Localization and Quantification of Magnetic Nanoparticles by Multichannel Magnetorelaxometry for *in vivo* Hyperthermia Studies in Carcinoma Models

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Abstract— **In magnetic fluid hyperthermia / thermal ablation (MFH), superparamagnetic iron oxide nanoparticles (MNP) exposed to an externally applied alternating magnetic field generate heat specifically in the tumor region, which inactivates cancer cells with minimal side-effects to the normal tissue. The quantity of MNP needs to be thoroughly controlled to govern adequate heat production in the carcinoma region. We demonstrate the capability of magnetorelaxometry (MRX) for the non-invasive quantification and localization of MNP accumulation in small animal models. The results of our advanced MRX measurements using a 304-multichannel biomagnetic SQUID system on two mice hosting two different carcinoma models (9L/lacZ, MD-AMB-435) are presented. The position and magnitude of the magnetic moment is reconstructed from the measured spatial magnetic field distribution by a magnetic dipole model fit applying a Levenberg-Marquadt algorithm. Therewith, the center of mass of the MNP accumulation in the tumor region of the mouse and moreover the amount of MNP in the determined region is quantified. This study shows that magnetorelaxometry is well suited for *in vivo* monitoring of MNP accumulation in hyperthermia application during cancer therapy.**

Keywords— **magnetic nanoparticles, magnetorelaxometry, quantification, localization, cancer therapy**

I. Introduction

Localized magnetic heating treatments (hyperthermia e.g. [11], thermal ablation e.g. [3]) using superparamagnetic iron oxide nanoparticles (MNP) continue to be an active area of cancer research and therapy, where cancer cells are inactivated by high temperatures with minimal side effects in healthy tissues. In magnetic fluid hyperthermia / thermal ablation (MFH) the heat-induction is initiated by an externally applied alternating magnetic field toggling the magnetization direction of the magnetic nanoparticles, which leads to a conversion of magnetic energy into thermal energy [8]. Magnetic nanoparticles are known to be more efficient to create adequate heating using low magnetic field strength than ferromagnetic or ferrimagnetic single or multidomain particles [8].

Current MFH phase II studies, post-mortem in brain tumors of patients, already found a minor uptake of intratumoral injected MNP in the tumors in contrast to a major macrophages uptake [12]. This finding emphasizes the need for *in vivo* localization and moreover quantification of MNP for magnetic heating treatments. One of the critical parameters is the accumulation of adequate amounts of MNP in cancer cells or in the tumor region that generate lethal doses of 55 to 60 °C [5]. *In vitro,* magnetic heating treatment studies [7] already indicate the advantage of an effective MNP labelling over 24 hours before magnetic heating. But little is known about the efficiency of intratumoral MNP accumulation *in vivo*. Here we suggest MRX as a proper tool for determining the intratumoral concentration of MNP *in vivo*. With this information, it should be possible to estimate if the accumulated MNP concentration is sufficient to generate heat of 55 to 60 °C that is necessary for tumor destruction.

In our pilot study, we examined two mice hosting different carcinoma models (9L/lacZ and MD-AMB-435), which were treated *in vivo* with an intratumorally injection of MNP suspension (fluidMAG-D, chemicell GmbH). The two different carcinoma models were chosen to study the influence of different grades of vascularization (9L/lacZ possesses low and MD-AMB-435 high vascularization). After 24 h of incubation time, i.e. shortly before usually thermal ablation will be applied, the mice were sacrificed and prepared for distinct measurements. Subsequently, multichannel magnetorelaxometry was used to examine the MNP accumulation in the two mice. Our study showed, that multichannel MRX is well suited to obtain knowledge about the accumulation`s center of mass and amount of MNP in the tumor, which presents essential information for a successful magnetic heating therapy.

