Journal of Magnetism and Magnetic Materials 380 (2015) 85–89

Contents lists available at ScienceDirect

Journal of Magnetism and Magnetic Materials

journal homepage: www.elsevier.com/locate/jmmm

The cytotoxicity of iron oxide nanoparticles with different modifications evaluated *in vitro*

Vlasta Zavisova [a,*], Martina Koneracka [a], Jozef Kovac [a], Martina Kubovcikova [a], Iryna Antal [a], Peter Kopcansky [a], Monika Bednarikova [b], Marta Muckova [b]

[a] Institute of Experimental Physics, Slovak Academy of Sciences, Watsonova 47, 040 01 Kosice, Slovakia
[b] hameln, rds a.s, Horna 36, 900 01 Modra, Slovakia

ARTICLE INFO

Article history:
Received 30 June 2014
Received in revised form
12 October 2014
Accepted 13 October 2014
Available online 17 October 2014

Keywords:
Magnetic fluid
Cytotoxicity
In vitro test
Melanoma
Dextran
Biocompatibility

ABSTRACT

The toxicity of magnetite nanoparticles modified with bioavailable materials such as dextran, bovine serum albumin, polyethylene glycol, and polyvinylpyrrolidone was studied in normal and cancer cells. The size distribution and magnetic properties of the modified magnetic nanoparticles were characterized by different techniques. Transmission electron microscopy showed a nearly spherical shape of the magnetite core with diameters ranging from 4 to 11 nm. Dynamic light scattering was employed to monitor the hydrodynamic size and colloidal stability of the magnetic nanoparticles: *Z*-average hydrodynamic diameter was between 53 and 69 nm and zeta potential in the range from −35 to −48 mV. Saturation magnetization of the modified nanoparticles was 55–64 emu/g_{Fe3O4}. Prepared biocompatible nanoparticles had no significant toxic effect on Chinese hamster lung fibroblast cell line V79, but they substantially affected mouse melanoma B16 cell line.

1. Introduction

Modern medicine is beginning to actively use nanotechnology in clinical diagnostics, targeted drug delivery, cancer treatment by hyperthermia and other fields [1]. Nevertheless, the toxicity of nanostructured materials is an open issue due to several factors: high reactivity, intrinsic toxicity of the material, and non-specific interactions with biological objects, that are determined by particle shape, size and structure. Biocompatibility, toxicity and ability to penetrate into cells are the main criteria that determine the effectiveness of nanoparticles in medicine [2].

One of the widely studied and currently used nanomaterials is the magnetite and magnetite-derived nanoparticles that possess stable magnetic characteristics. However, a lack of knowledge about the mechanism of magnetite (Fe_3O_4) nanoparticles penetration into tissues, organs and tumors, as well as the degree of their toxicity limits of their application [3–5].

To minimize biofouling and aggregation of magnetic nanoparticles, their escape from the reticuloendothelial system and to increase their circulation time, they are usually coated with a layer of hydrophilic and biocompatible polymers. Polymers based on poly(ethylene-*co*-vinyl acetate), polyvinylpyrrolidone (PVP), poly (lactic-*co*-glycolic acid), polyethylene glycol (PEG), poly(vinyl alcohol) are typical examples of synthetic polymeric systems [6,7]. The most commonly used natural polymers are gelatin, dextran (DEX), chitosan, and pullulan [8].

Iron oxide nanoparticles are generally coated to reduce aggregation and cytotoxicity [9]. DEX-coated iron oxide nanoparticles have been used as MRI contrast agents to investigate nanoparticle accumulation and cellular uptake in malignant neoplasms *in vivo*, and also to transform nanoparticles into active, targeted probes [10–12]. PEG is a stable, biocompatible hydrophilic polymer used in many drug and gene delivery applications [13]. In the study of Miao Yu [14], porcine aortic endothelial cells were exposed to iron oxide nanoparticles coated with either DEX or PEG. Results indicated that both coatings can reduce nanoparticle cytotoxicity, but different mechanisms may be important for different nanoparticle size. Cytotoxicity and cell uptake studies in VERO and MDCK cell lines showed low toxicity of PEG-coated superparamagnetic iron oxide nanoparticles and DEX-coated superparamagnetic iron oxide nanoparticles [15].

