



In situ measurements of magnetic nanoparticles after placenta perfusion



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ABSTRACT

Nanoparticles (NP) present promising tools for medical applications. However, the investigation of their spatial and temporal distribution is hampered by missing in-situ particle detection and quantification technologies. The placenta perfusion experiment represents an interesting model for the study of the particle distribution at a biological barrier. It allows the ex-vivo investigation of the permeability of the placenta for materials of interest. We introduce an approach based on a magnetic system for an in situ measurement of the concentration of magnetic NPs in such an experiment. A previously off-line utilized magnetic readout device (sensitivity of $\approx 10^{-8} \text{ Am}^2$) was used for long term measurements of magnetic NP of 100–150 nm size range in a closed circuit of a placenta perfusion. It represents a semiquantitative approach. The behavior of particles in the placenta and in the measurement system was studied, as well as the influence of particle surface modifications. The results suggest a transfer of a low amount of particles from the maternal to the fetal blood circuit.

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1. Introduction

Nanoparticles (NP) play an increasing role in our current society – both in daily life (toothpaste, cleaning agents, deodorant) [1] and biomedicine (cancer treatment, medical imaging) [2,3]. The different NP can be composed of a diversity of materials and can show quite different shapes and characteristics. The only given similarity of all these nanomaterials is their size, with one dimension not exceeding 100 nm [3]. NPs can serve to improve the application of medicals by increasing their half-life period or their specificity [4]. Particularly in medical imaging their use is very beneficial. Especially magnetic NPs are worth to be applied as a contrast agent in magnetic resonance tomography (MRT). Nevertheless the current lack of knowledge about their potential toxicity and the chance of spatial distribution in the human body require an excessive risk assessment before bringing these particles into broad use. For this purpose, the *ex vivo* placenta perfusion constitutes an approved method to illustrate the behavior and possible alterations of placental tissue due to a certain treatment

[5]. It allows investigating the behavior of agents of interest, like NPs, at the feto-maternal interface, the placental barrier, supplying helpful hints concerning a potential use during pregnancy [6]. Furthermore the human placenta represents a very suitable model of a humane organ that offers the option of the investigation of the behavior of interesting NPs without constituting ethical problems [7]. It allows analyzing the passage of NPs through human tissue as well as their retention at/inside the tissue [8] and their influence concerning different biological parameters like energy usage or the protein biosynthesis. To date, no simple standardized methods are available for quantification of NP in human tissue. Until now, the quantification of NPs in such experiments usually is performed in static samples [9], bringing up the problem of a delayed analysis period, the need of suitable sample treatment and the lack of possibilities to interact directly with the analysis system. We developed a setup to follow the magnetic signal in a floating fluid in-line by improving a given magnetic system that was designed for measuring the magnetic moment in static samples.

Aim of the investigations was to verify a potential transfer of magnetic particles across the placental barrier by means of a semi-quantitative magnetic in-line measurement in an *ex vivo* placenta perfusion experiment. To our knowledge, it is the first study of this kind.

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2. Materials and methods

2.1. Detection principle of the magnetic system

A measuring system for quantification of static magnetic beads (batch samples) was modified in order to measure particle concentrations in a flowing suspension. The original system, the Magnet Reader [10], is based on frequency composition and field strength at the non-linear magnetization curve of superparamagnetic samples (non-hysteretic). For details of the detection principle see [11,12]. The response signal generated at a frequency representing a linear combination of the two distinct excitation frequencies $f_1=49.4$ kHz ($H_{\max}=1.5$ mT) and $f_2=61$ Hz ($H_{\max}=4.5$ mT) is detected. In addition to the coaxial excitation coils two similar pick-up coils are mounted in one gauging head. These are used for measuring the difference between the magnetic field in the measuring coil (contains the sample) and in an empty coil. A linear measuring range is given for particle concentrations between 0.12 and 1300 $\mu\text{g}(\text{Fe})/\text{mL}$ [10] determined on superparamagnetic iron oxide particles. This lower limit corresponds with a magnetic moment of about 10^{-8} Am² and a particle concentration of about 1.7 $\mu\text{g}/\text{mL}$ in the cuvettes for static measurements. Theoretically the minimum detectable magnetic moment is 5×10^{-14} Am², calculated with an integration time of 10 s [10]. There is an offset in the signal coming from a non-perfect compensation/ adjustment of the coils of about 360 mV that has to be considered. After certain time intervals during the perfusion experiments the background was measured by removing the

cuvette and this signal (a function calculated by fitting a polynomial to these datapoints) was subtracted from the data measured with cuvette.

