Journal of Magnetism and Magnetic Materials 380 (2015) 72–77

Contents lists available at ScienceDirect

Journal of Magnetism and Magnetic Materials

journal homepage: www.elsevier.com/locate/jmmm

Micro-magnet arrays for specific single bacterial cell positioning

Jérémy Pivetal [a,*], David Royet [a], Georgeta Ciuta [b,c], Marie Frenea-Robin [d], Naoufel Haddour [a], Nora M. Dempsey [b,c], Frédéric Dumas-Bouchiat [e], Pascal Simonet [a]

[a] Ecole Centrale de Lyon, CNRS UMR 5005, Laboratoire Ampère, F-69134 Écully, France
[b] Univ. Grenoble Alpes, Inst NEEL, F-38042 Grenoble, France
[c] CNRS, Inst NEEL, F-38042 Grenoble, France
[d] Université de Lyon, Université Lyon 1, CNRS UMR 5005, Laboratoire Ampère, F-69622 Villeurbanne, France
[e] Univ Limoges, CNRS, SPCTS UMR 7513, 12 Rue Atlantis, F-87068 Limoges, France

ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form
12 September 2014
Accepted 29 September 2014
Available online 5 October 2014

Keywords:
Magnetic micropatterning
Magnetic bacterial cell labeling
Single cell micro-array
Microsystems
Single cell analysis

ABSTRACT

In various contexts such as pathogen detection or analysis of microbial diversity where cellular heterogeneity must be taken into account, there is a growing need for tools and methods that enable microbiologists to analyze bacterial cells individually. One of the main challenges in the development of new platforms for single cell studies is to perform precise cell positioning, but the ability to specifically target cells is also important in many applications. In this work, we report the development of new strategies to selectively trap single bacterial cells upon large arrays, based on the use of micro-magnets. *Escherichia coli* bacteria were used to demonstrate magnetically driven bacterial cell organization. In order to provide a flexible approach adaptable to several applications in the field of microbiology, cells were magnetically and specifically labeled using two different strategies, namely immunomagnetic labeling and magnetic *in situ* hybridization. Results show that centimeter-sized arrays of targeted, isolated bacteria can be successfully created upon the surface of a flat magnetically patterned hard magnetic film. Efforts are now being directed towards the integration of a detection tool to provide a complete micro-system device for a variety of microbiological applications.

1. Introduction

Since many years, single-cell analysis has become increasingly important in the field of microbiology [1]. Microbiological assays have been traditionally performed at the population scale, by probing a group of bacterial cells and averaging the values of the measured parameters. However, isolating and analyzing cells individually allows researchers to gain a deeper insight into cellular heterogeneities within populations, which is an important requirement for many biological applications. [2] For instance, studies involving antibiotic resistance analysis [3,4] or pathogen detection [5,6] are common examples where an accurate perception of cellular heterogeneities is needed, as such biological phenomena might often originate from rare bacteria randomly mutated in a whole population [7].

So far, microscopy imaging and flow cytometry have been the most popular methods to study cells individually [8]. However, recent advances in micro- and nano-fabrication technologies have provided new valuable tools for single cell studies [9,10]. Microsystems have emerged as powerful platforms for investigating the inherent heterogeneity of cellular systems. Their characteristic dimensions, adapted to bacterial cell size, offer the possibility to form large scale single-cell arrays on a substrate where a set of miniaturized tools can be integrated for high throughput chemical or physical analysis of individual cells [11].

Gaining the ability to place bacterial cells at defined positions on a substrate remains one of the main challenges in the development of such microsystems [12–14]. Several methods were investigated to achieve this goal [9]. For instance, many attempts have been made to build microwell arrays. Cells can be individually, simply and passively confined within uniform micron-sized cavities engraved in a substrate over a large area by using diverse microfabrication methods [15,16]. In other approaches, cells have also been arrayed using force-based methods such as dielectrophoresis [17], optical tweezers [18], or acoustic traps [19,20]. Selective cell separation or trapping can be achieved using these manipulation tools based on criteria such as size [21], dielectric properties [22,23], density [24] or refractive index [25]. However, such approaches are not adapted to the trapping of specifically targeted bacteria from a complex mixture containing unidentified microorganisms.

* Corresponding author.
E-mail address: jeremy.piv@netcmail.com (J. Pivetal).

http://dx.doi.org/10.1016/j.jmmm.2014.09.068
0304-8853/

One way to provide specificity in single-cell patterning is to graft specific cell-adhesion mediating biomolecules to the substrate. This approach typically relies on specific antibody–antigen interactions [26]. Such surface-chemistry based patterning methods have proven efficient for arraying individual cells of particular species on a substrate [26,27]. However, cells cannot be easily released from the traps if required and this approach often involves advanced surface chemistry steps, possibly difficult to implement.

