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Elimination of Magnetic Nanoparticles with Various Surface Modifications from the Bloodstream *in vivo*

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The magnetic nanoparticles with the core diameter 10 nm stabilized by sodium oleate and bovine serum albumin in phosphate buffer have been modified by different biocompatible substances such as poly(ethylene glycol) (PEG), dextran (DEX), and polyvinylpyrrolidone (PVP). Prepared biocompatible magnetic fluids were characterized to obtain the particle size distribution using scanning electron microscopy and the dynamic light scattering method. To study the elimination of different modified magnetic nanoparticles from bloodstream, the biocompatible samples were applied intravenously to the mice bloodstream with further blood specimens collecting in given time intervals. Magnetic moment of the lyophilized blood samples was measured by SQUID magnetometer. Time dependence of magnetic moment of magnetic nanoparticles and magnetic nanoparticles modified by DEX, PEG and PVP normalized to the Fe_3O_4 showed that the circulation time of magnetic nanoparticles in the bloodstream depends on the substance used for modification. The unmodified magnetic nanoparticles were trapped by reticuloendothelial system within 1 h while magnetic nanoparticles modified by DEX, PEG and PVP circulated in blood up to 3 h.

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1. Introduction

In recent years, great interest was attracted by magnetic nanoparticles (MNPs) due to their ferro- and superparamagnetic properties useful both for engineering and for the field of medicine. Particularly, magnetic fluids (MF) consisting of the magnetic nanoparticles and liquid carrier are known as a contrast agent in magnetic resonance imaging [1, 2], as mediators in magnetic hyperthermia for cancer treatment, etc. [3].

The important advantage of magnetic nanoparticles is their size ($\approx$ 10 nm), which enable them to penetrate through biological membranes [4]. This allows one to actively use nanoparticles as carriers for targeted drug delivery. Usually *in vivo* and *in vitro* investigations employ MNPs coated by biocompatible polymers [5]. Such systems can bypass the normal physiological processes of defence and, depending on the particle size and properties of bilayer, remain for a long time in bloodstream [6].

As is known [7], it is not possible to reduce systemic toxicity of the chemotherapy, this leads to sub-optimal dosing and insufficient effect. Therapy, which uses a method of targeted drug delivery, can enhance the therapeutic effect by increasing the drug concentration in the tumour [8].

Nanoparticles are generally cleared by the reticuloendothelial system (RES), in particular taken up by the Kupffer cells in the liver, limiting particle bioavailability and *in vivo* applications [9]. Our goal was to modify magnetic particle surface by appropriate biocompatible compounds that could decrease the RES clearance and prolong the circulation time of particles.

2. Experimental

2.1. Materials

Poly(ethylene glycol) with average molecular weight (Mw) 1000, dextran with average Mw 70 000 and bovine serum albumin were provided by Sigma Aldrich. Polyvinylpyrrolidone K30 with average Mw 40 000 was obtained from Fluka and sodium oleate ($C_{17}H_{33}COONa$) from Riedel-de Han. Typically, ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), ferrous sulphate heptahydrate ($FeSO_4{\cdot}7H_2O$) and ammonium hydroxide (NH_4OH) were used for magnetite synthesis.

2.1.1. Animals

The experimental animals were male mice, body weight of which was 24.6 ± 1.4 g. They were housed individually in a room cages with artificial 12/12 h light/dark cycle. Mice had free access to water and fed by MP-OS-06 (BIOFET, SR) ad libitum. Before sampling blood, mice were placed in desiccator filled by the diethyl ether (inhalation of it conducted light anesthesia).

2.2. Sample preparation and experimental details

MNPs were prepared by co-precipitation method of ferric and ferrous salts with a molar ratio 2:1. Under vigorous stirring, ammonium hydroxide solution was added

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into the flask with mixture of water solution of Fe^{3+} and Fe^{2+} and magnetite in the form of a black precipitate formed immediately. After washing the particles were isolated from water and sodium oleate was added at 50 °C to prevent agglomeration of the particles.

