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Research paper

Non-invasive *in vivo* evaluation of *in situ* forming PLGA implants by benchtop magnetic resonance imaging (BT-MRI) and EPR spectroscopy

Sabine Kempe [a], Hendrik Metz [a], Priscila G.C. Pereira [b], Karsten Mäder [a,*]

[a] Martin-Luther-University Halle-Wittenberg, Department of Pharmaceutics and Biopharmaceutics, Halle/Saale, Germany
[b] Universidade Estudial de Maringá, Departamento de Farmácia e Farmacologia, Paraná, Brazil

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ABSTRACT

In the present study, we used benchtop magnetic resonance imaging (BT-MRI) for non-invasive and continuous *in vivo* studies of *in situ* forming poly(lactide-co-glycolide) (PLGA) implants without the use of contrast agents. Polyethylene glycol (PEG) 400 was used as an alternative solvent to the clinically used NMP. In addition to BT-MRI, we applied electron paramagnetic resonance (EPR) spectroscopy to characterize implant formation and drug delivery processes *in vitro* and *in vivo*.

We were able to follow key processes of implant formation by EPR and MRI. Because EPR spectra are sensitive to polarity and mobility, we were able to follow the kinetics of the solvent/non-solvent exchange and the PLGA precipitation. Due to the high water affinity of PEG 400, we observed a transient accumulation of water in the implant neighbourhood. Furthermore, we detected the encapsulation by BT-MRI of the implant as a response of the biological system to the polymer, followed by degradation over a period of two months. We could show that MRI in general has the potential to get new insights in the *in vivo* fate of *in situ* forming implants. The study also clearly shows that BT-MRI is a new viable and much less expensive alternative for superconducting MRI machines to monitor drug delivery processes *in vivo* in small mammals.

1. Introduction

Injectable *in situ* forming implants (ISFI) based on biodegradable polymer solution have received considerable attention in the past years. They represent an alternative to common parental formulations such as implants or microspheres [1,2]. Injected as a liquid they subsequently solidify and form a subcutaneous depot. ISFI have several advantages compared to pre-shaped implants, as they are simple to fabricate and avoid invasive techniques such as surgery or the use of needles with large diameters for its implantation. The formation process of an *in situ* implant can be triggered via temperature and pH changes [3,4], ionic or chemical cross-linking [5,6] or solvent exchange [7]. So far only systems based on solvent exchange are commercially available products. Examples include Atridox® [8] which is used for the periodontal delivery of doxycycline and Eligard® [9], a subcutaneous depot for leuprolide. In both systems, poly(lactide-co-glycolide) (PLGA) polymers are dissolved in the water miscible, physiologically compatible organic solvent *N*-methyl-2-pyrrolidone (NMP). After injection, the polymer precipitates due to the solvent (NMP)/non-solvent (body fluids) exchange. As a result, an implant is formed at the injection site. It is obvious that the phase inversion dynamics of PLGA solutions are very critical for the drug release behaviour. A slow conversion of the polymer solution into a solid implant will cause an initial burst release. In order to control the burst effect, several formulation parameters have been examined to influence the rate of polymer precipitation. The concentration and type of PLGA, type of solvent or co-solvent and additives all influenced the *in vitro* drug release [10,11]. McHugh et al. developed a dark-ground video imaging technique to visualise and quantify the phase inversion dynamics of several PLGA solutions [12,13]. However, this technique is limited to *in vitro* experiments and the evaluation of thin films over a small time scale (minutes to few hours). Unfortunately, there is only very limited knowledge about the *in vivo* situation, due to the difficulty to study such complex systems. Most frequently applied analytical methods for the *in vivo* characterization of implants, such as chromatography, infrared spectroscopy and histological staining microscopy require the surgical extraction of the implants and special sample preparation that may lead to artefacts [14]. In addition, these procedures exclude continuous studies with the same sample on a single animal. Conventional *in vivo* experiments require a large number of animals, which does not comply with the international efforts to reduce the number of animal experiments. Because of their non-

* Corresponding author. Martin-Luther-University Halle-Wittenberg, Institute of Pharmacy, Department of Pharmaceutics and Biopharmaceutics, Wolfgang-Langenbeck-Str. 4, 06120 Halle/Saale, Germany. Tel.: +49 345 5525167; fax: +49 345 5527029.
E-mail address: karsten.maeder@pharmazie.uni-halle.de (K. Mäder).

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destructive nature, magnetic resonance techniques (e.g. magnetic resonance imaging (MRI) and electron paramagnetic resonance (EPR) spectroscopy) are well suited for serial and real time *in vivo* measurements [15,16]. By means of EPR spectroscopy, we were able to detect the replacement for the organic solvent NMP by water inside PLGA-implants online both *in vitro* and in living mice [17]. Furthermore, the precipitation of a model drug substance within the polymer was monitored. Both the kinetics of solvent/non-solvent exchange and polymer precipitation showed good *in vitro–in vivo* correlation. However, EPR spectroscopy gave no direct information about the response of the biological system to the implant (e.g. edema, inflammation or encapsulation). In addition, we got no spatial information about the shape and size of the formulation after injection and its disappearance from the injection site. To circumvent these limitations, we applied magnetic resonance imaging (MRI) as a complementary non-invasive method to EPR spectroscopy.

In general, ^{1}H-MRI is a non-invasive imaging method that detects the local concentration and physical state of protons. It has been used to monitor hydration, swelling and erosion of non-coated and coated tablets or implants [16,18,19]. Recently, it has been investigated to follow carbon dioxide formation in floating tablets [20] and the fate of several parental formulations such as oils, lipid emulsion and water solutions of block co-polymers after subcutaneous injection [21].