In an alternative approach, the selective isolation of magnetically labeled cells with magnets was investigated to pattern cells individually [28]. However, as regards cell manipulation using micro-magnetic devices, most studies reported in the literature were performed either on eukaryotic cells [29,30], of size typically ten times larger than bacterial cells, or on magnetotactic bacteria [31], which naturally synthesize intracellular chains of nano-magnets. Moreover, the micron scaled magnetic flux sources employed in these studies were either based on soft magnetic materials requiring external magnetic fields or on the use of micro-electromagnets requiring an external power supply and generating Joule heating.

In this work, we demonstrate that micro-patterned hard magnetic structures can be used to achieve selective individual patterning of magnetically labeled *Escherichia coli* bacteria ($\approx 2\,\mu m$ in size). These micron scaled magnetic flux sources, requiring neither an external magnetic field nor a power supply, have been used to fabricate compact autonomous devices for the trapping of magnetic particles [32] and of magnetically-labeled eukaryotic cells [33]. So as to be adaptable to several applications in the field of microbiology, two different strategies were employed to label cells magnetically and specifically using (1) immunomagnetic labeling, based on antibody–antigen interactions and (2) magnetic *in situ* hybridization relying on a specific DNA sequence detection [34]. In both cases, we show that selective arraying of individual bacteria can be achieved using micro-magnets of a size approaching that of bacteria.

2. Materials and methods

2.1. Micro-magnet design and fabrication

5 μm thick hard magnetic neodymium iron boron (NdFeB) films were sputtered on Si wafers covered by a 100 nm Ta buffer layer. A 100 nm Ta protecting overlayer was then deposited to prevent oxidation of the magnetic thin film. NdFeB films present a coercivity of around 2 T and a remanence up to 1.3 T. Micro-magnet arrays were obtained by the Thermo-Magnetic Patterning technique (TMP), which consists in exploiting the temperature dependence of coercivity to locally switch magnetization [35]. The magnetized hard magnetic films were irradiated with a KrF (248 nm) pulsed excimer laser, through a TEM grid (5 μm wide metal stripes defining an array of 7.5 μm × 7.5 μm holes), used as the contact mask for patterning. At the same time, an external magnetic field was applied opposite to the initial magnetization direction, which induced magnetization reversal in the areas heated up by the laser. The resulting structures consist of arrays of oppositely magnetized micromagnets. The stray magnetic field patterns above the micro-magnet array were visualized using Magnetic Force Microscopy. The magnetic field and magnetic field gradient at the surface of the micro-magnet array are maximum at the interfaces between oppositely magnetized regions [36].

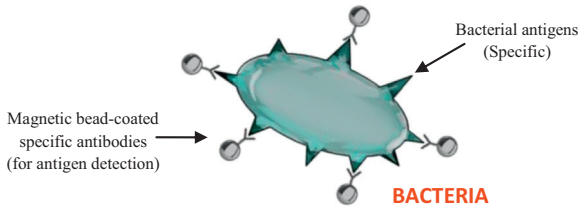

Fig. 1. Principle of bacterial cell labeling using immunomagnetic labeling.

2.2. Bacterial cell labeling

For all experiments, the organisms used were *E. coli* DH10β and *Acinetobacter sp.* ADP1 cultured in Luria Bertani (LB) broth at 37 °C. Bacterial cell labeling was performed using two different strategies: (1) immunomagnetic labeling and (2) magnetic *in situ* hybridization. Schematic illustrations of each labeling concept are shown in Figs. 1 and 2.

2.2.1. Immunomagnetic labeling

For immunomagnetic labeling, commercially available biotinylated *anti-E. coli* antibodies were purchased from AbD Serotec. A sample of 250 μl of cultured bacterial cells ($OD_{600nm}=0.25$) were washed 3 times and re-suspended in 500 μl of phosphate buffer saline (PBS: 130 mM NaCl, 10 mM sodium phosphate buffer, pH 7.2). A volume of 1 μl of biotinylated *anti Escherichia coli* antibodies (Serotec®, Rabbit *anti E. coli* antibody: Biotin) were then mixed with bacteria. The sample was incubated at room temperature for 1 h, washed 3 times and re-suspended in 500 μl of PBS. A volume of 25 μl of streptavidin-coated superparamagnetic beads (MiCroBeads, diameter 50 nm) was then added to the mixture before 1 h incubation at room temperature.