II. Materials and Methods

A. Magnetorelaxometry

Accomplishing MRX measurements, the magnetic relaxation of the MNP is measured by a highly sensitive biomagnetic measurement system [4]. We used the PTB superconducting 304-multichannel quantum interference device (SQUID) [9] housed in a Dewar vessel and operating in our magnetically shielded room BMSR-2 [10], which enables

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the detection of magnetic fields down to a few fT. During the measurements, the sample is positioned on a non-magnetic table with a slide-in tray with section paper. A small cylindrical coil with a stabilized power supply of a rectangular ac-current is used as a calibration coil to determine an absolute reference point relating the middle of the tray to the SQUID sensor's origin. Taking a measurement and reconstructing the magnetic source (see below) by employing the known magnetic moment of the coil of 17 nAm², we obtain the spatial zero position in (x, y, z). The z-distance between tray and the first SQUID layer was determined to be around d = 6 cm for the measurements of the two mice. Accounting for 2.9 cm cold-warm distance between Dewar bottom and lowest SQUID layer, considering a height of the mouse of about 2 cm, the mouse surface is actually placed about 1 cm underneath the Dewar. For our measurement, the mouse was fixed in a plastic foil, attached in an abdominal position on the tray. An external static magnetic field generated by a Helmholtz coil (d = 2r ≈ 85 cm) with homogenous magnetic flux density B = 1 mT is applied in z-direction to the sample for about 90 s outside the magnetically shielded room. Due to the superparamagnetic behavior of the particles, the magnetic moments of the MNP in the sample align along the applied magnetic field in z-direction. After switching off the magnetic field, the sample is rapidly transferred underneath the measurement device (this causes a delay of about 7 s) and the decaying magnetic induction B(t) in z-direction is measured for 70 s with a 250 Hz sampling rate. During the measurement the door of the magnetically shielded room is kept open.

Employing several magnetic field sensors at the same time, the localization of magnetic nanoparticle accumulations is feasible by reconstructing the magnetic source from a measured spatial magnetic field distribution. Applying a Levenberg-Marquardt algorithm we approximate the measured time-dependent magnetization of the sample to the field of a point-like magnetic moment $\vec{m}(t)$ at location $\vec{r}'$ of

$$\vec{B}(\vec{r},t) = \frac{\mu_0}{4\pi}\left(\frac{3(\vec{m}(\vec{r}',t)\cdot\vec{R})\vec{R}}{|\vec{R}|^5} - \frac{\vec{m}(\vec{r}',t)}{|\vec{R}|^3} \right) \qquad (1)$$

where $\vec{B}(\vec{r},t)$ is the magnetic induction and $\vec{R} = \vec{r} - \vec{r}'$.

The reconstructed dipole position at $\vec{r} = (x, y, z)$ represents the center of mass of the MNP accumulation. Its coordinates are related to the determined reference point and subsequently matched to the sample position. Note, that strictly speaking this fit is valid for a point-like dipole. However, in our case the dipole fit was assessed to be an adequate approximation.

The accuracy in spatial resolution of our measurements was estimated by performing repeated measurements of the calibration coil; we obtain $\triangle x = \triangle y = 0.5$ mm, were the error merely depends on the reproducibility of the measurement position.

Due to the physics of a SQUID sensor, merely field changes are detected, thus any remanent magnetization simply contributes to the offset. This means, that the measured signal amplitude of the relaxation curve scales with the MNP amount in the sample. Consequently, by measuring a reference sample of the originally administered MNP under the same experimental setup, again applying magnetic dipole fitting, a quantification is practicable. This additionally requires corresponding quality checks of the reference solution as well as the preparation of an appropriately immobilized reference.

B. In vivo carcinoma models

Two female immunodeficient mice that weighed 20.2 g and 21 g were obtained from the Institute of Animal Research of the Clinics of Friedrich Schiller University, Jena. The animals were group housed in a solid-floor cage (Ehret, Berlin) with adequate bedding (Altromin Tierlabor Service, Lage). Room temperature was controlled at 22 °C ± 1. A 14 hours of light, 10 hours of dark cycle was maintained. Animals received a diet of commercially available pellets (ssniff Spezialdiäten GmbH, Soest) and water ad libitum. Experimental tumors were grown after an injection of 200 µl of BD Matrigel™ Basement Membrane Matrix (Becton Dickinson GmbH, Heidelberg) subcutaneously between the shoulder blades containing 10^7 9L/lacZ cells (mouse A; rat gliosarcoma cell line, low vascularization, LGC Standards GmbH / American Type Culture Collection, Wesel) or 10^7 MDA-MB-435S cells (mouse B; human ductal breast carcinoma cell line, high vascularization, Cell Lines Service, Eppelheim), respectively. All experiments were approved by the regional animal care committee.

