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Effect of PEG functionalization on the saturation magnetization of magnetic nanoporous core-shell nanoparticles

T. Herrmann[a,*] · A. K. Schierz[a] · M. Prediger[b] · J. Reifenrath[c] · J. Meißner[d] · M. C. Wurz[e,f] · P. Behrens[a,f,g]

[a] Institute for Inorganic Chemistry, Leibniz University Hannover, Hannover, Germany
[b] Institute of Micro Production Technology, Leibniz University Hannover, Hannover, Germany
[c] Clinic for Orthopedic Surgery, Hannover Medical School, Germany
[d] Institute for Pharmacology, Toxicology and Pharmacy, University of Veterinary Medicine Hannover, Foundation, Germany
[e] DRL Institute for Quantum Technologies, Ulm University, Ulm, Germany
[f] Cluster of Excellence PhoenixD, Leibniz University Hannover, Germany
[g] Cluster of Excellence Hearing4all, Hannover, Germany
[*] Corresponding author, email: timo.herrmann@acb.uni-hannover.de

Abstract

The treatment of implant-associated infections is still a considerable issue in modern orthopaedical surgery. A promising candi-date to improve this situation are superparamagnetic, drug-loaded nanoparticles in combination with a magnetizable implant and an external magnetic field. This set-up can enhance the accumulation of the magnetic nanoparticles at the targeted implant, thus reducing the number of nanoparticles needed for a successful treatment. Important prerequisites for the superparamagnet-ic nanoparticles to be used are a high magnetization and a sufficiently long circulation time within the body. A poly(ethyleneglycol) (PEG) functionalization is widely used to increase the circulation time. Since the PEG functionalization adds mass to the nanoparticles and influences also other properties, we functionalized magnetic nanoporous silica nanoparti-cles (MNPSNPs) with PEG moieties of different chain length and studied the effect of the chain length on the saturation magnet-ization of the particles.

I. Introduction

The number of implant-associated infections, which can occur at different implantation sites, for example at dental or orthopaedic implants, is still rising, as the number of implantations is rising. The conventional treatment of such infections is carried out by the systemic application of antibiotics. To improve this situation, we are developing a targeted treatment of implant-associated infections by a special drug delivery system, where a combination of a magnetizable implant, magnetic nanoporous silica nanoparticles (MNPSNPs) and an external magnetic field is used.[1,2] The super-paramagnetic MNPSNPs are injected into the blood circulation system. An external magnetic field is applied at the infection site, which is enhanced by the magnetizable implant. The MNPSNPs are thus attracted to and accumulate at the implant. The nanoporous shell of the MNPSNPs shall be loaded with an antibiotic drug which is then released at the implant site, so that the infection is treated locally and effectively,

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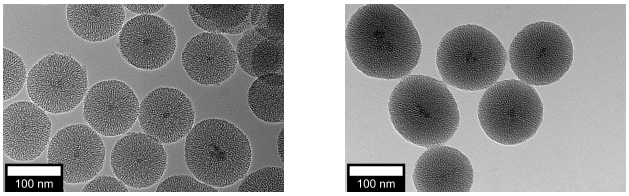

Figure 1: TEM images of unfunctionalized MNPSNPs (left) and 5kmPEG-RITC functionalized MNPSNPs (right).

avoiding side effects of the drug. This implant-directed magnetic drug targeting (IDMDT) thus represents an effective treatment compared to other drug delivery methods.[1,2]

To reduce the immunogenic reaction of the MNPSNPs, the particles shall be PEGylated, resulting in core-shell-shell nanoparticles. A PEG functionalization is known to mask nanoparticles, weakening the interactions between the nanoparticles and phagocytic cells. In this stealth effect, the chain length of the PEG moiety plays an important role. In general, it is observed that a longer PEG results in a better stealth effect.[3,4] Likewise, the magnetization of the MNPSNPs plays a decisive role. To accomplish a strong accumulation of the MNPSNPs onto the implant, a high magnetization is preferable. The magnetization of the MNPSNPs is strongly dependent on the amount of magnetic material within the nanoparticle. The nanoporous shell, needed for drug delivery, and the PEG functionalization further increase the size and the weight of these particles while being non-magnetic. Therefore, in this work we investigate the influence of the PEG chain length on the magnetic properties of the MNPSNPs.