Although MRI has proven to be useful in a number of biomedical applications and diagnostic tool in clinics, there are only a few reports dedicated to drug delivery research [16,22]. The main reasons for its rare application are the high installation and running costs of common superconducting MRI machines. An alternative option is the use of commercial, low-cost benchtop NMR systems with permanent magnets [23,24]. The successful implementation of BT-MRI for the *in vitro* characterization of tablets [20,25,26] and scaffolds [27] encouraged us to explore the possibility of BT-MRI for pre-clinical *in vivo* studies.

Contrast agents and markers are often used in biomedical and clinical MRI. But in drug delivery research their use has to be critically analysed. The addition of contrast agents may influence the general properties of the formulation, affecting stability, release rates or its biocompatibility. It was, therefore, the aim of the present study to evaluate the use of benchtop MRI for studying ISFI without the use of marker substances and contrast enhancing agents. We investigated the process of polymer precipitation and solvent/non-solvent exchange during implant formation in living mice. In addition, we followed the response of the biological system and implant degradation over a period of 2 months. Furthermore, we conducted an *in vivo*/*in vitro* correlation study of the solvent exchange process by EPR spectroscopy.

2. Materials and methods

2.1. Sample preparation

PLGA (Resomer RG503H, 50:50 M ratio, D,L-lactide/glycolide, Mw 34,000, Boehringer Ingelheim, Germany) was dissolved in *N*-methyl-2-pyrrolidone (Merck, Germany) or PEG 400 (Lutrol 400, BASF, Ludwigshafen, Germany) upon stirring at room temperature. The formed solution had a final concentration of 30% (w/v) PLGA. For EPR measurements, the lipophilic stable nitroxyl radical 4-benzoyloxy-2,2,6,6-tetramethylpiperidine-1-oxyl (Tempolbenzoate, TB, Sigma, Germany) was incorporated in the PLGA-solutions up to a final concentration of 5 mmol per kg PLGA.

For calibration measurements, TB was dissolved in mixtures of NMP/phosphate buffer or PEG 400/phosphate buffer (pH 7.4) at a concentration of 0.25 mM. Aliquots of 1 ml were taken and measured with a 1.1–1.3 GHz EPR spectrometer (MagnetTech, Germany). Recently, we presented an EPR method [17] to determine dissolution induced changes in micropolarity. Briefly, the values of the isotropic hyperfine-splitting $2a_N$ (distance first to third peak) were determined from the spectra and plotted against the concentration of NMP and PEG 400 in different NMP-buffer and PEG 400–buffer mixtures. The obtained calibration curves (Fig. 1) showed that the hyperfine-splitting parameter $2a_N$ corresponded to the NMP and PEG 400 concentrations in the dilution series. The regression coefficients for both calibration curves were $R = 0.99$. The EPR data also show that PEG 400 is slightly more polar compared to NMP.

2.2. In vitro EPR studies

For *in vitro* monitoring of the *in situ* forming implant formation 200 μl of the TB-containing polymer solution was injected through a 25 gauge needle into 50 ml of 0.1 M phosphate buffer (pH 7.4; 37 °C) placed in an incubation shaker (30 rpm). The polymer solutions were injected into perforated plastic capsules placed in the buffer solutions, where the implants formed immediately. At determined time points the implants were taken out the buffer, the capsules were dried with paper, wrapped into plastic foil to prevent drying and subsequently transferred to the EPR spectrometer. The EPR measurements were performed by an L-band spectrometer (MagnetTech, Germany) with a re-entrant resonator, operating at a microwave frequency of about 1.1–1.3 GHz. Measurement parameters were set to modulation amplitude 0.14 mT, scan width 10 mT, scan time 30 s, centre field 49.1 mT, conversion time 0.2 ms and number of accumulations 3. All measurements were taken in triplicate, data are reported as mean ± SEM. The "Cu3 v.6.1" program (MagnetTech, Germany) was used for data recording. The program "Analysis" (MagnetTech, Germany) served for calculation of polarity shifts inside the implants by determining $2a_N$. The proportion of immobilized TB inside the polymers was calculated from EPR spectra fitted with "Nitroxide Spectra Simulation—Freeware Version. 4.99–2005" ("Jozef Stefan" Institute, Department of Solid State Physics, Ljubjana, Slovenia) according to Kempe et al. [17].

2.3. In vivo MRI studies

The animal experiments protocol was approved by Animal Ethics Committee of the state Saxony-Anhalt, Germany. Female BALB/

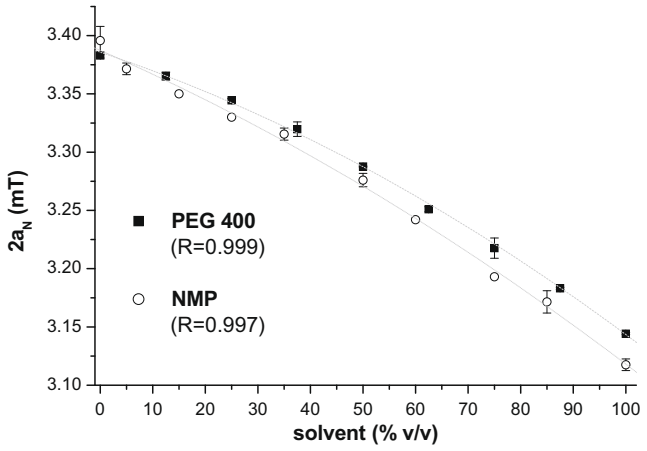

Fig. 1. Dependence of the isotropic hyperfine-splitting $2a_N$ in different NMP-buffer and PEG 400–buffer mixtures. The hyperfine-splitting parameter correspond to the NMP and PEG 400 concentrations and can therefore be used to determine phase inversion-induced changes in micropolarity inside the implants.