Experiments were started approximately 6 weeks after tumor implantation. Tumor volumes were calculated to be in the region of 200 mm³, as determined by using the formula V = π/6 x (product of three principal diameters) [6]. Prior to the experiments, the animals received an anesthetic gas (Isofluran, DeltaSelect GmbH, Pfullingen). On the basis of tumor volume, 40 mg of a magnetic fluid sample (fluidMAG-D, β = 200 mg/ml, d_{hydr} = 200 nm, chemicell GmbH, Berlin [1]) per 200 mm³ was injected into the tumor. X-ray images (Mobilett II, Siemens AG, Erlangen) were taken of the native mouse, right after intratumoral MNP injection as well as after 24 hours of incubation with MNP under general anaesthesia to determine the position of the tumor and MNP. After that, mice were sacrificed by CO_2 in order to preserve the condition of the onset of magnetic heating treatments and were fixed in 96 % ethanol.

III. RESULTS

C. Reference samples

A quality check of the reference material (fluidMAG-D) was carried out by investigating the scaling behavior of a dilution series 1:10, 1:100 in aqua dest, where 40 μl (fluid and immobilized) were measured in 150 μl vials by a single channel MRX device (see also [4]). Deviations from scaling in relaxation curves would indicate a possible aggregation or even precipitation of the MNP in the solution and subsequently also in the immobilized sample. We found excellent scaling in the diluted reference samples, see also figure 1 for immobilized samples.

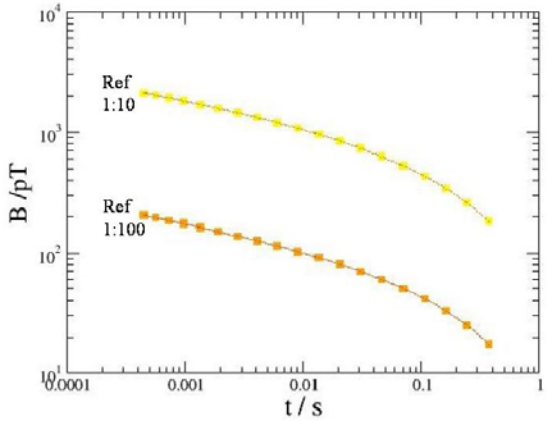

Fig. 1 Relaxation curves of reference samples, 40 μl immobilized.

To assemble a suitable reference sample for the multichannel MRX measurements, accounting for the mostly bound MNP in the tumor tissue, the MNP in the suspension need to be immobilized. The reference was prepared in a 1:10 dilution of fluidMAG-D with aqua dest and thereof 200 μl were immobilized in plaster placed in a 8 ml container. A comparison of the curve shapes of the reference and the mouse sample indicate good agreement.

D. Localization and quantification

In both measured carcinoma mice, mouse A (not depicted here) and mouse B (see Fig. 2 and 3), the localization of the center of mass of the MNP accumulation was explicitly obtained in the tumor region by MRX.

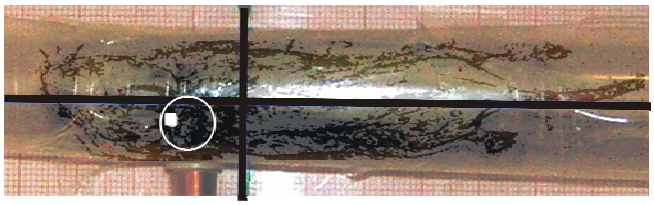

Fig. 2 Mouse B on experimental tray, head to the left, mouse`s contour is schematically indicated with tumor region (white circle). Cross hair indicates of zero position. Localization result for MNP accumulation denoted by a white square.