In our current work we focused on preparation of a stable, biocompatible magnetic fluid (MF) with low toxicity to normal cells. We also investigated the cytotoxicity of magnetite nanoparticles coated with bovine serum albumin (BSA), DEX, PVP, or PEG. We selected normal Chinese hamster lung fibroblast cell line

* Corresponding author.
E-mail address: zavisova@saske.sk (V. Zavisova).

http://dx.doi.org/10.1016/j.jmmm.2014.10.041
0304-8853/

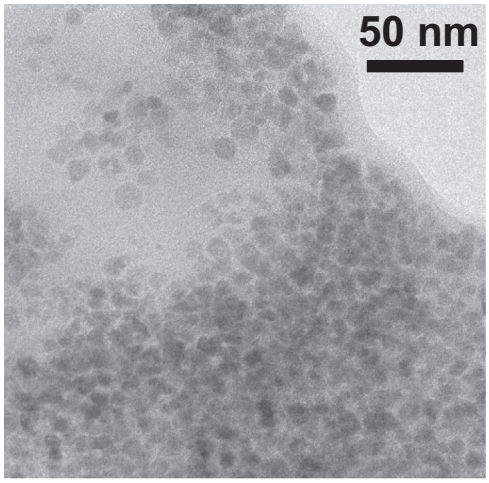

Fig. 1. TEM image of core magnetic particles in MF.

V79 and melanoma mouse cell line B16 to assess the cytotoxicity of prepared nanoparticles.

2. Materials and methods

Polyethylene glycol (average molecular weight (Mw) 400, 1000, 10,000, 20,000), dextran (average Mw 70,000), and bovine serum albumin were purchased from Sigma-Aldrich. Polyvinylpyrrolidone K30 (average Mw 40,000) was obtained from Fluka and sodium oleate from Riedel-de Haën. Typically ferric chloride hexahydrate ($FeCl_3 \cdot 6H_2O$), ferrous sulfate heptahydrate ($FeSO_4 \cdot 7H_2O$) and ammonium hydroxide (NH_4OH) were used for magnetite synthesis.

Dulbecco's modified Eagles medium (DMEM) and fetal bovine serum were from BioWhittaker. MTT salt, trypsin and EDTA were obtained from Sigma-Aldrich. Streptomycin and Penicillin G antibiotics were from AppliChem and Biotika, respectively.

V79 cell line was obtained from ECACC (European Collection of Cell Cultures, UK) and B16 cell line was obtained from CRI SAS (Bratislava, Slovakia).

The co-precipitation method of ferric and ferrous salts in an alkaline aqueous medium was used to prepare spherical magnetite particles. In a typical synthesis, an aqueous solution of Fe^{3+} and Fe^{2+} (molar ratio 2:1) was prepared by dissolving in deionized water. An excess of hydroxide ions was added to the mixture of Fe^{3+} and Fe^{2+} with vigorous stirring at room temperature to form a black precipitate of magnetite nanoparticles. After washing by magnetic decantation and heating up to 50 °C, the surfactant sodium oleate ($C_{17}H_{33}COONa$) was added to the mixture to prevent agglomeration of the particles. The mixture was then stirred and heated until the boiling point was reached. The obtained oleate bilayer stabilized magnetite particles were dispersed in water. Agglomerates were removed by centrifugation at 9000 rpm for 30 min. The particles prepared by this method are referred to as MF (magnetic fluid) hereafter.

To improve biocompatibility, the MF was further modified by coating with bovine serum albumin (BSA). BSA was dissolved in water and added to the MF at weight ratio BSA/Fe_3O_4= of 2 and stirred (200 rpm at 40 °C) for six hours. The pH of the obtained colloid was adjusted to 7.4 by addition of phosphate buffer. Magnetic fluid modified by BSA (MFBSA), prepared by described procedure, is an intermediate product between MF and next biocompatible compound modification below.

