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Nanoparticles for magnetic biosensing systems

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ABSTRACT

The further development of magnetic biosensors requires a better understanding of the interaction between living systems and magnetic nanoparticles (MNPs). We describe our experience of fabrication of stable ferrofluids (FF) using electrostatic or steric stabilization of iron oxide MNPs obtained by laser target evaporation. Controlled amounts of FF were used for *in vitro* experiments with human mesenchymal stem cells. Their morphofunctional responses in the Fe concentration range 2–1000 maximum tolerated dose revealed no cytotoxicity.

1. Introduction

Nanoparticles can enter the human body through the air, the gastrointestinal tract, or as nanoscale wears of implants and endoprosthesis. An accumulation of nanoscale objects and their degradation products in the tissues and biological fluids is fraught with a huge, insufficiently understood potential health hazard [1]. Nanoparticles with a diameter below 50 nm penetrate tissue barriers and can be toxic for parenchymal and stromal cells [2]. Magnetic nanoparticles (MNPs) are employed in magnetic biodetection widely requested in medicine and environmental control. A magnetic biosensor is a compact analytical device in which a magnetic transducer converts a magnetic field variation into a change of frequency, current, voltage. Different types of magnetic effects are capable of creating magnetic biosensors: magnetoelastic, Hall, inductive effects, anisotropic magnetoresistance, giant magnetoresistance and magnetoimpedance (MI) [3–5]. MI provides the highest sensitivity with respect to the applied field. The magnetic label detection principle is simple: the stray fields induced by the magnetic markers are employed as biomolecular labels providing a means for transfer of information about the concentration of magnetic labels and therefore the biocomponent of interest [4–6]. The sensitivity limit is related to the type of MNPs – the magnetic moment of an individual particle in the external magnetic field governs the stray fields and the biodetection limit. MNPs properties can vary from batch to batch, which is why finding fabrication techniques ensuring enhanced batches is of utmost importance. One of them is the laser target evaporation method (LTE) providing a production rate of 50 g/h [7,8].

Biomedical applications demand magnetic MNPs in the form of water-based ferrofluids (FF). LTE MNPs are adequate for the formation of water-based FF [8]. There are two ways of obtaining colloidal suspensions of de-aggregated particles: electrostatic and steric stabilization [9]. The stability of FF in physiological solutions characterized by a large concentration of salts is a parameter of major importance in biomedicine. An increase of the ionic strength of the biological medium makes the electrostatic stabilization of the suspensions less favorable. In a number of recent studies the natural polymer chitosan has been discussed as an effective electrosteric stabilizer [10] due to the presence of amino and hydroxyl groups in its elementary unit providing the specific interaction of chitosan macromolecules with the MNPs surface and leading to a suspension stabilization at low polymer concentrations [11]. The analysis of the morphofunctional response of living cells to the presence of different kinds of suspensions is missing in the literature but a better understanding of the interactions of the living systems and MNPs would be beneficial for the biosensing and drug

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Table 1
Comparison of calculated and experimental concentrations (mg/L) of iron determined by stripping voltammetry (SV).

Time of cell cultivation (h)	0 (before cultivation)	24		72		144	
		FF-1	FF-2	FF-1	FF-2	FF-1	FF-2
Calculated concentration of iron oxide MNPs corresponding to 100 MTD	–	30.0					
Iron concentrations measured by SV in intercellular liquids	< 0.1	23.2 ± 7.0	30.5 ± 1.0	31.5 ± 9.4	38.0 ± 11.4	32.7 ± 9.8	39.9 ± 12.0

Note: The data of 3 measurements are presented; maximum tolerated dose (MTD) was 0.3 mg/L iron ions in water solution.