II. Material and methods

All chemicals were used without further purification. Iron(II) chloride tetrahydrate (≥99%), iron(III) chloride tetrahydrate (99%), oleic acid (90%), chloroform (≥99%), cetyltrimethylammonium bromide (CTAB, ≥98%), Ammonium hydroxide solution (≥25% NH_3 in water), tetraethyl orthosilicate (TEOS, ≥99%), ethyl acetate (99.8%), 3-aminopropyl triethoxy silane (APT-ES, 99%), 3-aminopropyl trimethoxysilane (APTMS, 97%), rhodamine B isothiocyanate (RITC, mixed isomers), poly(ethyleneglycol) monomethylether (10kmPEG, Mw: 10 kDa) were purchased from Sigma-Aldrich Corporation (München, Germany). Poly(ethylene glycol) monomethylethers (2kmPEG, 5kmPEG, M_w: 2 or 5 kDa, resp.) were purchased from TCI Europe N.V. (Zwijndrecht, Belgium). The commercially available silane [Hydroxy(polyethyleneoxy)propyl] triethoxysilane (50% in ethanol, M_w: 575-750 g/mol), designated as csPEG, was purchased form Gelest (Morrisville PA, USA). Tosyl chloride (98%) was purchased from abcr GmbH (Karlsruhe, Germany). Tetrahydrofuran (99.8%) was purchased from Acros Organics (Geel, Belgium). Dichloromethane (DCM, ≥99,9%) was purchased from Honeywell GmbH (Seelze, Germany). $MgSO_4$ (99%) was purchased from ThermoFisher GmbH (Kandel, Germany). Ethanol (EtOH, absolute) was purchased from Merck (Darmstadt, Germany).

II.I. Synthesis of magnetic nanoporous nanoparticles (MNPSNPs)

First, the magnetic core was prepared as reported in the literature.[1] Next, the synthesis of the MNPSNP was performed following a published procedure with a slight modification.[1] In brief, the oleic acid-coated nanoparticles were transferred into the aqueous phase using the surfactant CTAB. After the phase transfer, water was added to the dispersion and it was allowed to stir for 30 min at 60 °C. Next, ammonium hydroxide, TEOS and ethyl acetate were added within 1 min and the reaction mixture was stirred for 3 h at 60 °C. The product was obtained after centrifugation, washing with ethanol and drying under vacuum. To remove the surfactant the obtained particles were calcined at 550 °C for 5 h with a heating rate of 5 °C min^{-1}.

II.II. Synthesis of mPEG-silanes with different molecular weight

mPEG-silanes were synthesized starting from the different mPEGs following a published procedure with some modifications.[5] In a typical synthesis, the mPEG was first tosylated using tosyl chloride in a THF/aqueous NaOH mixture at 4 °C. The tosylated product was extracted with 50 mL DCM and dried. The crude product was used in the next step without further purification. It was reacted with a stoichiometric amount of APTES in 20 ml chloroform for 17 h under N_2 and reflux. After solvent removal and drying the product was stored as a 50 wt% solution in ethanol.

II.III. Grafting of mPEG-silanes onto the MNPSNPs

For the grafting of the mPEG/PEG-silanes onto the MNPSNPs, 120 mg of the MNPSNPs were dispersed in 42 ml of EtOH. Next, 0.725 mmol of the appropriate mPEG-silane was added and the suspension was stirred for 24 h at 50 °C. To enable possible bioimaging, the fluorescent dye RITC was bound to the particles in addition. For this purpose, 25 mg RITC were stirred with 5.4 µL APTMS for 24 h in 2 mL EtOH and 502 µL of the obtained solution was also added to the MNP-SNPs.[1] The functionalized particles are obtained after centrifugation, washing with EtOH and drying under vacuum.