2.2.2. Magnetic in situ hybridization

2.2.2.1. Cell fixation. Two milliliters of cells in exponential growth phase (optical density=0.8–1) were collected by centrifugation (10 min at 2500 × *g*) and fixed in 500 μl of 3% paraformaldehyde solution (PFA) diluted in phosphate-buffered saline (130 mM NaCl, 10 mM sodium phosphate buffer, pH 7.2). The sample was then incubated 12 h at 4 °C. After incubation, cells were washed 3 times with PBS and re-suspended in 500 μl of the same buffer. For storage at −20 °C, 50% (v/v) absolute ethanol was added to samples.

2.2.2.2. Preparation of rDNA templates for in vitro transcription. E. coli DH10β genomic DNA was extracted using the NucleoSpin® Tissue kit (Macherey-Nagel) following the manufacturer's instructions. PCR amplification of 23S RNA gene fragments encoding the variable region of domain III was then performed with the *E. coli* DH10β DNA. The nucleotide sequences of the primers used were 5′-MADGCGTAGBCGAWGG-3′ ($1900V^{37}$) and 5′-**TAATACGACTCACTATAGGG**GGACCWGTGTCSGTTTHBGTAC-3′

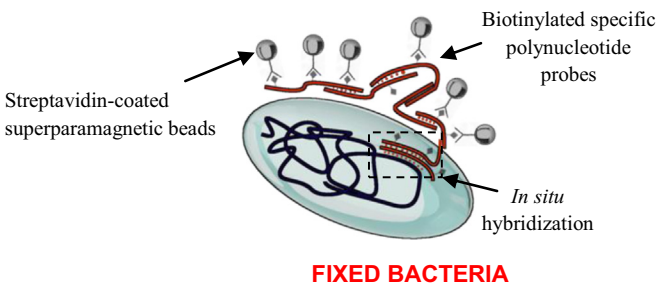

Fig. 2. Principle of bacterial cell labeling using magnetic *in situ* hybridization.

(317RT[38]). The latter primer contained the T7-RNA polymerase promoter sequence (underlined) required for the *in vitro* transcription. PCR assays were performed with Illustra Hot Start Mix RTG PCR beads from GE Healthcare following the supplied protocol. The cycling parameters for PCR were 95 °C for 10 min, followed by 35 cycles at 95 °C for 30 s, 53 °C for 60 s and 72 °C for 90 s, followed by 72 °C for 10 min. The resulting rDNA fragments with an expected size of about 236 nucleotides were controlled by agarose gel electrophoresis (1% (w/v)) stained with ethidium bromide (BET) (0.1 mg/ml). The purified PCR products (GFX PCR DNA and Gel Band purification Kit, GE Healthcare) obtained were then used as templates for *in vitro* transcription.

2.2.2.3. Preparation and labeling of transcript probes. Transcipts of 23S rDNA fragments were obtained using an RNA transcription kit (Invitrogen). The resulting RNA probe products were purified using the NucleoSpin® RNA purification kit (Macherey-Nagel) and subsequently labeled with a biotin labeling kit (MIRUS®) which offered a labeling density of about 1 labeled nucleotide every 50 nt.

2.2.2.4. Magnetic labeling of bacterial cells. A volume of 10 μl of PFA-fixed cells was washed with 200 μl of PBS (130 mM NaCl, 10 mM sodium phosphate buffer, pH 7.2), centrifuged for 3 min at 10,000 × *g*, and re-suspended in 30 μl of a hybridization buffer (100 mM NaCl, 0.01% SDS, 20 mM Tris–HCl (pH 8.0), 5 mM EDTA (pH 8.0) containing 80% formamide and 1 μg of labeled transcript probes). The solution was then incubated at 80 °C for 20 min and hybridization was performed at 53 °C for 4 h. After cell hybridization, 60 μl of PBS (130 mM NaCl, 10 mM Na_2HPO_4/NaH_2PO_4, 0.5 M EDTA, pH 7.2) and 10 μl of streptavidin-coated superparamagnetic beads (Miltenyi Biotec MicroBeads, diameter 50 nm) were added to 40 μl samples. An incubation step was then performed overnight at 4 °C.

2.3. Single-cell micro-patterning

Cell suspensions from labeled samples were stained with 1 μl of ethydium bromide (BET) (0.1 mg/mL) for subsequent microscopic observation. Ten microliters of this suspension were placed upon magnetically patterned hard magnetic films. We used an epifluorescence microscope (Carl Zeiss AxioImager/AxioCam HSm camera) to visualize the emergence of the magnetic patterns, revealed by the attraction of bacteria to the regions of highest stray magnetic field.

3. Results and discussion

Although microsystem technologies are providing new opportunities for single cell analysis in microbiology, a key step in their development is to be able to micro-pattern bacterial cells selectively and individually upon defined areas of a substrate. In this paper, we propose the use of a magnetic micro device as a tool to individually micro-pattern bacterial cells selectively. As a first proof of concept, we used *E. coli* bacterial cells as a model. We demonstrate that *E. coli* can be magnetically labeled selectively and subsequently micro-patterned upon a flat array of 7.5 × 7.5 μm^2 magnets.