c mice (20–25 g) were housed under controlled conditions (12 h light/dark schedule, 24 °C) and received mice chow and tap water ad libitum. About 200 μl of the polymer solution or PEG 400, as control, was injected in to the lower back of the mice. The mice were anesthetized prior to injection and each measurement by inhalation of isoflurane (Forene®, Abbott, Germany) (initial 4 l/min; maintain 2 l/min mixed with oxygen 4 l/min by a Dräger Vapour System, Dräger, Germany). MRI measurements were carried out at regular intervals after every 10 min within the first hour after injection and then after 2 h, 3 h, 6 h and 24 h. The subsequent measurements were performed once a week up to 2 months. Two 50 μl glass capillaries filled with PEG 400 were used as a standard. All measurements were performed in triplicate.

For the MRI measurements, a 20 MHz NMR benchtop system (prototype based on Maran DRX2, Oxford Instruments Molecular Biotools, UK) was used. The system is capable of T1 and T2 relaxation time measurements, determination of diffusion coefficients and 3D imaging. The BT-MRI machine is equipped with 23 mm sample access, suitable for small animals and keeps a constant temperature of 37 °C inside the coil. The mice were placed on glass slides with a fixed inhalation mask for anesthetization. A standard spin-echo sequence was applied with a spin-echo time (TE) of 9.8 ms and a repetition time (TR) of 178 ms. Sixteen averages were used resulting in an acquisition time of about 352 s for each image. The field of view was 40 mm × 40 mm with a resolution of 128 × 128 points. Five slices with a thickness of 3 mm and a separation of 3.5 mm were obtained.

3. Results and discussion

Although NMP is used in commercial products, its use is controversially discussed. NMP is described as a mild irritant to the eyes, the mucous membranes and the skin, and harmful to muscles [28,29]. Beyond that the solvent NMP facilitated the degradation of proteins and polymers based on lactic and glycolic acid [30,31]. Therefore, we used polyethylene glycol (PEG) 400 as well-tolerated non-toxic solvent for PLGA [32] and compared it to NMP. PEG 400 is considered to be among the safest organic solvents and has been used for injectable formulations of lorazepam (Antivan®) [33]. Low molecular weight PEGs have already been used as a solvent or co-solvent for ISFI [32,34]. The addition of PEG 400 into PLGA/NMP-based formulations suppressed the initial burst release of aspirin, but did not prolong the total release period [34].

3.1. In vitro EPR studies

EPR spectroscopy provides unique information in several fields of pharmacy [15]. The measurements of micropolarity and microviscosity can be used to characterize the direct environment to which potential drugs are exposed prior to their release from biodegradable polymers. The hyperfine coupling parameter ($2a_N$) and the rotational correlation time (t_c) are typical parameters that could be obtained from EPR spectra. The rotational correlation time describes the tumbling behaviour of spin markers, such as nitroxyl radicals, and can directly be related to the microviscosity of the surrounding system [35]. In high viscous or solid media, the rotational motion is restricted resulting in higher t_c values compared to low viscous media, where the radicals can tumble free. The hyperfine coupling parameter depends on the polarity of the direct nitroxide environment. Polar environments (e.g. high water content) cause high $2a_N$ values (Fig. 1). Therefore, EPR is capable to detect polarity shifts due to solvent/non-solvent exchange inside the implant.

The polymer solutions were injected into buffer solutions and formed immediately an implant. After injection the solvents diffused into the surrounding aqueous environment, while water diffused into the PLGA matrix. NMP posses a good water solubility and is described as typical fast inverting system [10]. Within 1 h, water replaced about 49.5 ± 0.5% of the NMP inside the implant (Fig. 2). On the other hand, PEG 400 showed a faster solvent/non-solvent exchange. After 1 h, the PEG 400 content inside the implants decreased to 28.3 ± 0.24%. After 6 h, the PEG 400 was completely replaced by water. This fast and complete exchange was not observed for PLGA/NMP implants, where 42.4 ± 0.3% NMP was still present in the implant after 6 h, although the initial exchange was also very fast (Fig. 2). The faster PEG exchange was not unexpected, because NMP is a better solvent for PLGA than PEG 400. At high polymer concentrations, as in our study, the viscosity is lower in good solvents than in poor solvents [36]. The spin probe TB tumbles more slowly in PLGA/PEG 400 solutions (TB rotation correlation time t_c = 1.222 ± 0.001 ns) compared to PLGA/NMP solutions (t_c = 0.095 ± 0.015 ns), which reflects a higher viscosity. In addition, PEG 400 as polar protic solvent has a strong hydrogen bond capacity and therefore a better water affinity than the polar aprotic solvent NMP [30].

Two major diffusional processes play a major role in the formation of *in situ* forming implants, the diffusion of the solvent out of the polymer/solvent system and the diffusion of the non-solvent (buffer, biological fluid) into the PLGA/solvent system. The solvent outflow results in an increase in the polymer concentration. At a certain concentration, phase separation occurs and the polymer precipitates. A faster polymer precipitation for the PLGA/PEG 400 systems compared to NMP was observed. This phenomenon can also be explained by the high water affinity and lower PLGA solubilising capacity of PEG 400. After 2 h, 99.0 ± 0.01% of the TB was immobilized in the precipitated PLGA/PEG 400 implants (Fig. 3 top). Whereas only 95.6 ± 1.0% of the TB precipitated inside the PLGA matrix within 24 h from PLGA/NMP solutions. The residual 1–4% corresponds to a compartment with a viscosity comparable to aqueous solutions of TB, reflecting water entrapped in the interconnecting pores of the PLGA network. After two weeks, we observed a steady increase in this mobile compartment (Fig. 3 bottom), indicating further water penetration inside the implant accompanied by TB solubilisation and starting polymer degradation. Similar results we obtained from pre-shaped PLGA implants, where after a lag time the contribution of the mobile part to the EPR spectrum increased steadily with time [18]. No EPR signals were detectable after 2 months, which indicate a complete TB release within this time period.