For measurements of flowing samples (integration time: ≤ 1 s) a lower sensitivity, i.e. a higher upper limit of the linear range, is expected. The noise of the measurement decreases with the square root of the increasing integration time. Since the sample cuvettes have to be modified, the change in the sample volume inside the measuring coil and a change of the exposure time of particles (in the order of 0.1 s maternal and 0.5 s fetal) caused by the flow rate (see Section 2.2) will alter the limits. In the case that a fraction of NPs is non-superparamagnetic only a semi-quantitative result can be given.

2.2. Ex vivo placenta perfusion model

The employed system of *ex vivo* placenta perfusion was adopted from the setup established by Panigel et al. in 1967 [13] and further optimized by Schneider et al. in 1972 [14] (Fig. 1). Apart from the installation of the MagnetReader and non-magnetic stirrers, the setup was not modified for the magnetic measurements.

In the perfusion experiments, the placenta was connected with the pumping and measuring system by cannulae. Before the measurement the placenta was rinsed and reoxygenated for 30 min with perfusion fluid to remove remaining blood and further perfused for 2 h as a control phase without nanoparticles, to detect possible leaks and check stability of the system. Perfusion

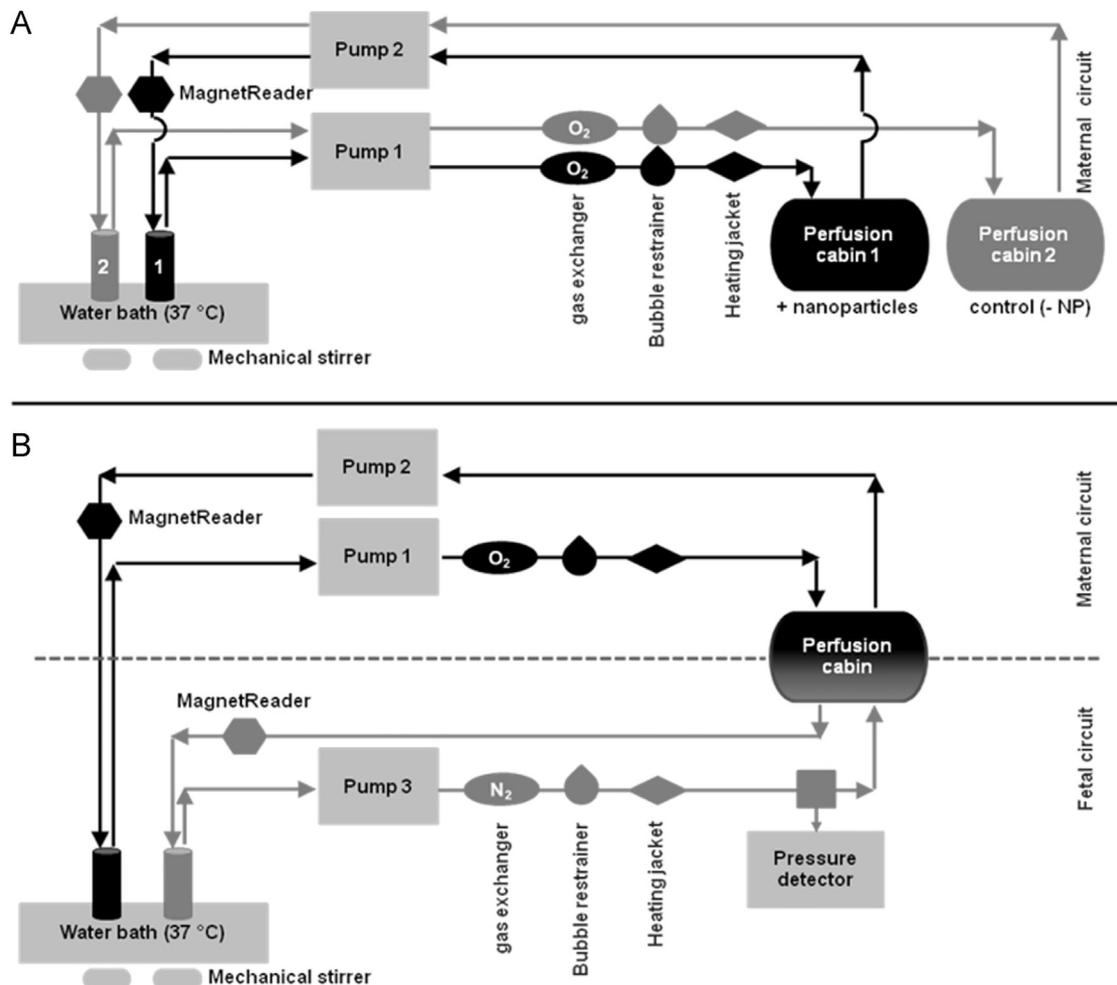


Fig. 1. The setup of the experiment. A: for evaluation of the measuring system B: for the double sided perfusion experiments.

fluid is diamagnetic and contains NCTC-135 Medium, Earle's buffer (solution of alkaline and alkaline earth salts), BSA, Dextran, D-Glucose, Amoxicillin and Heparin. The pH was adjusted to pH = 7.4 with NaOH prior to filtration using a 0.8/0.2 μm filter and freezing to $-20\text{ }^{\circ}\text{C}$ until usage. The flow rate in the maternal circuit was 12 mL/min (± 0.2 mL/min) and in the fetal circuit 3 mL/min (± 0.1 mL/min), chosen for biological reasons [15]. The maximum flow velocity in the cuvette (maternal circuit) is about 65 mm/s, the minimum (fetal circuit) about 6 mm/s that is a comparable range of velocities of blood in small vessels given in [16].