After that suspension was heated up to reach the boiling point. Then undesirable agglomerates obtained during this process were removed by centrifugation with 9000 rpm for 30 min (Hettich universal 32, Germany). To improve the biocompatibility of magnetic particles, bovine serum albumin (BSA) in weight ratio of $BSA/Fe_3O_4 = 2$ was used for modification. The mixture was incubated in horizontal shaker (Heidolph promax 1020) for 6 h at 40 °C and 200 rpm. Then, pH of the colloid was adjusted to value 7.4 by phosphate buffer. Further on, the resulting sample consisting of magnetic fluid modified by BSA is referred to as MFBSA. MFBSA was afterwards mixed with DEX (MFBSADEX), PEG (MFBSAPEG) and PVP (MFBSAPVP) water solutions and incubated in a horizontal shaker for 24 h at 40 °C and 200 rpm. The PEG/magnetite and PVP/magnetite weight ratios were each equal to 0.25, while the DEX/magnetite weight ratio was 3.

Examination of the size and morphology of all prepared MF samples was done using transmission electron microscopy (TEM, Tesla BS 500 microscope, operated at 90 kV with 80 000 magnification) and scanning electron microscopy (SEM, JEOL 7000F microscope). The particle size distribution was measured by dynamic light scattering (DLS) technique (Zetasizer NanoZS, Malvern, UK), which measures the velocity of particles' Brownian motion. The stability of the samples was studied by the measuring of Zeta potential using laser Doppler velocity. The magnetic moment measurements were conducted by SQUID magnetometer (Quantum Design MPMS 5XL).

To study different surface modified MNPs' elimination from the bloodstream, the prepared biocompatible samples as well as the unmodified MF were diluted in water for injections (1:1) and administered intravenously to mice's tail vein. The blood was collected after 15 min, 30 min, 1, 2, 3, 4, 5, and 24 h, respectively. The blood was then frozen and after lyophilisation the magnetic moment of the samples was measured at room temperature.

3. Results and discussion

The mean core diameter of magnetic particles in unmodified MF obtained by TEM was found to be 10 nm (Fig. 1a). SEM observations confirmed roughly spherical shape of nanoparticles and the average MFs diameters are summarized in Table I. As one can see from Table I, as well as from Fig. 1b–d, increase of hydrodynamic diameter of modified magnetic nanoparticles in comparison with the diameter of unmodified magnetic particles indicates the presence of biocompatible shells.

A complementary technique for particle size distribution determination is DLS. Table I shows results of the z-average hydrodynamic diameters of all studied

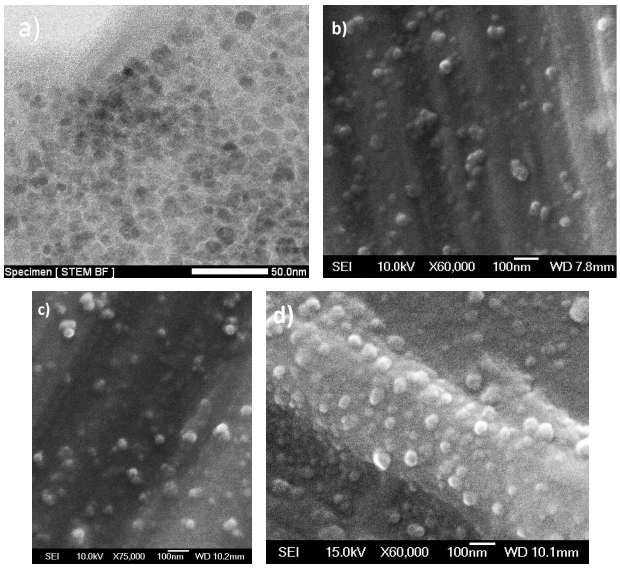

Fig. 1. (a) TEM image of magnetic nanoparticles in MF; SEM image (b) MFBSAPEG, (c) MFBSAPVP, (d) MFBSADEX.

TABLE I

Comparison of the MNP diameters and Zeta potentials (ZP) of prepared MFs obtained by SEM and DLS.

Method	MFBSA	+PEG	+PVP	+DEX
D_{SEM} [nm]	28	29	34	33
D_{DLS} [nm]	53	63	59	69
ZP [mV]	-19	-39	-49.8	-47

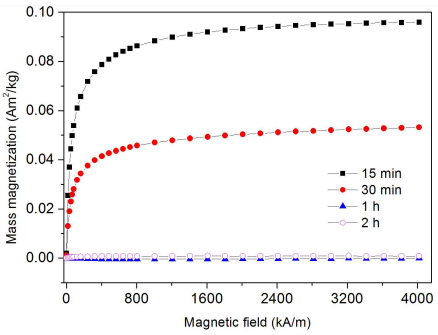

Fig. 2. Magnetization curves of MFBSA.

