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Biological evaluation of surface-modified magnetic nanoparticles as a platform for colon cancer cell theranostics

Maksym Moskvin[a], Michal Babič[a], Salette Reis[b], M. Margarida Cruz[c], Liliana P. Ferreira[c,d], Maria Deus Carvalho[e], Sofia A. Costa Lima[b,*], Daniel Horák[a,*]

[a] Institute of Macromolecular Chemistry, Academy of Sciences of the Czech Republic, Heyrovského Sq. 2, 162 06 Prague 6, Czech Republic
[b] UCIBIO-REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira, 228, 4050-313 Porto, Portugal
[c] BioISI, Biosystems and Integrative Sciences, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal
[d] Department of Physics, University of Coimbra, 3004-516 Coimbra, Portugal
[e] CQB, Centro de Química e Bioquímica, Faculdade de Ciências, Universidade de Lisboa, Campo Grande, 1749-016 Lisboa, Portugal

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ABSTRACT

Magnetic nanoparticles offer multiple possibilities for biomedical applications. Besides their physicochemical properties, nanoparticle-cellular interactions are determinant for biological safety. In this work, magnetic nanoparticles were synthesized by one-shot precipitation or two-step reaction and coated with biocompatible polymers, such as poly(L-lysine) and poly(*N*,*N*-dimethylacrylamide-*co*-acrylic acid), and carbohydrates, like L-ascorbic acid, D-galactose, D-mannose, and sucrose. The resulting magnetic nanoparticles were characterized by dynamic light scattering, FT-Raman spectroscopy, transmission electron microscopy, SQUID magnetometry, and Mössbauer spectroscopy. Ability of the nanoparticles to be used in theranostic applications was also evaluated, showing that coating with biocompatible polymers increased the heating efficiency. Nanoparticles synthesized by one-shot precipitation were 50% larger (~13 nm) than those obtained by a two-step reaction (~8 nm). Magnetic nanoparticles at concentrations up to 500 μg mL^{-1} were non-cytotoxic to L929 fibroblasts. Particles synthesized by one-shot precipitation had little effect on viability, cell cycle and apoptosis of the three human colon cancer cell lines used: Caco-2, HT-29, and SW-480. At the same concentration (500 μg mL^{-1}), magnetic particles prepared by a two-step reaction reduced colon cancer cell viability by 20%, affecting cell cycle and inducing cell apoptosis. Uptake of surface-coated magnetic nanoparticles by colon cancer cells was dependent on particle synthesis, surface coating and incubation time.

1. Introduction

Magnetic nanoparticles, due to their magnetic field-responsive properties, have attracted considerable attention for several biomedical applications, such as biosensors, drug delivery systems, hyperthermia (cancer therapy), and magnetic resonance imaging (MRI) [1,2]. These applications require intravenous administration of the particles and their guidance to the targeted tissue is done either passively, through the enhanced permeation and retention effect, or actively, by a ligand molecule [3].

Uncoated nanoparticles exhibit inferior colloidal stability and are rapidly removed from the blood circulation through the reticuloendothelial system [4]. Surface modifications can render the magnetic particles biocompatible, colloidally stable [5], and allow drug delivery [6]. Poly(ethylene glycol) (PEG) is commonly used as a non-ionogenic and hydrophilic agent to modify the nanoparticle surface through covalent binding, improving particle biocompatibility, and reducing immunogenicity [7,8]. Also, ionogenic polymers containing amino- or carboxyl groups can be anchored on the particle surface enhancing the particle uptake by cells due to increased surface charge. Optionally, poly- and monosaccharides, including sucrose, D-galactose, D-mannose, and L-ascorbic acid, have been demonstrated to be good particle stabilizers due to complex formation with iron oxides [9,10]. There are many other types of coatings reported, including polyanhydride, dendrimers, citrate, or dimercaptosuccinic acid [11–13].

Though magnetic nanoparticles have proved to be safe in experimental models [14], the development of new modified particles

* Corresponding authors.
E-mail addresses: slima@ff.up.pt (S.A.C. Lima), horak@imc.cas.cz (D. Horák).