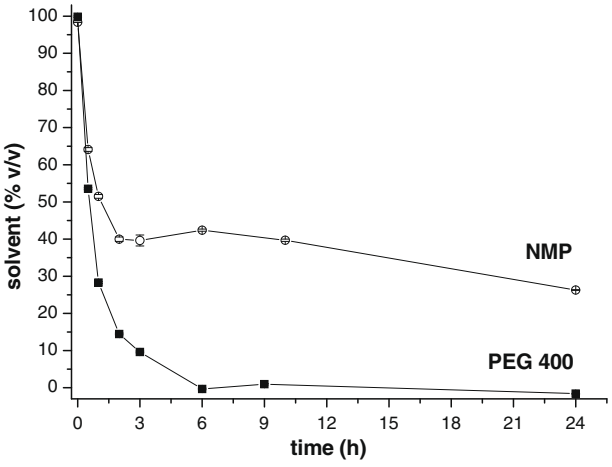

Fig. 2. *In vitro* kinetics of the solvent/non-solvent exchange inside the implants (obtained by EPR spectroscopy).

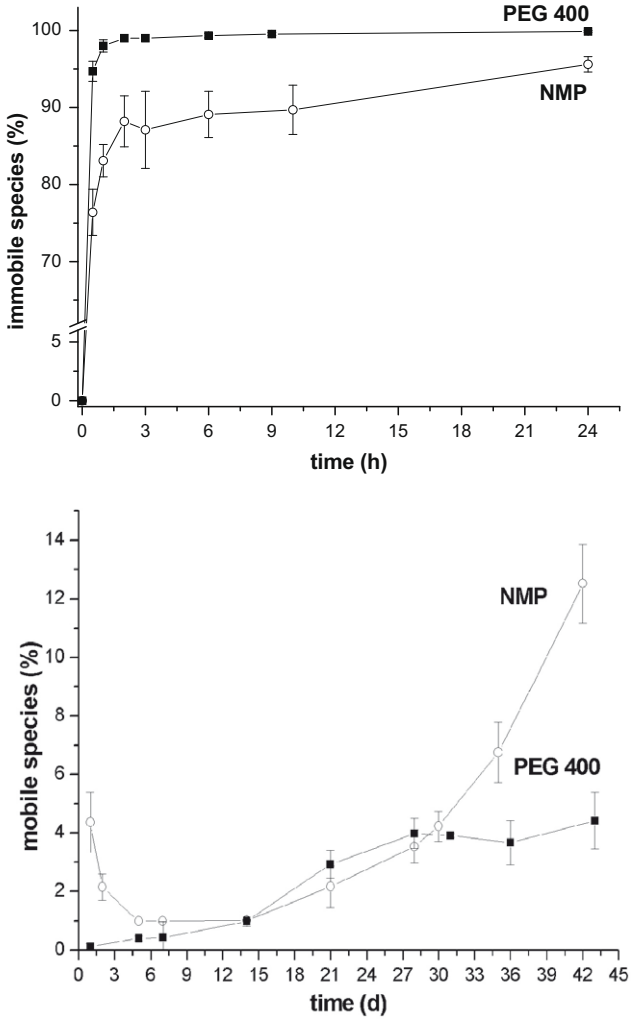

Fig. 3. Top: *in vitro* distribution of precipitated TB in the implant during implant formation within in the first 24 h, as a marker for the PLGA precipitation. Bottom: *in vitro* distribution of mobile TB inside the implants. The slight increase in the mobile compartment inside the implants after 2 weeks indicates water diffusion inside the implant.

3.2. In vivo MRI studies

The MRI signal intensities depend on the total amount of protons in the sample, their relaxation times and the MRI parameters. All MR images were acquired in a T1 weighted mode. NMP and water posses nearly equal T1 times and give, therefore, similar contrast in MRI (Table 1). In contrast to NMP, PEG 400 possesses a much lower T1 relaxation time (Table 1) and is, therefore, quite distinguishable from water till concentrations of 25% (Fig. 4). Under the present conditions, the imaging sequence is finished before the magnetization of the free water proton is able to return to equilibrium and therefore water appears dark. PEG 400 with its lower relaxation time appears brighter in the images than water. Thus, the MRI experiments were only performed with PEG 400 as solvent. Also in mice we can easily distinguish the PLG/PEG 400 implant solution from the surrounding tissue, since the contrast and signal intensities were very good (Fig. 5). The polymer solution with nearly the same relaxation times as subcutaneous fat appears bright. It can easily be detected under the skin and distinguished from subjacent muscle tissue, which appears darker in the MRI image. In addition to the location of the implant, anatomical details can be visualised. One can differentiate skin from back and leg muscles, subcutaneous fat and the urine filled bladder (Fig. 5). All formulations were injected in the lower back of the mice in position of the bladder to easily relocate the implants during the repeated measurements. In initial trials, we injected pure PEG 400 to get more information about the solvent distribution and disappearance from the injection site. MR images were taken repeatedly every 10 min within the first hour after injection and then after 2, 3, 6 and 24 h (Fig. 6). The PEG 400 spread rapidly after injection under skin and dissipated over the whole lower back. PEG 400 attracts water, which caused a subcutaneous edema. The edema was already detectable after 30 min and had a maximum size after 1 h. Between 1 and 3 h it could easily be localized macroscopically

Table 1
T1 relaxation times in different tissues and samples measured at 20 MHz.