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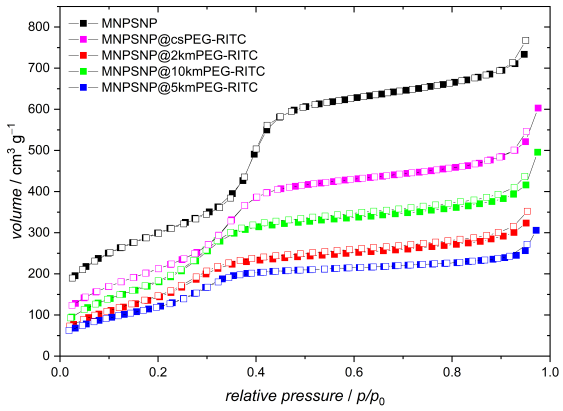

Figure 2: Nitrogen physisorption isotherms of unfunctionalized and functionalized MNPSNPs; filled squares: adsorption, empty squares: desorption.

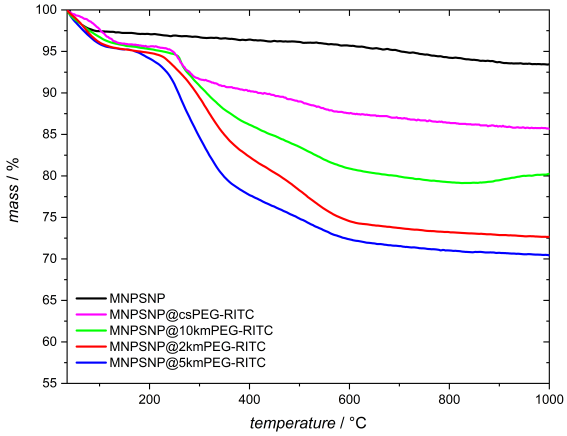

Figure 3: Thermogravimetric curves of unfunctionalized and functional-ized MNPSNPs.

Table 1: Calculated values for BET surface area (BET), pore diameter d and pore volume V of MNPSNPs and functionalized MNP-SNPs.

functionalization	MNP-SNP	csPEG-RITC	2k mPEG-RITC	5k mPEG-RITC	10k mPEG-RITC
BET / $m^2\ g^{-1}$	1080	840	550	550	700
d / nm	4.2	4.0	3.5	3.8	3.8
V / $cm^3\ g^{-1}$	1.1	0.8	0.5	0.4	0.6

III. Results and discussion

The synthesized MNPSNPs show a spherical shape and a uniform size distribution with a diameter of 108 nm ± 10 nm as determined by TEM, shown in Fig. 1. The dark spots visible within the particles represent the Fe_3O_4 core-NPs, embedded within the nanoporous silica shell, proving a successful formation of the MNPSNPs. After functionalization of the MNPSNPs with different PEG chain lengths, the spherical shape and the pore system of the particles were preserved as shown in Fig. 1 on the right-hand side for the 5kmPEG modification.

Unmodified MNPSNPs show a BET surface area of 1080 $m^2\ g^{-1}$, a pore diameter of 4.2 nm and a pore volume of 1.1 $cm^3\ g^{-1}$ as determined by nitrogen physisorption measurements. After functionalization these values decreased depending on the PEG chain length. For the MNPSNP@csPEG with the shortest chain length, a BET surface of 840 $m^2\ g^{-1}$, a pore diameter of 4.0 nm and a pore volume of 0.8 $cm^3\ g^{-1}$ were obtained. The values obtained for the samples functionalized with other PEG chains are listed in Table 1. Noticeably, there is a trend for the BET surface area and pore volume to decrease with increasing PEG chain length with the exception of the sample modified with the longest PEG chain 10kmPEG. This has quite high porosity parameters which might be explainable by the lower amount of the 10kmPEG bound to the surface.