3.1. Micro-magnet array for single cell micro-patterning

Micromagnetic patterns (Fig. 3a) produced by applying the TMP technique to a flat hard magnetic film can be visualized using Magnetic Force Microscopy. Fig. 3b shows the reconstructed image obtained from the measurement of the interaction force between a magnetized tip and the magnetic stray field of the 7.5 × 7.5 μm² patterns. Fig. 3c. and d show, respectively, the norm of the magnetic flux density (B) and the modulus of the magnetic field gradient along the z-axis ($\partial B/\partial z$) calculated 1 μm above such a patterned film (COMSOL). The most intense gradients (2.5 × 10^5 T/m) are found at the boundary separating two regions of

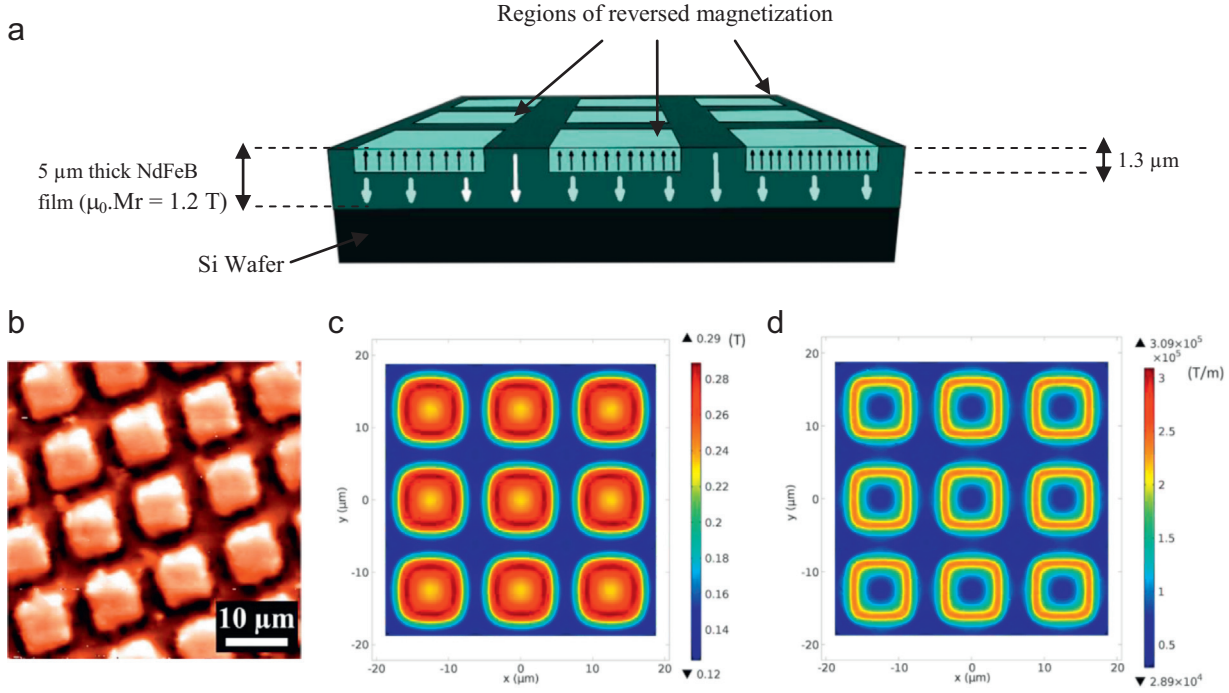

Fig. 3. Micro-magnet arrays: (a) schematic of a Thermo-Magnetically Patterned (TMP) hard magnetic film, (b) MFM (Magnetic Force Microscope) image of an array of 7.5 × 7.5 μm^2 micro-magnets produced by TMP, (c) magnetic flux density norm 1 μm above the micro-magnet array (COMSOL) and (d) magnitude of the magnetic field gradient ($\partial B/\partial z$).

opposite magnetization. It should be noted that the strong field gradients obtained with such micro-magnets are required to trap small size bacteria labeled with magnetic nanoparticles [39]. These high field gradients should maintain the trapped cells immobilized when performing long-term experiments such as cell culture.

3.2. Selective labeling of bacterial cells

Prior to placement upon the micro-magnet array, cells were specifically marked with magnetic nanoparticles using two different strategies. The first strategy was based on immunomagnetic labeling while the second one relied on magnetic *in situ* hybridization.