After a control phase of 2 h, the fluid reservoirs (maternal and fetal) were replaced by fresh ones. The total volume amounts to 160 mL, including the volume of the cuvettes and hoses and of the fluid flowing in the placenta estimated by a rinsing experiment. For the measurement 50 μL of the magnetic particles (fluidMag-D) were added to the maternal reservoir. This volume of particle solution was adjusted according to the application recommendations of the formerly commercially available ferric oxide based contrast agent Resovist[®], in assumption of a body weight of over 60 kg during pregnancy. Then the perfusion experiment was run for further 4 h. The initial particle concentration in the maternal circuit is one order of magnitude higher than the lower limit of the linear concentration range (see above). The magnetic moment of such a sample is more than three orders higher than the given limit of 10^{-8} Am^2 . At certain time intervals (30 min) small amounts (4 mL or 5 mL) of fluid were taken from both reservoirs for biological investigations (glucose and lactate concentration, beta-hCG concentration, cytokine concentration etc., see Section 3.2). The extraction of these samples leads to an overestimation of the fetal signal of about 12% in case there is a constant transfer (particles/time) and has to be considered. If the transfer shows a kind of saturation effect (rate decreases with time) the overestimation is weaker. The particle concentrations of the maternal as well as the fetal circuit were measured alternately with one measuring system by changing the measuring cuvette. Differences in the signals of both circuits caused by the different cuvette volumes (typical size: 60 μL) were corrected. Because of the expected very low concentration of particles in the fetal circuit, a slightly bigger cuvette was used here. Changes in the fluid volume could be detected in the reservoir with an accuracy of about 1 mL. For simplicity reasons the first investigations for the evaluation of the magnetic measuring system, e.g. the influence of the cuvette material, were done in single side perfusion experiments, i.e. the setup was as described above, however only the maternal side was measured with the MagnetReader. There was no need to change the cuvettes. In some cases a control experiment in a second perfusion circuit without particles was running simultaneously (Fig. 1a). Double-sided perfusion experiments were carried out in a setup according to Fig. 1b.

The offset (see Section 2.1) in the first experiment was measured before particles were added and extrapolated to higher measuring times. In the double-sided experiments it was measured at every change of the cuvettes to improve the precision, i.e. the polynomial fit.

