International Journal on Magnetic Particle Imaging
Vol 10, No 1, Suppl 1, Article ID 2403032, 4 Pages

Infinite Science Publishing

Proceedings Article

Biocompatibility of sulfobetaine and PEG 25 kDa functionalized synomag®-D

B. Kunkel[a] · V. Khaimov[b] · T. Stahnke[b] · S. Großmann[b] · P. Stoer[a,*]

[a]micromod Partikeltechnologie GmbH, Rostock, Germany
[b]Institute for ImplantTechnology and Biomaterials (IIB) e.V., Rostock, Germany
*Corresponding author, email: stoer@micromod.de

Abstract

The method of magnetic particle imaging relies crucially on the accessibility of adequate tracer materials. Those have to fulfill different technical requirements like high signal strength but also high biocompatibility, in particular hemocompatibility, and high blood circulation time since magnetic nanoparticles are applied intravascular in most applications. In order to further improve these properties, high-performing synomag®-D was optimized within the present study by coupling of sulfobetaine or PEG 25 kDa-OMe. Relative cell viability of these functionalized magnetic nanoparticles was found to be 8 to 18% higher compared to commercially available Resotran® while observed activation of the coagulation system was slightly faster. Thus, sulfobetaine and PEG 25 kDa-OMe functionalized synomag®-D present promising candidates for further investigations with respect to their behavior in vivo.

I. Introduction

Magnetic particle imaging (MPI) as a relatively new and growing diagnostic tool opens up various unprecedented possibilities. However, for the exploitation of the full potential of the method, accessibility towards safe and high-performing tracer materials is essential. Herewith, research focuses mainly on iron oxide based magnetic nanoparticles (MNPs) due to their combination of non-toxicity, stability, and signal strength.

During the last two decades, various types of MNPs were approved for application in humans, mainly as contrasting agents for magnetic resonance tomography, but the fact that most manufacturers have been shutting their production necessitates the need for new available tracer materials. For many years, Resovist® (Bayer Schering, Germany) was used as the standard tracer for MPI, which utilized ferucarbotran, but since it was also removed from the market in 2008, the need for new tracer materials is further intensified.

Another drawback for MPI arises since many of those previous MNP systems suffer under low blood half-life time, especially due to clearance by macrophages in liver and spleen. Therefore, the possible applications are rather restricted to observations of these organ systems.

As shown previously [1], synomag®, a multicore maghemite MNP, represents a promising candidate for a new generation of MPI tracers with outstanding signal quality as well as excellent biocompatibility [2]. Thus, we utilized this type of MNP for the further optimization of the particle functionalization. Within this study, two types of functionalization which are promising candidates for exhibiting enhanced blood half-life times were examined with regard to their implications for biocompatibility:

1. PEGylation: It was demonstrated that PEGylation of synomag®-D leads to MNPs, which can readily be encapsulated in human blood cells possibly resulting in prolonged blood circulation times.[3-4]
2. Coupling of sulfobetaine analogue: It was demon-

strated that polybetaine-coating results in a reduction of unspecific protein adsorption due to its zwitterionic character improving blood half-life time.[5] Hence, we investigated the influence of coupling sulfobetaine monomers onto the surface of synomag®-D.

II. Material and methods

II.I. Magnetic nanoparticles

Magnetic nanoparticles investigated in the present study were based on commercially available synomag®-D (micromod Partikeltechnologie GmbH, Germany) with a nominal hydrodynamic diameter of 70 nm. Those multicore maghemite (γ-Fe_2O_3) particles coated with dextran were tested without and with further functionalization by PEGylation, referred as synomag®-D PEG 25 kDa-OMe, and by coupling of a sulfobetaine analogue ((2-Hydroxyethyl)dimethyl(3-sulfopropyl) am-monium hydroxide), referred as synomag®-D sulfobetaine. Furthermore, synomag®-D PEG 25 kDa-OMe particles were formulated with mannitol to achieve an isotonic suspension (osmolalilty of 324 mOsmol·kg^{-1}) and autoclaved at 121 °C for 20 min (referred as synomag®-D PEG 25 kDa-OMe form.). Ferucarbotran-based Resotran® (b.e.imaging GmbH, Germany) was utilized as reference in biocompatibility studies.