	T1 (s)
Buffer	2.95 ± 0.026
NMP	2.19 ± 0.034
PEG 400 (50%)	0.60 ± 0.12
Muscle	0.57 ± 0.92
Fat	0.15 ± 0.70
PEG 400	0.13 ± 0.026

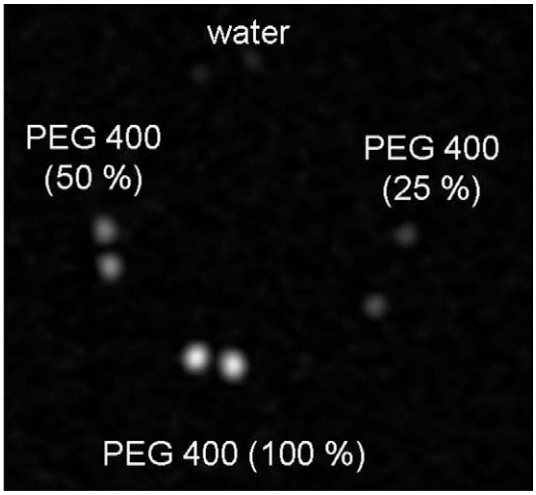

Fig. 4. *In vitro* MRI image of capillaries filled with different PEG 400–water mixtures showing the dependency of the MRI signal intensity on the PEG 400 content.

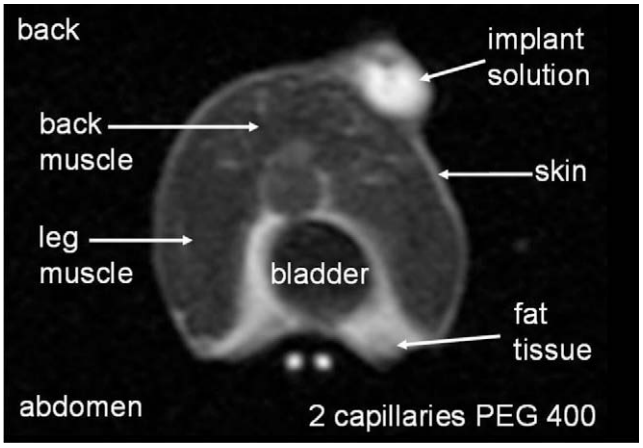

Fig. 5. *In vivo* MRI image 5 min after s.c. injection of PLGA/PEG 400 implant solution, showing the location of the implant and anatomical details of the mice. Two capillaries filled with PEG 400 placed under the abdomen of the mice served as internal standard.

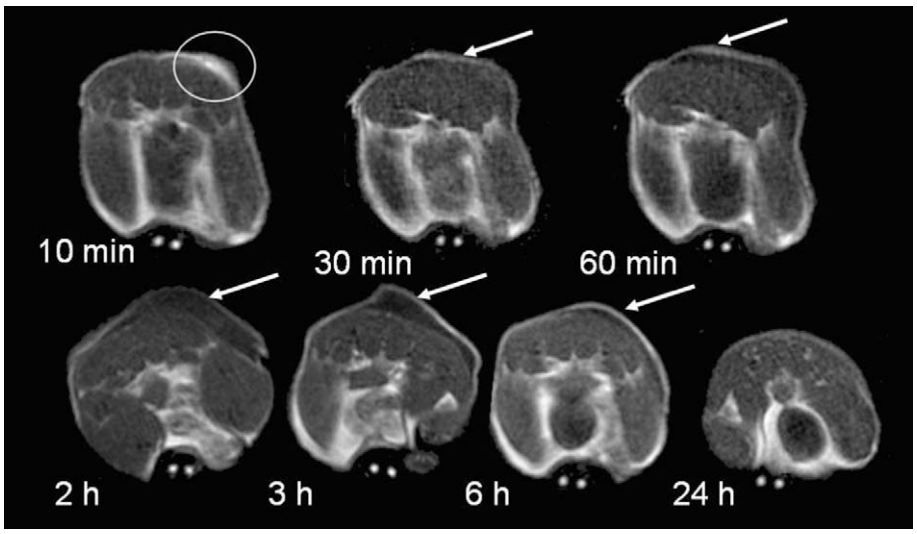

Fig. 6. *In vivo* MRI images of the distribution and disappearance of PEG 400 after s.c. injection. White circle mark the point of injection, white arrows indicate subcutaneous edema. Two capillaries filled with PEG 400 placed under the abdomen of the mice served as internal standard.