2.3. Magnetic nanoparticles

Two types of particles with different coatings were used in the experiments. Commercially available superparamagnetic iron oxide particles in aqueous suspension (50 mgNP/mL) with polyethylene imine coating and a diameter of approximately 100 nm (fluidMAG-PEI, chemicell GmbH, Berlin) were compared to starch coated particles with a hydrodynamic size of 150 nm (fluidMAG-D, chemicell GmbH, Berlin), (data according to the suppliers

information, www.chemicell.com). The value of mass concentration of nanoparticles includes the coating and contains 75% magnetite.

3. Results and discussions

3.1. Characteristics of the measuring system

At first, investigations to check the performance of the measuring system with flowing samples were carried out in a set-up without placenta tissue.

For flowing samples new cuvettes had to be constructed from a non-porous, machinable (sufficient stiffness), diamagnetic and chemically resistant material (for cleaning). A drift of the measuring value was observed in longterm measurements. Experiments have shown that there is a small thermal drift depending on the warming of the measuring system, but the main influence is caused by adhesion of magnetic material inside the cuvette, which occurs particularly under the influence of a magnetic field. This effect is strongly dependent on the cuvette material and the organic coating material of the magnetic particles. We tested cuvettes made from polyoximethylen (POM) and glass, respectively.

The influence of different coating materials of the magnetic particles can be seen in Fig. 2 for different particle concentrations, each measured in a POM cuvette. PEI coated particles (positively charged) obviously adhere partly to the cuvette which leads to a drift of the signal during the particle flow and a remaining signal even after dilution of the particle-containing fluid in three steps (Fig. 2a).

The same dilution procedure was done with polyelectrolyte (starch) coated particles (Fig. 2b). Here there is almost no drift. The dilution steps can be seen clearly. Please note that the "jump" in the signal at 130 s is caused by an air bubble. Based on these results, further studies were conducted with starch-coated particles. Measurements over a longer period (30 min) with a POM cuvette revealed even in the case of starch-coated particles a small drift of about 0.2 mV/min. There was no influence of the flow rate of the suspension (up to 12 mL/min).

An improved drift behavior was observed with a glass cuvette. The influence of the flow rate (mean non-corrected values of a one-minute measurement) on the signal can be seen in Fig. 3. The constant value indicates that there is no significant adhesion of particles to the glass cuvette. The variance of the data is about 1.8 mV for an integration time of 1 s per data point.

Despite the absence of an influence of the flow rate on the signal, the limit of detection was checked using a glass cuvette and starch-coated particles (Fig. 4). The fluid was pumped through the maternal circuit (without placenta). A linear measuring range was observed for particle concentrations between about 3 and 160 μg nanoparticles/mL assuming an accuracy of the measurement of ≈ 1 mV. The mass concentration of NPs includes the coating.

In placenta experiments, the background signal had to be measured in each experiment separately because of influences of the temperature and possible external electrical disturbances.

3.2. Influence of leaks in the tissue

Because of damages of the placenta or leaks at the placenta-cannula junctions, perfusion fluid of the fetal circuit could pass over to the maternal circuit causing a continuous increase of the fluid volume in the maternal reservoir. In such a case the MagnetReader detects a decreasing particle concentration in the maternal circuit that is not only caused by particle diffusion but as