II.II. Cell viability assay

Biocompatibility studies of synomag®-D and derived particles, as well as Resotran®, were performed via cell viability analysis according to ISO 10993-5. Cell culture reagents were obtained from PAN-Biotech (Aidenbach, Germany). Human endothelial cells (EA.hy926, ATCC, USA) were incubated in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin/streptomycin at 37 °C and 5% CO_2. Cells were seeded with a density of 1.5·10^4 cells·cm^{-2} in a 96-well plate (Greiner Bio-One, Germany) and cultured for 24 h. Thereafter, nanoparticles were added to endothelial cells with a resulting iron concentration of 100 µg·ml^{-1}. After further 24 h, cell viability was determined under usage of a resazurin-based assay (CellQuanti-Blue™, BioAssay Systems, USA) and a microplate reader (FLUOstar OMEGA, BMG Labtech, Germany) at ex544/em590. For every nanoparticle system, three independent experiments were performed. The obtained average values were normalized to an untreated control.

II.III. Coagulation studies

Nanoparticles were mixed with pooled human plasma (VisuCon-F frozen unassayed normal control plasma,

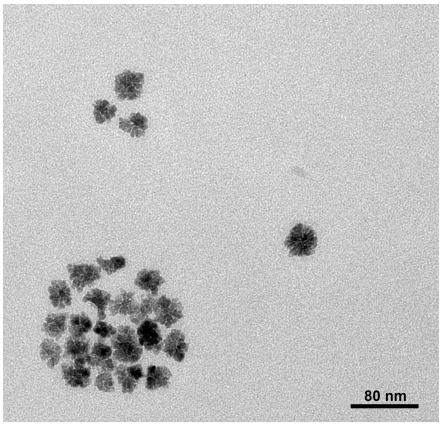

Figure 1: TEM image of synomag®-D PEG 25 kDa-OMe form.

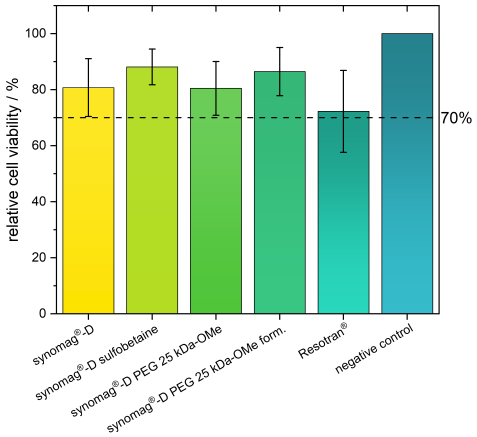

Figure 2: Relative viability of human endothelial cells loaded with functionalized and unfunctionalized synomag®-D nanoparticles and Resotran® for 24 h. Values are normalized to unloaded cells (negative control). Iron concentration was 100 µg·ml^{-1}, respectively.

Affinity Biologicals, Canada) in PBS with a resulting iron concentration of 100 µg·ml^{-1}. The obtained mixtures were further incubated at 37 °C for 30 min. Thereafter, the suspensions were mixed in a rotating cuvette with a steel ball inside the coagulometer (MC4, Merlin medical, Germany) in a ratio of 2:1 with 25 mM $CaCl_2$, and the clotting times were measured. For the positive control Dapttin® TC (Haemochrom Diagnostica, Germany) was added to plasma instead of nanoparticle suspensions.