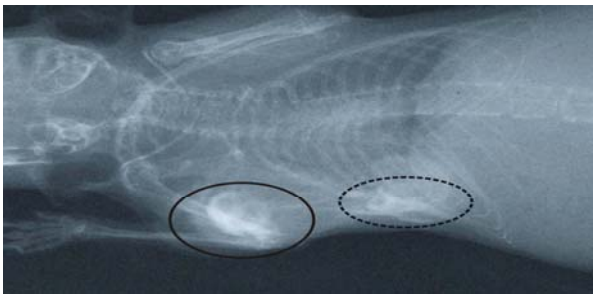

Fig. 3 X-ray image of mouse B, 24h after intratumoral MNP injection. Tumor region (solid line) and MNP loss in surrounding tissue (dashed line) are indicated.

Fig. 2 illustrates the localization result for mouse B, where a photo of the mouse in measurement position on the experimental tray is overlayed with a schematic contour of the mouse (not exact measurement position). The tumor region in measurement position is indicated by a white circle. A cross hair marking the zero-position determined by the calibration coil is given; also a marker coil in the lower left quadrant is depicted. The determined center of mass position is marked as a white square. Note, that in mouse B the tumor was located more towards the left shoulder blade. The localization well approves the center of mass of the MNP accumulation in the tumor region.

The quantification results in the localized regions of the two mice were obtained using the immobilized 1:10 dilution reference sample. We yield 39 mg total iron amount in the tumor region of mouse A, for mouse B, however, we obtain 30 mg total iron amount. The quantification uncertainty is about 7 %, as approximated by repeated measurements.

Additional X-ray images (Mobilett II, Siemens AG, Erlangen) taken of the native mouse, right after intratumoral injection of the MNP and after 24 hours incubation time (the latter see Fig. 3) indicate, that a small fraction of the MNP suspension may also be lost to the surrounding tissue (see dashed oval). Note, that the MRX multichannel measurement setup is presently not allowing for a separation of magnetic sources with a distance like resolved in the

X-ray image. Future work for an improved experimental setup to enable higher spatial resolution is on its way.

IV. Conclusions

Our pilot study demonstrates that MRX is well-suited for the monitoring of nanoparticle accumulation for magnetic heating applications in a carcinomal region. Using multichannel MRX in interplay with magnetic dipole fitting using a Levenberg-Marquardt algorithm, we were able to localize and moreover quantify MNP accumulation in the tumor region of two mice. The results show, that the center of mass accumulation of the MNP was clearly determined in the tumor region of each mouse; even for the off-centered tumor of mouse B the location was located well in the tumor region. The quantification results show that after a 24 hours incubation 75 % of MNP were regained in the highly vascularized tumor (mouse B, MD-AMB-435) and 98 % in the low vascularized tumor (mouse A, 9L/lacZ). The discrepancy is most likely a result of the experimental multiple injection procedure (leftovers in the injection needle). The finding that 75 - 98 % of the originally administered MNP are accumulated in the tumor region is encouraging, because this implies that, for further magnetic heating treatments, 30 to 40 mg of MNP material should be enough for generating lethal doses [6].

In our study the mice had to be sacrificed and preserved due to planned supplementary measurements of our research consortium. But note, that our non-invasive MRX not only enables the fast and contactless location of magnetic nanoparticle accumulations, but in fact the quantification of nanoparticle concentrations on *in vivo* animal models is feasible.

Advantageously, MRX is an integral method where all magnetic moments of the nanoparticles in the sample add to the magnetic field signal, thus a very sensitive detection of MNP on hand [13]. However, the amount of MNP accumulated in the tumor of the mouse was fairly high, so the high sensitivity was not fully exploited. We extrapolate, that a 400 times smaller amount of MNP can still be detected with our current measurement setup.

Continuative work of *in vivo* MRX monitoring on studies in the prior and post magnetic heat treatment phase of carcinoma mice will be performed to gain a statistically relevant basis.

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