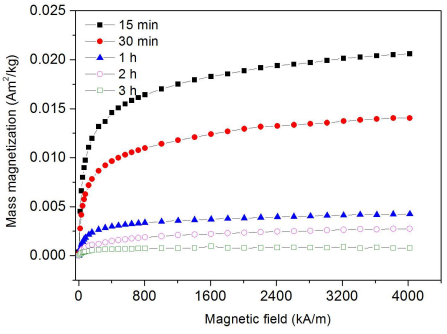

Fig. 3. Magnetization curves of MFBSAPEG.

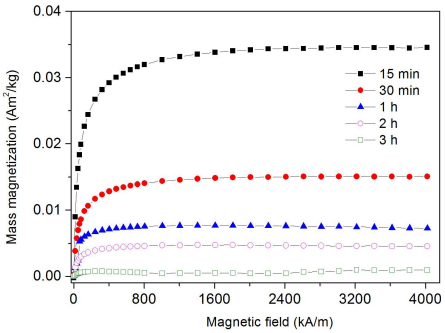

Fig. 4. Magnetization curves of MFBSAPVP.

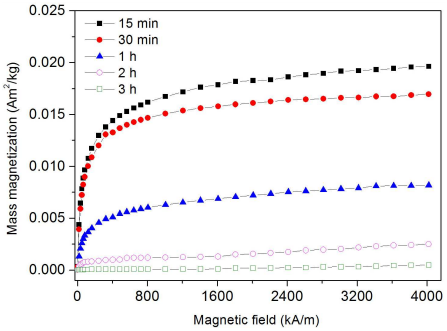

Fig. 5. Magnetization curves of MFBSADEX.

samples (MFBSA, MFBSADEX, MFBSAPEG, MFBSAPVP). These values are larger than those measured by SEM. This fact can be explained by the use of different techniques working on various principles. Regarding Zeta potential measurement, obtained results indicated good colloidal stability of studied MFs.

The magnetite concentration in MFBSA used for intravenous application was 1.025 mg Fe_3O_4/mouse, for MFBSA modified by PEG, PVP and DEX was 0.492 mg Fe_3O_4/mouse.

The following figures show the magnetization curves of the lyophilized blood containing MFBSA (Fig. 2), and MFBSA modified by PEG (Fig. 3), PVP (Fig. 4), and DEX (Fig. 5).

Figure 6 shows magnetic moments time dependence of unmodified and modified magnetic particles in the dried blood samples normalized to the Fe_3O_4 weight.

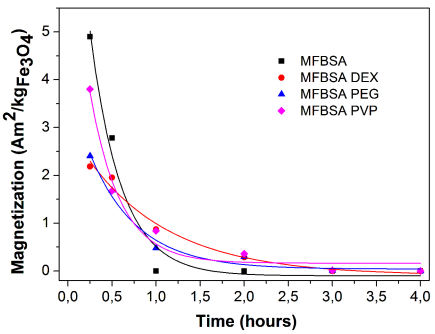

Fig. 6. Time dependence of magnetization of different modified MNPs in bloodstream.

In all samples magnetization of the nanoparticles decreased with time. The magnetization of unmodified MNPs in dried blood samples dropped to zero after 1 h after administration. In the case of magnetic particles modified by DEX, PEG, and PVP the magnetization was higher than zero even 3 h after. The obtained results showed that the circulation time of MFBSA in the bloodstream depends on the substance used for modification.

4. Conclusions

The influence of surface modification of magnetic nanoparticles on the circulation time in bloodstream was studied. The obtained results showed that all MNPs of MFBSA were trapped by reticuloendothelial system within 1 h, while in the case of MNPs modified by DEX, PEG and PVP nanoparticles stayed in bloodstream up to 3 h. Nanoparticle surface modifications decreased the RES clearance and prolonged the nanoparticles circulation time. Considering the importance of this parameter for the *in vivo* targeting efficiency, further investigations on different nanoparticles' modifications are required.

Acknowledgments

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