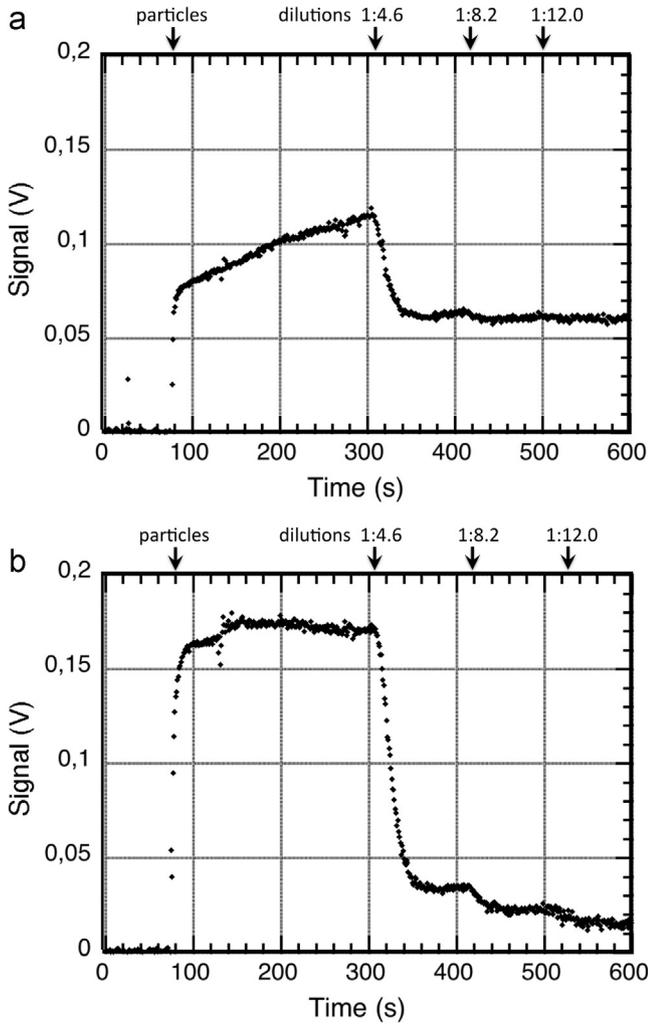


Fig. 2. Offset-corrected magnetic signal of particle suspensions of different coatings in a phantom system with POM cuvette at different dilutions. a: PEI particles and b: starch particles. Time steps: injection of particles at 80 s, dilution steps at 310, 420 and 530 s by a factor of 4.6, 8.2, and 12 from an initial concentration of 50 µg particles/mL.

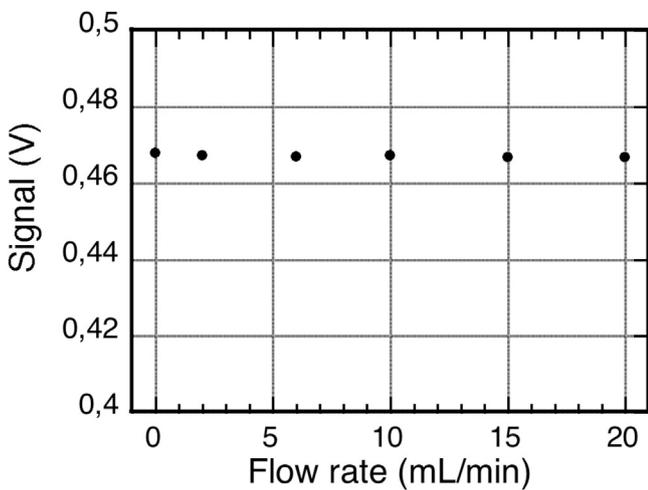


Fig. 3. Non-offset corrected magnetic signal (mean values integrated over 1 min) of particle suspension in the perfusion system with glass cuvette at different flow rates. There is no detectable drift.

well by dilution of the fluid. Changes of the reservoir volumes were measured (see above) and the signal of the maternal circuit was corrected accordingly. Due to the direct connection of the

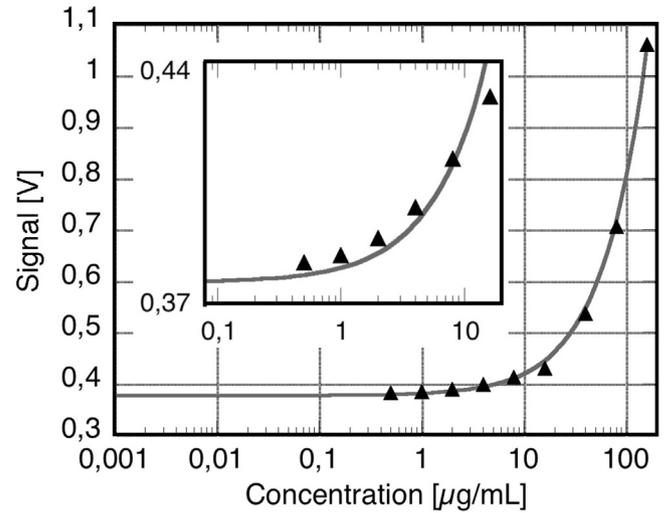


Fig. 4. Magnetic signal of particle suspensions in the perfusion system with glass cuvette at different dilutions of starch coated particles. Inset: Enlargement of the low-concentration range.

tubes via thin cannulae to the tight fetal vessels, the internal pressure of the fetal circuit is much higher compared to that of the maternal circuit. In the maternal circuit, the artery tube is just introduced into the intervillous space by careful penetration of the decidua whereas the venous tube is not connected to the tissue at all, but collects only perfusion solution running out of the maternal side of the cotyledon by surface leaks or formerly vessels. Hence there should be no transfer of fluid from the maternal to the fetal side even if there was a leakage. The data of the fetal circuit are not affected. Nevertheless, the pressure of the perfusion fluid was measured in the fetal circuit between pump and placenta using a disposable transducer for the continuous measurement of physiological pressure (B. Braun Melsungen AG, Germany) in order to detect the occurrence of a leak.