Each strategy has its own advantages and limitations that can meet different requirements depending on the study carried out in microbiology. Immunomagnetic labeling is a technique based on antibody–antigen recognition. In this work, targeted *E. coli* bacteria were labeled using a biotinylated antibody directed against those cells, onto which magnetic nanoparticles were subsequently attached. The main advantage of this strategy is its high specificity and the fact that antibody-based cell manipulation tools preserve cell viability [40], which allows to study complex biological responses of bacteria to a wide range of environments, stressors, and growth conditions. On the other hand, the use of immunolabeling techniques in microbiology is limited by the need to recover enough antigenic molecules to produce antibodies. This will generally restrict its application to bacteria than can be grown in a laboratory, which represents a low percentage of bacteria ($\approx$ 1–10%). Consequently, this approach is mostly employed for the detection of well-characterized bacteria [41].

Magnetic *in situ* hybridization (MISH) is a molecular biology technique that allows to identify specific cells through nucleic acid sequences (DNA or RNA) [34]. This method is based on the specific base pairing interaction between nucleic acids. After identifying a specific bacterial nucleic acid sequence, a complementary nucleotide probe is synthesized and directed against the cells of interest. In this study, biotinylated polynucleotide transcript probes were used. These probes, once hybridized to their target sequences, allowed an anchorage of streptavidin-coated superparamagnetic beads [38]. The polynucleotide probes used for *in situ* hybridization were RNA transcripts from PCR-amplified fragments of *E. coli* DNA corresponding to a specific part of the 23S rDNA domain III. The 23S rDNA domain III being considered as a phylogenetic marker [37], probes used are specific to the strain from which they originate. Here the probes specifically targeted *E. coli*. However, the method described in this paper can be used to specifically label other target cells from a complex mixture. In MISH, prior isolation of targeted microorganisms in pure culture is not required, which broadens the application to unculturable bacteria. A DNA sequencing step is generally performed to obtain the DNA (or RNA) template which will be used to synthesize the probe *in vitro* [42]. Cells labeled by this method are not viable due to the fixation step performed with a chemical fixative (here paraformaldehyde) to preserve cell integrity. This fixation step aims at denaturing the bacterial cell wall and achieving crosslinking of proteins [43]. Nevertheless, fixed bacteria remain whole cells genetically viable for subsequent genomic applications [44,45].

3.3. Selective micro-patterning of single bacterial cells upon a micro-magnet array

After magnetic labeling of target cells, micro-patterning experiments were performed. Before attraction experiments, bacteria samples were stained with ethidium bromide to facilitate their observation and consequently displayed orange fluorescence under an excitation wavelength of 524 nm. Fig. 4 and Fig. 5 show results of experiments carried out using immunomagnetic labeling and magnetic *in situ* hybridization, respectively.

Trapping of target cells resulted in fluorescent patterns revealing the magnetization patterns of the NdFeB film. Bacterial cells were attracted towards magnetic field maxima and for an initial concentration of 10^9 bacteria/mL, small groups (≤ 10 bacteria) were localized at each micro-magnet (Fig. 4c). For a concentration of 10^8 bacteria/mL, bacteria were overall individually positioned onto $7.5 \times 7.5\ \mu m^2$ sites (Fig. 5 c–f), but it could happen that two cells were captured in a single trap. When the labeling step was skipped, no fluorescent pattern could be observed (Figs. 4a and 5a). In a second control experiment, to confirm the specificity of *E. coli* DH10β micro-patterning, we used another bacteria strain, *Acinetobacter sp.* ADP1, as a negative control. *E. coli* cells were therefore replaced with *Acinetobacter* cells. The random positioning of the *Acinetobacter sp.* ADP1 bacteria on the micro-magnet array confirms trapping specificity with both labeling techniques used. (Figs. 4b and 5b). Those results show that the use of micro-magnets, combined with flexible labeling approaches, ensure proper bacterial cell organization, in which precisely positioned cells are sufficiently isolated from their neighbors.

4. Conclusion

In this study, we demonstrated the formation of a large 2D array of single bacterial cells upon a magnetically patterned hard magnetic film. For this purpose, flat micro-magnets of size approaching that of bacteria were designed. *E. coli* bacterial cells were selected as a model in our study. However, labeling