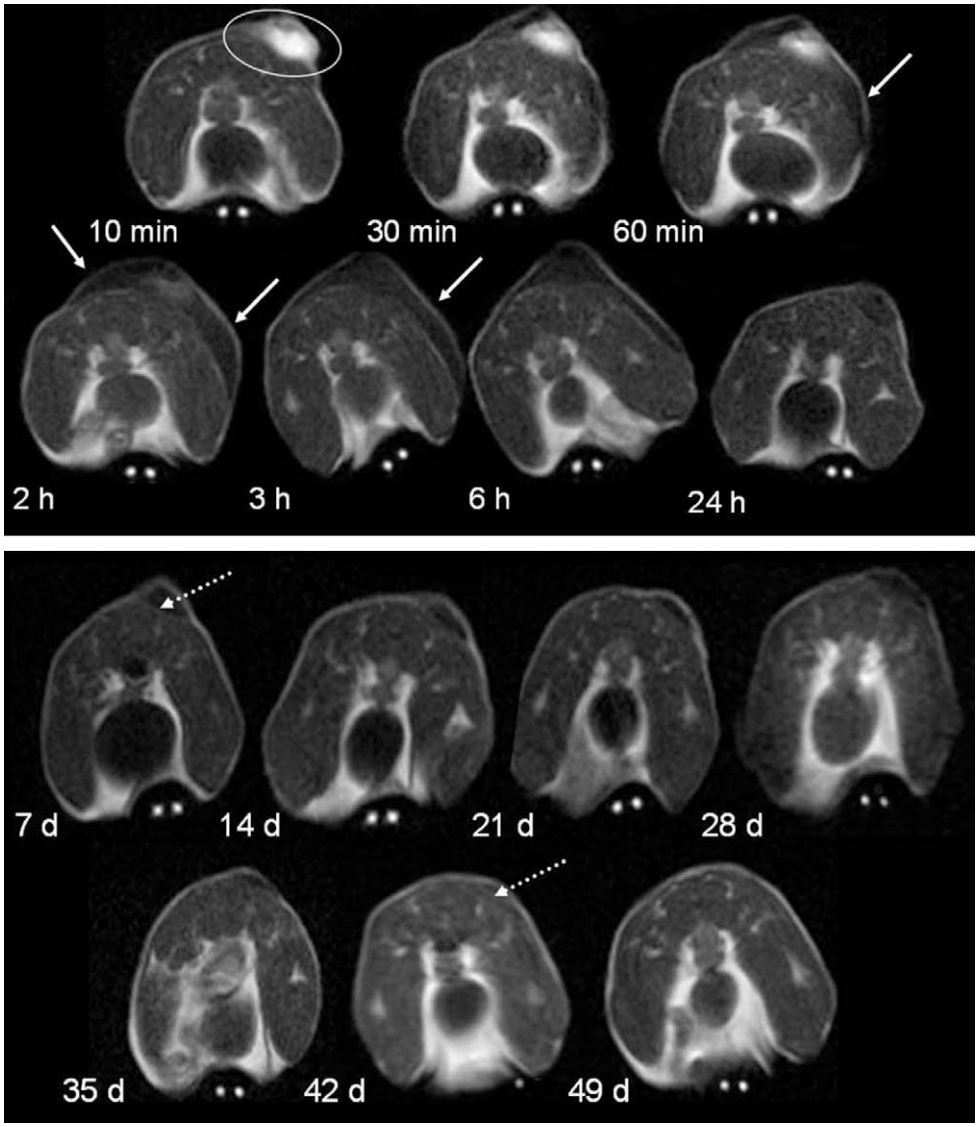

Fig. 7. *In vivo* MRI images of the distribution and disappearance of PLGA/PEG 400 implant solution after s.c. injection. White circle mark the point of injection, solid white arrows indicate subcutaneous edema and dashed white arrows point to connective tissue adhesion on implant surface. Two capillaries filled with PEG 400 placed under the abdomen of the mice served as internal standard.

and was palpable under the skin. The water/PEG 400 mixture was eliminated within few hours. After 6 h, the edema consisting only of water was solely detectable by MRI. After 24 h, it disappeared completely. The edema formation can be attributed to local conditions and the physical properties of PEGs. The subcutaneous space offers a reduced volume flow away from injection site compared to i.v. or i.m. injection [33]. PEGs are known to be osmotically active. This effect is widely used in medicine to stimulate the gastrointestinal mobility [37]. All the mice gained weight steadily during the study. Macroscopic evaluations at the injection sites showed no untoward local reaction. We do not suppose edema formation for NMP, as NMP poses skin permeation enhancement properties [38] and rapidly diffuses from injection site [17]. In a recent study, we could not observe any edema formation for the PLGA/NMP solutions [17]. Studies on rats and dogs showed also no vasodilatation, erythema or edema [39].

In contrast to PEG 400, the polymer solution did not disperse rapidly after injection. The polymer concentrated locally at the point of injection (Fig. 7). The precipitated PLGA appeared dark in the image due to the shorter relaxation times of the solid polymer [18]. A part of the solvent is entrapped in the core of the forming implant. The bright intensity of the implants' core faded with time. Water penetrated inside the implant and replaced the PEG 400. The PEG 400 dispersed in the surrounding subcutaneous space and caused the same edema as observed for the pure solvent. The largest extent was also detectable after 1 and 2 h by MRI and is visible and palpable externally. After 3 h, the edema diminished and was no more detectable after 24 h. Like in the *in vitro* EPR measurements PEG 400 was eliminated after 6 h. Within the first week, the size and shape of the PLGA implant did not change remarkably. Similar results were obtained from subcutaneous implanted PLGA tablets [18]. But we observed the development of a bright ring around the implant, with a good contrast to the subjacent darker muscle tissue. The bright ring can be attributed to the encapsulation of the implant which was verified by visual examination after sacrificing at the end of the experiment. The formation of connective tissue capsules as biological response to polymers based on lactic acid is a common observed phenomenon. Fibrous capsule formation or the association of connective tissue bands have already been reported as well as with common pre-shaped implants of PLA and PLGA [14,18], microparticles of PLGA–PEO–PLGA [40] and ISFI of PLGA/NMP [39] and poly(lactide-co-caprolactone)/NMP [41]. The onset of the decrease in the implant size after 2 weeks corresponds to the increase in the mobile compartments detected by EPR. The degradation occurred faster *in vitro* than *in vivo*. The implant was no more detectable after 7 weeks by MRI and after 8 weeks by visual histological examination, respectively. The faster *in vivo* degradation may have several reasons. On the one hand, the development of a more acidic microenvironment *in vivo,* due to the autocatalytic degradation mechanism of PLGA and different buffer capacities *in vitro* and *in vivo,* has been reported to cause faster degradation of PLGA scaffolds *in vivo* [14]. On the other hand, additional factors like enzymatic degradation *in vivo* or different implant shapes and therefore different surface areas might also contribute.