III. Results and discussion

Prior to cell viability and coagulation experiments, synomag®-D and derived MNPs were characterized with respect to physicochemical properties. Furthermore, commercially available and approved Resotran®, which bases on ferucarbotran like Resovist®, was utilized as reference. Table 1 summarizes the hydrodynamic di-

Table 1: Properties of investigated nanoparticles.

Nanoparticle	z_{av}* [nm] / polydispersity index [-]	pH of Isoelectric point** [-]
synomag®-D	65.9 / 0.06	6.1
synomag®-D sulfobetaine	83.3 / 0.10	4.5
synomag®-D PEG 25 kDa-OMe	87.7 / 0.16	5.7
synomag®-D PEG 25 kDa-OMe form.	76.1 / 0.12	5.8
Resotran®	66.4 / 0.19	2.2

*average hydrodynamic diameter; determined by dynamic light scattering
**determined by electrophoretic light scattering

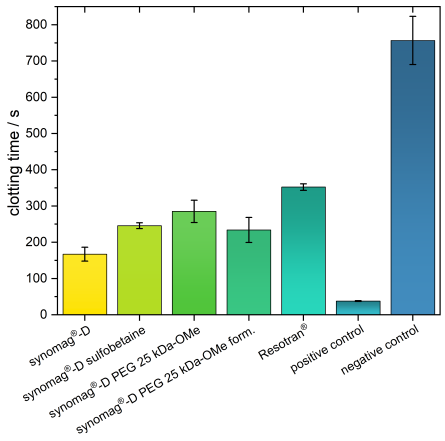

Figure 3: Influence of functionalized and unfunctionalized synomag®-D nanoparticles and Resotran® on clotting time. Iron concentration was 100 µg·ml^{-1}, respectively. PBS-buffer and Dapttin® TC were utilized as negative control and positive control.

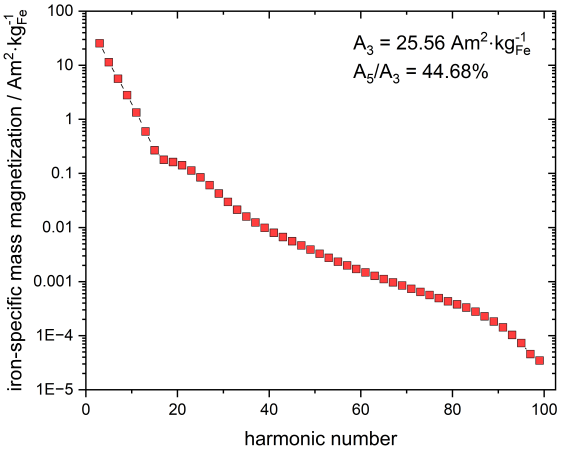

Figure 4: Magnetic particle spectrum of synomag®-D PEG 25 kDa-OMe form. measured at 25 mT. Only odd harmonics are given.

ameters and isoelectric points of the investigated particle systems. All nanoparticles exhibit comparable hydrodynamic diameters from 66 nm (synomag®-D and Resotran®) to 88 nm (synomag®-D PEG 25 kDa-OMe). It is worth mentioning that differences in size of synomag®-D PEG 25 kDa-OMe and synomag®-D PEG 25 kDa-OMe form. arise as a result of lot to lot variations.

The formulation and autoclavation procedure itself lead only to minor changes in particle size (reduction of the hydrodynamic diameter from 77 to 76 nm). Due to the coating of the underlying iron oxide core with carboxydextran, Resotran® shows the lowest isoelectric point of all nanoparticle systems at a pH-value of 2.2 followed by synomag®-D sulfobetaine at 4.5 and the remaining synomag®-D nanoparticles at ∼6. The typical multicore nanoflower structure of synomag®-D based particles is shown in the transmission electron microscopy (TEM) image in Fig. 1 for synomag®-D PEG 25 kDa-OMe form. as representative example. Due to the dextran-based coating, only the synomag® cores are imaged with diameters around 50 nm.