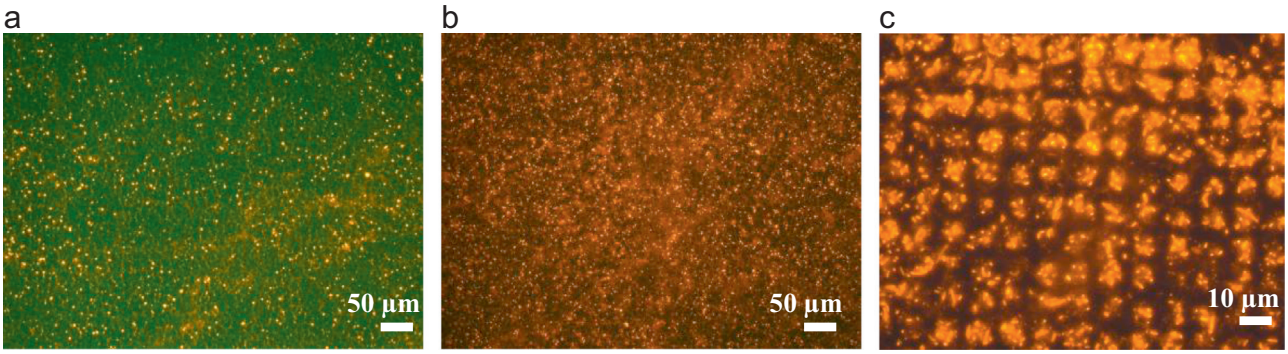

Fig. 4. Micro-patterning of *E. coli* bacterial cells magnetically labeled by immunomagnetic labeling upon an array of $7.5 \times 7.5\ \mu m^2$ magnets: (a) control showing that non-labeled *E. coli* are randomly positioned upon the micro-magnet surface; (b) negative control showing the randomly positioning of *Acinetobacter sp.* ADP1 exposed to the same antibody and (c) micro- patterning of *E. coli* in small groups (concentration: 10^9 cells/mL).

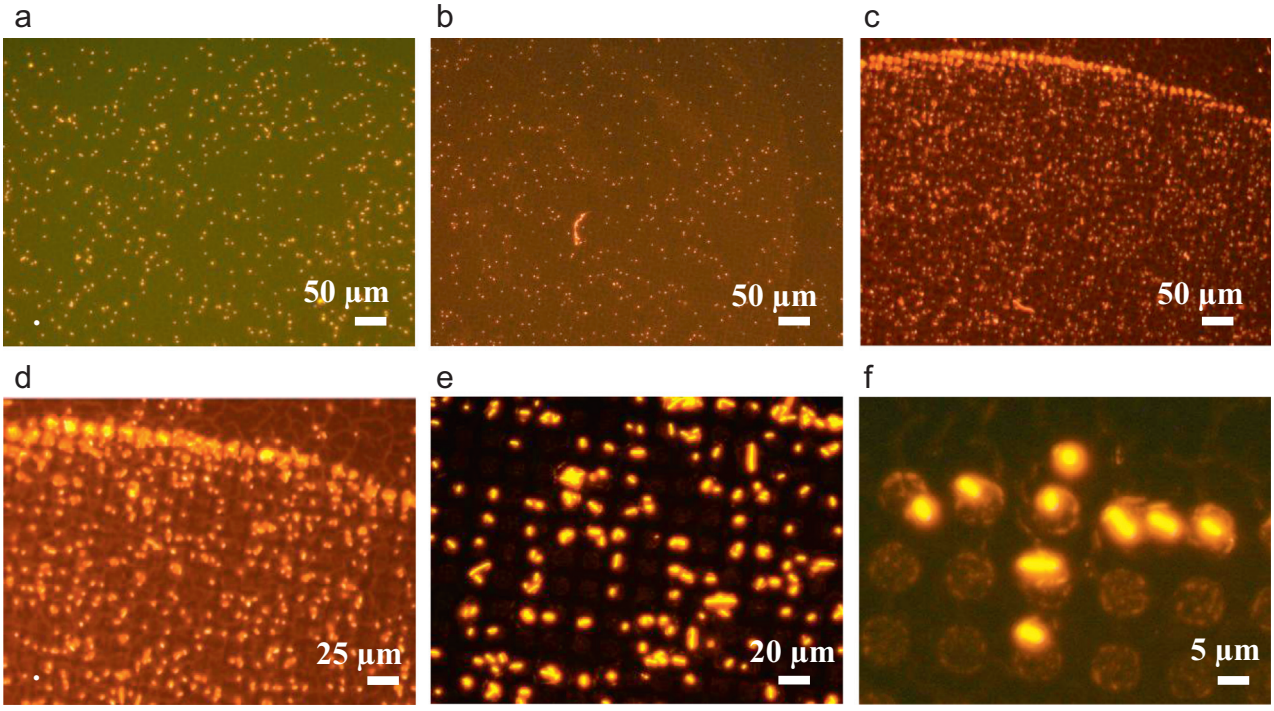

Fig. 5. Micro-patterning of *E. coli* bacterial cells magnetically labeled by magnetic *in situ* hybridization upon an array of $7.5 \times 7.5\ \mu m^2$ magnets: (a) control showing that non-labeled *E. coli* were randomly positioned upon the micro-magnet array, (b) negative control showing the random positioning of *Acinetobacter sp.* ADP1 exposed to the probes selected for labeling of *E. coli*, (c–f) progressively zoomed views showing *E. coli* individually trapped upon $7.5 \times 7.5\ \mu m^2$ micro-magnets (concentration: 10^8 cells/mL). In pictures e and f, it can be noticed that free biotinylated RNA probes linked to streptavidin-coated superparamagnetic nanoparticles and labeled by ethidium bromide reveal the magnetic pattern.