4. Conclusions

This is the first MRI study which monitors the formation and disappearance of *in situ* forming implants. It was conducted with a newly developed *in vivo* benchtop MRI Imager without the use of contrast agents in a non-invasive and continuous manner. PEG 400 provided an excellent MRI signal and contrast to the biological tissue. Hence, we were able to follow the process of solvent/non-solvent exchange and polymer precipitation. The results were in good agreement with previous EPR measurements. Due to the high water affinity of PEG 400, we observed the formation of subcutaneous edema. Even though it is regarded as a well-tolerable and biocompatible solvent, the local injection of high amounts has to be critically revised. Recently, Schoenhammer et al. reported that low molecular PEGs accelerate the degradation of PLGA in solution as a result of transesterification. So the stability of PLGA in PLGA/PEG 400 solutions may be another obstacle in its use as alternative solvent. Beside the formation of the transient edema, we observed encapsulation of the implant as a response of the biological system to the polymer, followed by degradation over a period of two months. We could show that MRI has the potential to get new insights in the *in vivo* fate of *in situ* forming implants as well as parenteral formulations. The obtained results with the prototype of the Benchtop apparatus are a proof that BT-MRI is a new alternative for superconducting MRI machines for *in vivo* MRI on mammals. We anticipate that due to its low installation and running costs, BT-MRI will be used more frequently in pre-clinical research.

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References

[1] A. Hatefi, B. Amsden, Biodegradable injectable in situ forming drug delivery systems, Journal of Controlled Release 80 (2002) 9–28.
[2] C.B. Packhaeuser, J. Schnieders, C.G. Oster, T. Kissel, In situ forming parenteral drug delivery systems: an overview, European Journal of Pharmaceutics and Biopharmaceutics 58 (2004) 445–455.
[3] E. Ruel-Gariepy, J.C. Leroux, In situ-forming hydrogels – review of temperature-sensitive systems, European Journal of Pharmaceutics and Biopharmaceutics 58 (2004) 409–426.
[4] M.D. Determan, J.P. Cox, S.K. Mallapragada, Drug release from pH-responsive thermogelling pentablock copolymers, Journal of Biomedical Materials Research Part A 81A (2007) 326–333.
[5] A.K. Poshusta, J.A. Burdick, D.J. Mortisen, R.F. Padera, D. Ruehlman, M.J. Yaszemski, K.S. Anseth, Histocompatibility of photocrosslinked polyanhydrides: a novel in situ forming orthopaedic biomaterials, Journal of Biomedical Materials Research Part A 64A (2003) 62–69.
[6] J. Berger, M. Reist, J.M. Mayer, O. Felt, N.A. Peppas, R. Gurny, Structure and interactions in covalently and ionically crosslinked chitosan hydrogels for biomedical applications, European Journal of Pharmaceutics and Biopharmaceutics 57 (2004) 19–34.
[7] W.J. Lambert, K.D. Peck, Development of an in situ forming biodegradable poly-lactide-co-glycolide system for the controlled release of proteins, Journal of Controlled Release 33 (1995) 189–195.
[8] D. Steinberg, M. Friedman, Dental drug delivery devices: local and sustained release applications, Critical Reviews in Therapeutic Drug Carrier Systems 16 (1999) 425–459.
[9] O. Sartor, Eligard: leuprolide acetate in a novel sustained-release delivery system, Urology 61 (2003) 25–31.
[10] A. McHugh, The role of polymer membrane formation in sustained release drug delivery systems, Journal of Controlled Release 109 (2005) 211–221.
[11] X. Luan, R. Bodmeier, Influence of the poly(lactide-co-glycolide) type on the leuprolide release from in situ forming microparticle systems, Journal of Controlled Release 110 (2006) 266–272.
[12] P.D. Graham, K.J. Brodbeck, A.J. Mchugh, Phase inversion dynamics of PLGA solutions related to drug delivery, Journal of Controlled Release 58 (1999) 233–245.
[13] K.J. Brodbeck, J.R. DesNoyer, A.J. Mchugh, Phase inversion dynamics of PLGA solutions related to drug delivery – Part II. The role of solution thermodynamics and bath-side mass transfer, Journal of Controlled Release 62 (1999) 333–344.
[14] E. Pamula, E. Menaszek, In vitro and in vivo degradation of poly(l-lactide-co-glycolide) films and scaffolds, Journal of Materials Science – Materials in Medicine 19 (2008) 2063–2070.
[15] D.J. Lurie, K. Mäder, Monitoring drug delivery processes by EPR and related techniques – principles and applications, Advanced Drug Delivery Reviews 57 (2005) 1171–1190.