3.3. Investigations of a placenta system

The results of two independent double-sided perfusion experiments are revealed in Figs. 5 and 6. Both figures show values that are offset corrected and corrected with respect to the transfer of perfusion of fluid from the fetal circuit to the maternal circuit. The “leak rate” of the maternal circuit in the first and second

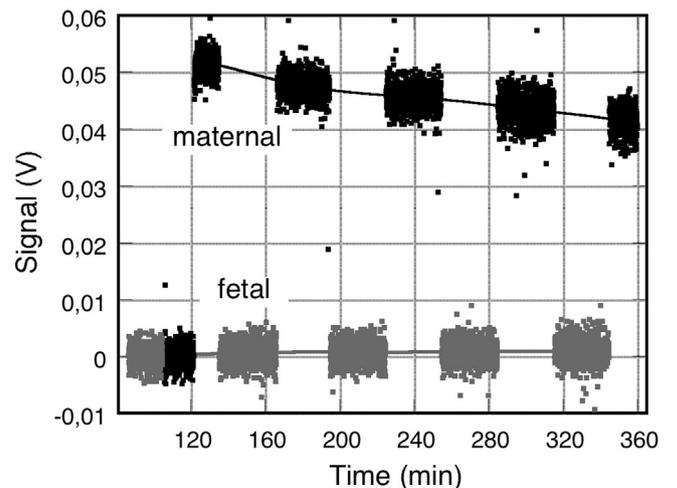


Fig. 5. Longterm measurements of the magnetic signal of particle suspensions in the placenta perfusion system with glass cuvette. The data are offset corrected and fluid volume corrected and contain as well the polynomial fit.

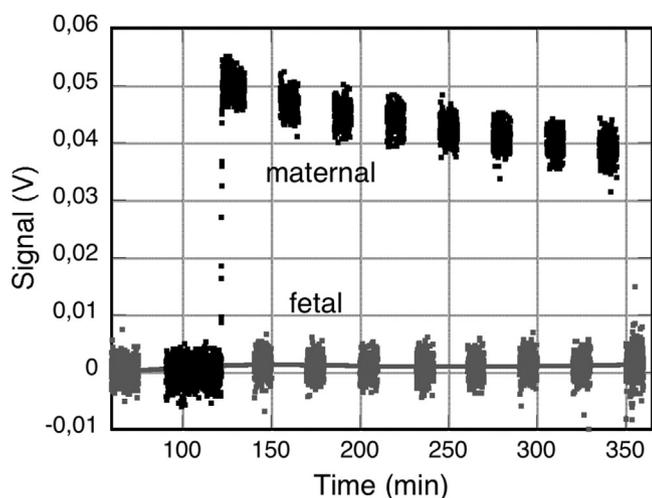


Fig. 6. Independent longterm measurements of the magnetic signal of particle suspensions in the placenta perfusion system with glass cuvette. The data are offset corrected and fluid volume corrected and contain as well the polynomial fit.

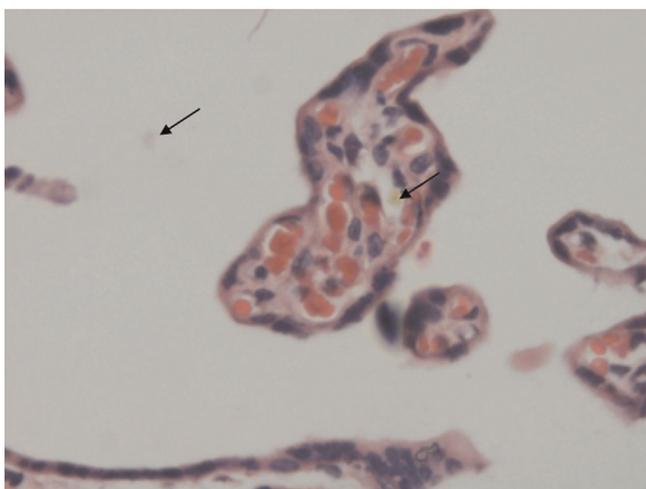


Fig. 7. Histological analysis of placental tissue after *ex vivo* single sided placenta perfusion with FluidMag-D nanoparticles. The tissue was stained with standard hematoxylin/eosin staining, whereas visualization of nanoparticles (indicated by arrows) was performed by Prussian blue reaction [17].