techniques used here could be easily applied to any other cell type. This improvement in cell array technology provides new perspectives for the development of innovative microsystem chips. We have recently demonstrated that micro-magnets could be integrated within microfluidic channels for future lab-on-chip applications [32,34]. This microfluidic integration can be exploited to elute non-target cells, while keeping the others at their individual trapping site. We are now working on improving the system design through the integration of detection tools to provide a complete micro-system device for a variety of microbiological applications.

Acknowledgements

The authors thank the Région Rhône-Alpes for the Ph.D. grant of J. Pivetal. This work was supported by the French National Research Agency (ANR 09-CESA-013) and by the labex iMUST of Université de Lyon.

References

[1] B.F. Brehm-stecher, E.A. Johnson, Single-cell microbiology: tools, technologies, and applications, Microbiol. Mol. Biol. Rev. 68 (2004) 538–561.
[2] H.M. Davey, D.B. Kell, Flow cytometry and cell sorting of heterogeneous microbial populations: the importance of single-cell analyses, Microbiol. Rev. 60 (1996) 641–696.
[3] K.S. Williamson, et al., Heterogeneity in *Pseudomonas aeruginosa* biofilms includes expression of ribosome hibernation factors in the antibiotic-tolerant subpopulation and hypoxia-induced stress response in the metabolically active population, J. Bacteriol. 194 (2012) 2062–2073.
[4] M.A. Sánchez-Romero, J. Casadesús, Contribution of phenotypic heterogeneity to adaptive antibiotic resistance, Proc. Natl. Acad. Sci. U. S. A. 111 (2014) 355–360.
[5] H.C. Van der Mei, H.J. Busscher, Bacterial cell surface heterogeneity: a pathogen's disguise, PLoS One 8 (2012).
[6] S. Helaine, D.W. Holden, Heterogeneity of intracellular replication of bacterial pathogens, Curr. Opin. Microbiol. 16 (2013) 184–191.
[7] a J. Link, K.J. Jeong, G. Georgiou, Beyond toothpicks: new methods for isolating mutant bacteria, Nat. Rev. Microbiol. 5 (2007) 680–688.
[8] S. Lindström, H. Andersson-Svahn, Methods In Molecular Biology, Humana Press (2012) 13–15.
[9] H. Andersson, A. van den Berg, Microtechnologies and nanotechnologies for single-cell analysis, Curr. Opin. Biotechnol. 15 (2004) 44–49.
[10] W.M. Weaver, et al., Advances in high-throughput single-cell microtechnologies, Curr. Opin. Biotechnol. 25 (2014) 114–123.
[11] J. El-Ali, P.K. Sorger, K.F. Jensen, Cells on chips, Nature 442 (2006) 403–411.
[12] J. Kim, et al., Direct-write patterning of bacterial cells by dip-pen nanolithography, J. Am. Chem. Soc. 134 (2012) 16500–16503.
[13] H.S. Hwang, et al., Dry photolithographic patterning process for organic electronic devices using supercritical carbon dioxide as a solvent, J. Mater. Chem. 18 (2008) 3087.
[14] M. Veiseh, O. Veiseh, M.C. Martin, F. Asphahani, M. Zhang, Short peptides enhance single cell adhesion and viability on microarrays, Langmuir 23 (2007) 4472–4479.
[15] S. Lindström, H. Andersson-Svahn, Miniaturization of biological assays – overview on microwell devices for single-cell analyses, Biochim. Biophys. Acta 308–16 (1810) 2011.
[16] T. Saeki, et al., Digital cell counting device integrated with a single-cell array, PLoS One 9 (2014) e89011.
[17] M. Frénéa, S.P. Faure, B. Le Pioufle, P. Coquet, H. Fujita, Positioning living cells on a high-density electrode array by negative dielectrophoresis, Mater. Sci. Eng. C 23 (2003) 597–603.
[18] H. Zhang, K.-K. Liu, Optical tweezers for single cells, Interface 5 (2008) 671–690.
[19] X. Ding, et al., On-chip manipulation of single microparticles, cells, and organisms using surface acoustic waves, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 11105–11109.
[20] J. Shi, et al., Acoustic tweezers: patterning cells and microparticles using standing surface acoustic waves (SSAW), Lab Chip 9 (2009) 2890–2895.
[21] H. Tsutsui, C.-M. Ho, Cell separation by non-inertial force fields in microfluidic systems, Mech. Res. Commun. 36 (2009) 92–103.
[22] X. Hu, et al., Marker-specific sorting of rare cells using dielectrophoresis, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 15757–15761.
[23] S. Bhattacharya, et al., Selective trapping of single mammalian breast cancer cells by insulator-based dielectrophoresis, Anal. Bioanal. Chem. 406 (2014) 1855–1865.
[24] R. Guldiken, M.C. Jo, N.D. Gallant, U. Demirci, J. Zhe, Sheathless size-based acoustic particle separation, Sensors, 12, 905–922.