[16] J.C. Richardson, R.W. Bowtell, K. Mäder, C.D. Melia, Pharmaceutical applications of magnetic resonance imaging (MRI), Advanced Drug Delivery Reviews 57 (2005) 1191–1209.
[17] S. Kempe, H. Metz, K. Mäder, Do in situ forming PLG/NMP implants behave similar in vitro and in vivo? A non-invasive and quantitative EPR investigation on the mechanisms of the implant formation process, Journal of Controlled Release 130 (2008) 220–225.
[18] K. Mäder, G. Bacic, A. Domb, O. Elmalak, R. Langer, H.M. Swartz, Noninvasive in vivo monitoring of drug release and polymer erosion from biodegradable polymers by EPR spectroscopy and NMR imaging, Journal of Pharmaceutical Sciences 86 (1997) 126–134.
[19] C.A. Fyfe, A.I. Blazek-Welsh, Quantitative NMR imaging study on the mechanism of drug release from swelling hydroxypropylmethylcellulose tablets, Journal of Controlled Release 68 (2000) 313–333.
[20] S. Strübing, T. Abboud, R.V. Contri, H. Metz, K. Mäder, New insights on polyvinylacetate based coated floating tablets: characterization of hydration and CO_2 generation by benchtop MRI and its relation to drug release and floating strength, European Journal of Pharmaceutics and Biopharmaceutics 69 (2009) 708–717.
[21] B. Madhu, I. Elmroth, A. Lundgren, B. Abrahamsson, B. Soussi, A novel evaluation of subcutaneous formulations by in vivo magnetic resonance imaging (MRI), Pharmaceutical Research 45 (2002) 207–212.
[22] H. Metz, K. Mäder, Benchtop-NMR and MRI – A new analytical tool in drug delivery research, International Journal of Pharmaceutics 364 (2008) 170–175.
[23] P. Cornillon, L.C. Salim, Characterization of water mobility and distribution in low- and intermediate-moisture food systems, Magnetic Resonance Imaging 18 (2000) 335–341.
[24] G.E. Schaumann, E. Hobley, J. Hurraß, W. Rotard, H-NMR relaxometry to monitor wetting and swelling kinetics in high-organic matter soils, Plant and Soil 275 (2005) 1–20.
[25] S. Strubing, H. Metz, K. Mader, Characterization of poly(vinyl acetate) based floating matrix tablets, Journal of Controlled Release 126 (2008) 149–155.
[26] V. Malaterre, H. Metz, J. Ogorka, R. Gurny, N. Loggia, K. Mäder, Benchtop-magnetic resonance imaging (BT-MRI) characterization of push–pull osmotic controlled release systems, Journal of Controlled Release 133 (2009) 31–36.
[27] H. Nitzsche, A. Noack, A. Lochmann, C. Oliveira, A. Besheer, H. Metz, A. Bernstein, G. Hause, T. Groth, K. Mader, Scaffold properties and interaction with cells investigated by magnetic resonance methods, Tissue Engineering Part A 14 (2008) 345.
[28] J.P. Moreau, P.J. Vachon, M.C. Huneau, Elevated glycemia and local inflammation after injecting N-methyl-2-pyrrolidone (NMP) into the marginal ear vein of rabbits, Journal of American Association for Laboratory Animal Science 40 (2001) 38–40.
[29] J.P. Payan, D. Beydon, J.P. Fabry, I. Boudry, B. Cossec, E. Ferrari, Toxicokinetics and metabolism of N-[14C]methylpyrrolidone in male sprague-dawley rats. A saturable NMP elimination process, Drug Metabolism and Disposition 30 (2002) 1418–1424.
[30] W.Y. Dong, M. Körber, V. Lopez Esguerra, R. Bodmeier, Stability of poly(DL-lactide-co-glycolide) and leuprolide acetate in-situ forming drug delivery systems, Journal of Controlled Release 115 (2006) 158–167.
[31] K. Schoenhammer, H. Petersen, F. Guethlein, A. Goepferich, Injectable in situ forming depot systems: PEG–DAE as novel solvent for improved PLGA storage stability, International Journal of Pharmaceutics, in press, doi: 10.1016/j.ijpharm.2008.12.019.
[32] M. Dittgen, S. Fricke, H. Gerecke, I.P. Möller, C. Völkel, Injection Implant, Germany Patent WO/1998/030245, 1998.
[33] R.G. Strickley, Solubilizing excipients in oral and injectable formulations, Pharmaceutical Research 21 (2004) 201–230.
[34] Y. Tang, J. Singh, Controlled delivery of aspirin: effect of aspirin on polymer degradation an in vitro release from PLGA based phase sensitive systems, International Journal of Pharmaceutics 357 (2008) 119–125.
[35] S. Ullrich, H. Metz, K. Mäder, Sucrose ester nanodispersions: microviscosity and viscoelastic properties, European Journal of Pharmaceutics and Biopharmaceutics 70 (2008) 550–555.
[36] H. Kranz, R. Bodmeier, Structure formation and characterization of injectable drug loaded biodegradable devices: in situ implants versus in situ microparticles, European Journal of Pharmaceutical Sciences 34 (2008) 164–172.
[37] D.A.I. Ashiru, R. Patel, A.W. Basit, Polyethylene glycol 400 enhances the bioavailability of a BCS class III drug (ranitidine) in male subjects but not females, Pharmaceutical Research 25 (2008) 2327–2333.
[38] P.J. Lee, R. Langer, V.P. Shastri, Role of N-methyl pyrrolidone in the enhancement of aqueous phase transdermal transport, Journal of Pharmaceutical Sciences 94 (2005) 912–917.
[39] H.B. Ravivarapu, K.L. Moyer, R.L. Dunn, Sustained suppression of pituitary–gonadal axis with an injectable in situ, forming implant of leuprolide acetate, Journal of Pharmaceutical Sciences 89 (2000) 732–741.
[40] T. Kissel, Y. Li, F. Unger, ABA-triblock copolymers from biodegradable polyester A-blocks and hydrophilic poly(ethylene oxide) B-blocks as a candidate for in situ forming hydrogel delivery systems for proteins, Advanced Drug Delivery Reviews 54 (2002) 99–134.
[41] M.A. Royals, S.M. Fujita, G.L. Yewey, J. Rodriguez, P.C. Schultheiss, R.L. Dunn, Biocompatibility of a biodegradable in situ forming implant system in rhesus monkeys, Journal of Biomedical Materials Research 45 (1999) 231–239.