The amount of PEG bound to the surface was investigated by thermogravimetric analysis (TGA) in air (Fig. 3). During TGA, the organic moiety is decomposed and some water is released from the dehydroxylation of silanol groups; the inorganic residue is left over. Thus, the smaller the relative residual mass is, the higher is the organic content of the sample. Here, it is observable that the residual mass of the functionalized MNP-SNPs decreased with increasing PEG chain length. Unfunctionalized MNPSNPs showed a weight loss of 6% caused by the loss of adsorbed water and the dehydroxylation of the silica material. This weight loss is increased, as the PEG chain length increases from the csPEG via the 2kmPEG to the 5kmPEG functionalization to 15%, 27% and 30%, respectively. Therefore, with increasing length of the PEG chain, the total mass of the particles also increases when the same amount of the respective PEG chain is bound to the surface. An exception for this trend is again observed for sample with the 10kmPEG functionalization. Here, a higher residual mass of 20% is obtained, corresponding to a smaller organic content.

This further confirmed the assumption of a lower functionalization density of the longest PEG chain as a lower amount of organic material is present. Since only one end of the polymer chain is modified to react with the silica surface; a lower PEG density with the longest PEG chain maybe caused due to an entropic limitation during the modification of the PEG or during the grafting process.

In Fig. 4 the room-temperature magnetization curves of the functionalized particles are shown. In all magnetization curves no remanence is visible, hence all particles show a superparamagnetic behavior. It can be observed that with increasing PEG chain length, the satura-

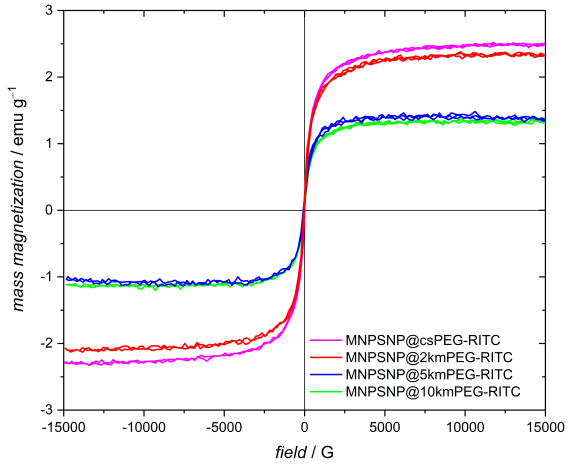

Figure 4: Room-temperature magnetization curves of functionalized MNPSNPs obtained with a vibrating sample magnetometer.

tion magnetization decreases from 2.47 emu g^{-1} for the shortest and lightest PEG chain to 1.33 emu g^{-1} for the longest and heaviest PEG chain (10kmPEG). The values for the 2kmPEG and 5kmPEG functionalization are between these two with a saturation magnetization of 2.33 emu g^{-1} and 1.40 emu g^{-1}, respectively. This trend can be explained by the amount of magnetic material per MNPSNP. While the MNPSNPs gain weight by attaching the PEG, the amount of magnetic material stays constant. This weight gain of a particle is higher for longer PEG chains, thus resulting in a lower saturation magnetization for a functionalization with longer PEG chains. Again, the sample with the 10kmPEG functionalization is an exception; its magnetization is about as high as that of 5kmPEG-modified MNPSNPs.

IV. Conclusions

Different PEG moieties were successfully modified with APTES in a two-step synthesis. Afterwards these PEG-silanes, as well as a commercially available PEG silane with small molecular mass, were grafted onto the surface of MNPSNPs. The amount of PEG on the surface increased, as the chain length increased. An exception was observed with the longest PEG chain. Here the amount decreased, which might be explained by entropic limitations. The functionalized MNPSNP show superparamagnetic behavior with a saturation magnetization of 2.47 emu g^{-1} for the shortest PEG chain used in this work. Here a correlation between the chain length of the attached PEG and the mass magnetization of the MNPSNPs was observed, as the magnetization decreases with an increasing chain length.

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Author's statement

Conflict of interest: Authors state no conflict of interest. Informed consent: Informed consent has been obtained from all individuals included in this study. Ethical approval: The research related to human use complies with all the relevant national regulations, institutional policies and was performed in accordance with the tenets of the Helsinki Declaration, and has been approved by the authors' institutional review board or equivalent committee.

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