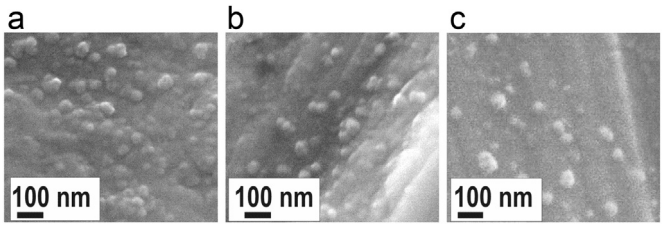

Fig. 2. SEM image of modified nanoparticles in MFDEX (a), MFPVP0.25 (b) and MFPEG1000 (c).

MFBSA was mixed with water solutions of DEX, PVP or PEG and stirred in a horizontal shaker (200 rpm and 40 °C) for 24 h to obtain magnetic fluids modified by dextran (MFDEX), PVP (MFPVP) and PEG (MFPEG). For functionalization of MFBSA by PEG, four different PEG molecular weights were used: 400, 1000, 10,000 and 20,000 g/mol at the constant PEG/Fe_3O_4 weight ratio = 1. In case of MFPVP, three samples with PVP/Fe_3O_4 weight ratios of 0.25, 0.5, 1 were prepared. DEX/Fe_3O_4 weight ratio of 3 was used to prepare MFDEX.

The prepared magnetic fluids were examined by transmission electron microscopy (TEM, JEOL-TEM 2100F microscope operated at 90 kV) under 80,000x magnification by the replication technique. Briefly, a drop of MF sample diluted in water was deposited on the 400 mesh copper grid and air dried before the picture was taken. Scanning electron microscopy (SEM, JEOL 7000F microscope) was used to evaluate the morphology and microstructure of the coated nanoparticles in the prepared MF samples. The colloidal dispersion was first diluted in water (typically $1{:}10^6$ dilution), and one droplet was deposited on an aluminum grid and dried under vacuum prior sputtering with carbon and subsequent observation.

To determine the particle size distribution the samples were measured by Dynamic light scattering (DLS) using Zetasizer Nano ZS (Malvern Instruments). The zeta potential was estimated using Laser Doppler Electrophoretic measurement technique with a scattering angle of 173° at 25 ± 0.1 °C. DLS evaluates the intensity fluctuation of scattered light reflected from nanoparticles in suspension. The fluctuation is resulting from the "Brownian motion" that keeps the particles in steady movement.

The complementary technique used to determine particle size distribution in the prepared samples was Differential Centrifugal Sedimentation (DCS). DCS enables to measure particle size by measuring the time required for the colloidal particles to settle in a density gradient in a disk centrifuge. The DC24000 UHR disk centrifuge (CPS Instruments, Inc.) was used to perform sedimentation based size distribution measurements.

Magnetic properties of the prepared samples were studied by MPMS XL-5 (Magnetic properties measuring system, SQUID magnetometer), which supplied magnetic fields with maximum intensity $\mu_0 H = 5$ T at temperature 290 K.

In vitro cytotoxicity of MFs was investigated on mouse melanoma cells B16 and Chinese hamster lung fibroblast cells V79 by colorimetric cell viability MTT assay. Cells were cultivated in DMEM supplemented with 10% fetal bovine serum, glucose (4 g/L), L-glutamine, penicillin (100 units/mL), and streptomycin (100 mg/mL) in a humidified atmosphere of 95% air and 5% CO_2 at 37 °C in sterile tissue culture dishes. Cells were treated with indicated 4–6 dilutions of MFs or buffer (untreated cells) for 24 h. MTT_{50} parameter for every tested sample is expressed as represents the Fe_3O_4 concentration (μg/mL) that reduces absorbance of MTT salt in tested cells by 50% compared to control untreated cells.