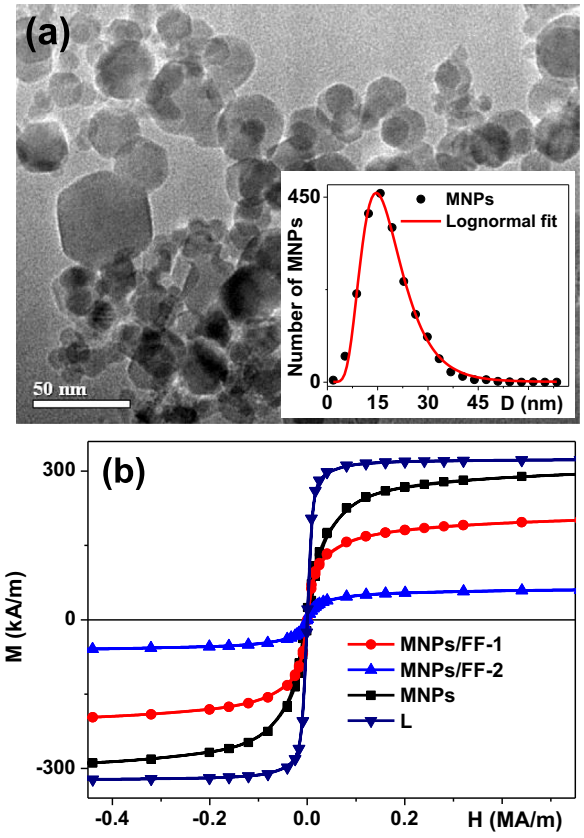

Fig. 1. TEM image of iron oxide LTE MNPs. Inset shows size distribution (a). Hysteresis loops of MNPs, FF-1, FF-2 and Langevin fit for MNPs curve at $T=20$ °C (b).

delivery.

Both the magnetic marker detection by the analysis of the impedance in the presence of magnetic markers and the detection of MNPs after intracellular uptake have been successfully demonstrated in MI sensor prototypes [4,12]. Meanwhile, one of the greatly demanded applications for cancer therapies, the detection of the MNPs incorporated into biological tissues, is still under development. The main obstacle is the difficulty to obtain reliable sampling. Biological and especially tumor tissues present a huge variety of morphologies. To overcome this issue, the possibility of substituting biological samples at the first stage of the magnetic biosensor development by a synthetic hydrogel with a certain amount of MNPs (ferrogel) has been studied and was even recently demonstrated [6]. Ferrogels to a certain extent mimic many functional properties of natural tissues [13]. In this respect, the synthesis of ferrogels based on sterically or electrostatically stabilized suspensions of iron oxide LTE MNPs needs to be performed within the limits of MNP concentration bearable for living cell cultures.

In this work we fabricated stable colloidal suspensions using electrostatic or steric stabilization of iron oxide MNPs obtained by LTE. Controlled amounts of ferrofluids were employed for *in vitro* experiments with human mesenchymal stem cells in order to examine their morphofunctional response.

2. Experimental methods

In the laser target evaporation method, the solid target is overheated by the high-power pulse of a focused laser beam. The introduced energy provides rapid evaporation in the laser spot area. The laser beam energy is transferred into the kinetic energy of the vapors, ejected away from the surface. The LTE target was made of the commercial Fe_2O_3 powder (Alfa Aesar). More details on the LTE method and the experimental setup used in the present study are given elsewhere [14]. The X-ray diffraction (XRD) studies were performed with DISCOVER D8 Bruker diffractometer. TOPAS-3 software with Rietveld full-profile refinement was employed for the quantitative analysis. Transmission electron microscopy (TEM) was performed for MNPs morphology evaluation (JEOL JEM2100). The specific surface area (S_{sp}) of the MNPs was measured by the low-temperature sorption of nitrogen [14]. Magnetic measurements were carried out with MPMS XL-7 SQUID-magnetometer. Ferrofluids and biological samples were dried prior to measurement and polymer capsule contribution was carefully subtracted.

Before the preparation of the suspension for biological testing, the iron oxide MNPs were dry-heat sterilized with Binder FD53 (Binder GmbH, Tuttlingen, Germany) at 180 °C for 1 h. Electrostatically stabilized water based ferrofluid (FF-1) was prepared using sodium citrate. De-aggregation was insured by ultrasound treatment on Cole-Parmer CPX-750 homogenizer operated at power output 300 W. Hermle Z383 centrifuge was used to remove large aggregates. A ferrofluid with sterical stabilization (FF-2) was prepared by the two-step modification of FF-1. First, FF-1 was mixed with chitosan solution (20 g/L concentration) in 0.2 N hydrochloric acid at pH = 2, and then phosphate buffered saline (PBS) with pH = 6.86 was added drop-wise under vigorous stirring. Hydrodynamic diameters of the MNPs and aggregates were measured by dynamic light scattering (Brookhaven ZetaPlus). The electrokinetic zeta-potential of the suspensions was measured by electrophoretic light scattering using the same instrument.

2.1. Electrochemical and biological testing

Concentrations of iron oxide MNPs in biological liquids were confirmed by stripping voltammetry (SV) of iron ions [15]. Calculated maximum tolerated doses (MTDs) in medium corresponded to those estimated by SV (Table 1).

2.2. Cell culturing in vitro

The primary culture of post-natal adipose-derived multipotent mesenchymal stromal cells (AMMSCs) was prepared from human fate tissue after processing of lipoaspirates (Permission No. 4 from 23.10.2013 of Local Ethics Committee of Innovation park of Immanuel Kant Baltic Federal University) [16]. The AMMSCs suspension was freshly prepared with a concentration of 5×10^4