experiment is 14 mL/h and 1 mL/h, respectively. However, the signal in the maternal and in the fetal circuit cannot be compared directly because of the 14% difference in size of the cuvettes (hand-made). The maternal data are normalized to the values at the starting time (addition of particles).

In both experiments a strong decrease of the particle concentration in the maternal circuit of about 20% (Fig. 5) and 25% (Fig. 6) was observed after 4 h perfusion. In the fetal circuit a very small increase of the signal of about 1.5–1.8% of the initial maternal signal was detected after the perfusion, i.e. the signal is definitely bigger than the initial one that is zero. Since the signal is close to the limit of the linear measuring range the concentration of nanoparticles can only be estimated.

In order to prove the reliability of the magnetic measurements in the fetal circuit a measurement of iron concentration in the fetal perfusion liquid after a 4 h-experiment was carried out by ICP-MS. The measured Fe-concentration was 526 $\mu\text{g/L}$ that corresponds with 752 $\mu\text{g Fe}_2\text{O}_3/\text{L}$. The reader should keep in mind that the cotyledon in question was rinsed and should not contain blood anymore.

Histological investigations (Fig. 7) after a single sided perfusion showed particles in the maternal (intervillous space) as well as in the fetal tissue, and in the villous stroma, fetal blood vessels and capillaries of the placenta. This suggests a particle transfer at the placental barrier. Over the time of perfusion usual quality parameters were observed in the perfusion liquid half-hourly. These have been the pH-value, the glucose/lactate ratio representing the energy consumption of the tissue and the protein biosynthesis by means of β -hCG production. Apart from those parameters the formation of certain cytokines was analyzed to estimate the level of tissue damage caused by the presence of the NPs (in addition to the histological analysis). Creatinin was considered as a diffusion marker with a surface- and flow-dependent diffusion profile as expected from the NPs. The discussion of the described as well as further *ex vivo* placenta perfusion experiments with diverse NPs will follow in a further publication.

Comparing our magnetic measurements with other methods ICP-MS is more sensitive but needed relatively high volumes of perfusion solution. The samples had to be taken at certain time intervals, that might cause a big amount of fluid loss during the experiment. This “fluid problem” exists as well at the magnetic quantification methods magneto-relaxometry and magnetic particle spectrometry, two methods that are very sensitive but more expensive and sophisticated in the handling. Based on this, the in-line measurements of the MagnetReader are offering big advantages. This user-friendly technology does not need extra sampling of the fluid or the tissue itself, thus it reduces the need of further analysis for the precious samples. These benefits cause a broad field of possible biological/medical applications, like further studies on the behavior of magnetic particles. In addition the MagnetReader could be applied to in-vitro experiments, e.g. in cell culture, as well as in *ex-vivo* experiments based on other model organs or comparable 3D models. Nevertheless, the system could be improved by using standardized cuvettes.

4. Summary

In summary, our modified measuring setup for magnetic floating samples allows the determination of linear produced standard sample with particle concentrations of about 3–160 μg nanoparticles/mL. With regard to application, we could not only measure magnetic moments in the higher concentrated maternal fluids during the *ex vivo* placenta perfusion, but also in the low concentrated fetal fluids. Furthermore, our data also suggests a passage of NPs from the maternal to the fetal circuit over the placental barrier. This could also be underlined with additional histological findings of NPs in the intervillous space as well as the villous stroma and the lumen of fetal vessels. This retention of NPs in the placental tissue also explains the big difference between the big loss of signal on the maternal side and the slight increase in the fetal signal. In this paper we wanted to focus on the characteristics of the detection system that might offer a variety of applications in biology and medicine in future.

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