter or multi-capillary structures. Different capture antibodies were immobilized on the large surface (up to 40 cm^2) of each structure. The scheme permits one to realize fast and effective 3D immunofiltration of antigens. The developed reader can interrogate five zones simultaneously. Besides, such multicomponent assays were tested by shifting of the flow channel with multiple assay zones through single interrogating system (Fig. 1b). These approaches are promising for biosensing of samples with practically unlimited volume, e.g. for testing extracts from food to detect pathogens and toxic chemicals, for inspection of transport visiting infected regions by testing washing liquids, etc. The next developed multi-analyte setup is based on recording of parallel immunoreactions on the surface of 3D filters or multi-capillary structures inside special immunosyringes (Fig. 1c). The tracer antibodies and magnetic nanolabels are delivered using an automated liquid handling system based on step motors. This setup can be quickly configured for detection of the expected antigens by installation of a proper set of prepared in advance immunosyringes. The developed multicomponent bioassay platforms are promising for medical and veterinary diagnostics, point of care, food inspection, environmental and security monitoring, etc.

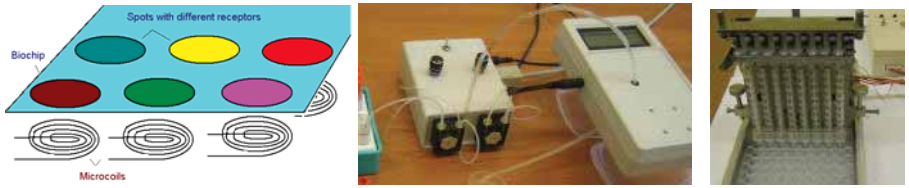

Fig. 1: a) planar MIA chip, b) bioassays on sequentially separated recognition zones, c) immunosyringes.

[1]. P.I. Nikitin, P.M. Vetoshko, Patent RU 2166751 (2000), EP 1262766 publication (2001).
[2]. P.I. Nikitin, P.M. Vetoshko, T.I. Ksenevich. Sensor Letters, 5 (2007) 296.
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Rapid biosensing using magnetic nanoparticles

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A biosensor is a system for the rapid measurement of a concentration of biological molecules in a complex fluid such as blood or saliva. In our investigations and developments we focus on technologies suitable for testing within one minute. The one-minute criterion is important because the testing should seamlessly fit into the medical workflow and should support the 'quality time' between doctor and patient.

We will present a biosensor technology based on superparamagnetic nanoparticles. The use of such nanoparticles enables fast single-step assay formats without any fluid wash steps, detection in raw samples, and integration into a miniaturized system. The detection technology will be described as well as biological performances for a range of biological assays (competition immunoassays, sandwich immunoassays, and DNA assays).

Reference

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Micromagnetic Simulations on Detection of Magnetic Labeled Biomolecules Using MR Sensors

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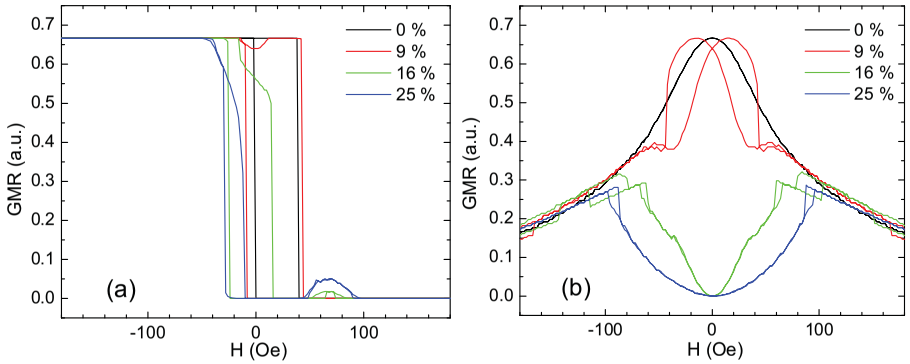
Lab-on-a-chip devices are very attractive because they allow shrinking entire chemical or biochemical assays down to small microfluidic chips. Detection of small magnetic fields generated by magnetic particles encapsulated in plastic, carbon or ceramic spheres which are coated with chemical or biological species such as DNA or antibodies that selectively bind to the target analyte is made using giant magnetoresistive effect (GMR) or Hall effect sensors. Results from theoretical modelling, as well as laboratory tests, show that GMR detectors can resolve single micrometer-sized magnetic beads.

In this work we present a study of the interaction between the magnetic particles and the GMR sensor. The fractional change in resistance, and hence the sensitivity, will be maximized by matching, as far as possible, the size of the sensor to the size of the bead and by reducing the distance between the sensor and the bead. We found, by micromagnetic simulations, that the amount of the surface coverage with magnetic particles may affect the magnetization curve of the sensor and will change the field dependence of his GMR response.



The field dependence of the GMR effect for different values of surface coverage with magnetic particles when the magnetic field is applied in the film plane (a) parallel with the easy axis and (b) perpendicular to the easy axis of magnetization.

In these simulations the total thickness of the layers (immobilization layer and protection layer, Si_3N_4), that give the distance between the magnetic beads and the GMR sensor, is 0.2 μm. The GMR sensors consist of multilayered structures as FeMn/NiFe(2 nm)/Cu(1 nm)/NiFe(2 nm) for which we assumed a small positive coupling between the magnetic layers through the nonmagnetic layer, H_{coupl}=70 Oe and a uniaxial anisotropy field H_K=20 Oe. This positive coupling is often present in real structures due to the small iregularities of the surfaces giving the so called orange-peel coupling. In order to obtain the GMR effect in these structures, the magnetization of bottom layer is pinned by exchange interaction, using a layer of FeMn. The pinninig field was set at 200 Oe, a value which is consistent with experimental data. To extract from the GMR signal the contribution corresponding to the total magnetic moment of the beads and to avoid the influence of the external magnetic fields and thermal fluctuations we designed a differential measurement system. Aspects as the field scanning method and the response of this system, according to proposed physical and electronic setup, are discussed in this paper.