Fig. 2 demonstrates the results of the cell viability assay. Values range from 72% relative viability for Resotran® to 90% for synomag®-D sulfobetaine. Hence, all investigated magnetic nanoparticles lie above the critical threshold of 70% for biocompatibility of medical products (ISO 10993-5).

Activation of the coagulation cascade releases platelet-activating factors and leads to thrombosis. In order to investigate the thrombogenic potential of MNPs in the bloodstream, activation of the coagulation system by the various nanoparticle systems was studied using an *in vitro* testing system based on human pooled plasma. The results of these experiments are given in Fig. 3. The clotting time was 756 s for the negative control (PBS-buffer). In the course of the addition of nanoparticles, clotting time was decreased between 167 s for synomag®-D and 353 s for Resotran® but still significantly slower than the clotting of the positive control (38 s, Dapttin® TC). Moreover, it was demonstrated that functionalization resulted in prolonged clotting times.

synomag®-D PEG 25 kDa-OMe form. was also characterized by means of magnetic particle spectroscopy allowing conclusions on their applicability as MPI tracer material (see Fig. 4). The iron mass normalized third harmonic A_3 was 25.56 Am^2·kg$_{Fe}{}^{-1}$ and the ratio of the fifth to third harmonic A_5/A_3 was about 45% reproducing the high performance of synomag® as compared to previous assessments [1].

Cell viability revealed superior biocompatibility of synomag®-D derived particle systems. Additionally, coagulation experiments demonstrated a low thrombogenicity, that was ∼70% of the time required for clot-

ting of the approved reference product for functionalized synomag®-D and clearly above that of the positive control. Moreover, these results were also obtained for autoclaved and formulated synomag®-D PEG 25 kDa-OMe which maintained the high performance of synomag®. Thus, the foundation is laid for further toxicity and biocompatibility assessments, which are subject of current research.

Acknowledgments

Authors are grateful for MPS spectra measured by Jochen Franke (Bruker BioSpin). Research funding: Financial support by the State Ministry of Economics, Employment and Health of Mecklenburg-Vorpommern within the project “NanoVis” (grant number TBI-V-1-318-VBU-111) is gratefully acknowledged.

Author’s statement

Conflict of interest: B.K., and P.S. are employees of micromod Partikeltechnologie GmbH. Informed consent: Informed consent has been obtained from all individuals included in this study.

References

[1] Vogel, P.; Kampf, T.; Rückert, M.; Grüttner, C.; Kowalski, A.; Teller, H.; Behr, V., Synomag®: The new high-performance tracer for magnetic particle imaging. International Journal on Magnetic Particle Imaging IJMPI 2021, 7(1).

[2] Matschegewski, C.; Kowalski, A.; Müller, K.; Teller, H.; Grabow, N.; Großmann, S.; Schmitz, K.-P.; Siewert, S., Biocompatibility of magnetic iron oxide nanoparticles for biomedical applications. Current Directions in Biomedical Engineering 2019, 5(1), 573-576.

[3] Antonelli, A.; Scarpa, E. S.; Di Corato, R.; Abdalla, P.; Thieben, F.; Grüttner, C.; Knopp, T.; Magnani, M., Encapsulation in human and murine erythrocytes of the Synomag®-D-PEG-OMe tracer for MPI application. International Journal on Magnetic Particle Imaging IJMPI 2022, 8(1).

[4] Gerosa, M.; Ren, G.; Zhang, Y.; Goodwill, P.; Mansfield, J.; Marzola, P.; Wintermark, M., Preliminary results: Imaging of in situ labeled tumor-associated macrophages with Magnetic Particle Imaging. International Journal on Magnetic Particle Imaging IJMPI 2020, 6(2).

[5] Jiang, S.; Cao, Z., Ultralow-fouling, functionalizable, and hydrolyzable zwitterionic materials and their derivatives for biological applications. Advanced materials 2010, 22(9), 920-932.

10.18416/ijmpi.2024.2403032