[25] M.P. MacDonald, et al., Cell cytometry with a light touch: sorting microscopic matter with an optical lattice, J. Biol. Regul. Homeost. Agents 18 (2004) 200–205.
[26] Z. Suo, X. Yang, R. Avci, M. Deliorman, P. Rugheimer, Antibody selection for immobilizing living bacteria, Anal. Chem. 81 (2010) 7571–7578.
[27] J. Yu, et al., A polyethylene glycol (PEG) microfluidic chip with nanostructures for bacteria rapid patterning and detection, Sens. Actuators A Phys. 154 (2009) 288–294.
[28] I. Safarík, M. Safaríková, Use of magnetic techniques for the isolation of cells, J. Chromatogr. B. Biomed. Sci. Appl. 722 (1999) 33–53.
[29] K. Ino, et al., Cell culture arrays using magnetic force-based cell patterning for dynamic single cell analysis, Lab Chip 8 (2008) 134–142.
[30] W. Liu, et al., A novel permalloy based magnetic single cell micro array, Lab Chip 9 (2009) 2381–2390.
[31] H. Lee, A.M. Purdon, V. Chu, R.M. Westervelt, Controlled assembly of magnetic nanoparticles from magnetotactic bacteria using microelectromagnets arrays, Nano Lett. 4 (2004) 995–998.
[32] L.F. Zanini, N.M. Dempsey, D. Givord, G. Reyne, F. Dumas-Bouchiat, Autonomous micro-magnet based systems for highly efficient magnetic separation, Appl. Phys. Lett. 99 (2011) 232504.
[33] O. Osman, et al., Microfluidic immunomagnetic cell separation using integrated permanent micromagnets, Biomicrofluidics 7 (2013) 054115.
[34] J. Pivetal, et al., Selective isolation of bacterial cells within a microfluidic device using magnetic probe-based cell fishing, Sens. Actuators B Chem. 195 (2014) 581–589.
[35] F. Dumas-Bouchiat, et al., Thermomagnetically patterned micromagnets, Appl. Phys. Lett. 96 (2010) 102511.
[36] M. Kustov, et al., Magnetic characterization of micropatterned Nd–Fe–B hard magnetic films using scanning Hall probe microscopy, J. Appl. Phys. 108 (2010) 063914.
[37] W. Ludwig, S. Dorn, N. Springer, G. Kirchhof, K.H. Schleifer, PCR-based preparation of 23S rRNA-targeted group-specific polynucleotide probes, Appl. Environ. Microbiol. 60 (1994) 3236–3244.
[38] M. Stoffels, W. Ludwig, K.H. Schleifer, rRNA probe-based cell fishing of bacteria, Environ. Microbiol. 1 (1999) 259–271.
[39] N. Pamme, Magnetism and microfluidics, Lab Chip 6 (2006) 24–38.
[40] D. a Veal, D. Deere, B. Ferrari, J. Piper, P.V. Attfield, Fluorescence staining and flow cytometry for monitoring microbial cells, J. Immunol. Methods 243 (2000) 191–210.
[41] R.I. Amann, W. Ludwig, K.H. Schleifer, Phylogenetic identification and in situ detection of individual microbial cells without cultivation, Microbiol. Rev. 59 (1995) 143–169.
[42] R. Amann, B.M. Fuchs, Single-cell identification in microbial communities by improved fluorescence in situ hybridization techniques, Nat. Rev. Microbiol. 6 (2008) 339–348.
[43] J.M. Nowacek, Fixation and Tissue Processing, Special Stain H&E, 2010, Chap. 16, pp. 141–152.
[44] G. Bussolati, L. Annaratone, E. Medico, G. D'Armento, A. Sapino, Formalin fixation at low temperature better preserves nucleic acid integrity, PLoS One 6 (2011) e21043.
[45] N. Masuda, T. Ohnishi, S. Kawamoto, M. Monden, K. Okubo, Analysis of chemical modification of RNA from formalin-fixed samples and optimization of molecular biology applications for such samples, Nucl. Acids Res. 27 (1999) 4436–4443.