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requires the identification of potential risks and toxicity effects as the particle ability to interact with cells remains unknown [15,16]. *In vitro* models are reliable tools to unravel the effects of particles in the human body, allowing to estimate their toxicity and biocompatibility [17]. The high surface/volume ratio of the particles enables cell and tissue accumulation but makes the particles quite reactive [18], which may result in toxicity. Among several factors, magnetic nanoparticle cellular toxicity depends on the particle size, shape, porosity, surface charge, chemical composition, and colloidal stability [19]. Moreover, the particle/cellular interactions and responses differ, depending on the cell type [20–22]. Hence, it is important to analyze the interactions of the nanoparticles with a specific cell line. Magnetic nanoparticles with potential biomedical applications may then proceed towards *in vivo* validation and biodistribution assays.

In the present work, a systematic study was conducted to assess the *in vitro* magnetic nanoparticle toxicity on colon carcinoma cell lines for theranostic applications. The effect of surface coating was evaluated by synthetizing uncoated and polymer- and saccharide-coated γ-Fe_2O_3 nanoparticles. As a polymer coating, poly(L-lysine) (PLL) and poly(*N,N*-dimethylacrylamide-*co*-acrylic acid) (PDM) were selected, while saccharides included sucrose (SC), D-galactose (GA), D-mannose (MAN), and L-ascorbic acid (ASA). The magnetic properties of the nanoparticles were characterized and their ability to operate as nanoheaters for magnetic hyperthermia was evaluated. To characterize the biocompatibility of the nanoparticles obtained by both procedures, cell viability of three mammalian colon cancer cell lines was assayed. The cells included human epithelial colorectal adenocarcinoma Caco-2, HT-29, and SW-480 cells, and mouse fibroblasts (L929). The cells were incubated with the various γ-Fe_2O_3 nanoparticles, and the particle cell uptake kinetics, as well as the effects on cell viability, cell cycle, and apoptosis were quantitatively evaluated. According to our results, the biological effect of the particles was influenced by both synthesis procedure and surface coating. Surface-coated magnetic nanoparticles displayed different behaviour in terms of cell uptake and low toxicity, which is of great interest for further biomedical applications in colon cancer theranostics.

2. Materials and methods

2.1. Materials

$FeCl_2 \cdot 4H_2O$ (98%), $FeCl_3 \cdot 6H_2O$ (98%), L-ascorbic acid (ASA; 98%), poly(L-lysine) hydrobromide (PLL; M_w = 70,000–150,000), *N,N*-dimethylacrylamide (DMA), acrylic acid (AA), propidium iodide (PI), and 2,2′-azobis(2-methylpropionitrile) (AIBN; recrystallized from ethanol) were obtained from Sigma-Aldrich (St. Louis, USA). Sodium hypochlorite was obtained from Bochemie (Bohumín, Czech Republic). Hydrochloric acid (35%), ammonium hydroxide (25%), hydrogen peroxide (30%), tetrahydrofuran, toluene, crystalline sucrose (SC; 98%), and D-galactose (GA; 97%) were obtained from Lach-Ner (Neratovice, Czech Republic). D(+)-mannose (99%) was obtained from Acros Organics (Geel, Belgium). Ultrapure Q-water from a Milli-Q Gradient A10 system (Millipore, Molsheim, France) was used throughout the experiments. All other reagent grade chemicals were purchased from Sigma-Aldrich and used as received.

2.2. Preparation of poly(N,N-dimethylacrylamide-co-acrylic acid)

AA (0.3 g) and DMA (3 g) were dissolved in tetrahydrofuran/toluene mixture (3.5 mL/3.5 mL). After addition of AIBN (10 mg), the mixture was purged with nitrogen for 10 min and the polymerization was started by heating at 70 °C for 8 h. Resulting poly(*N,N*-dimethylacrylamide-*co*-acrylic acid) (PDM) was dissolved in ethanol and precipitated three times in diethyl ether. Dried polymer was dissolved in water (8.8 mg mL^{-1}) and filtered through syringe with poly(vinylidene fluoride) membrane (Millipore; 0.22 μm pores).

2.3. Preparation of γ-Fe_2O_3 nanoparticles and their surface modification

The first set of saccharide-modified particles (Run I/2-4; Table 1), such as SC-, GA-, and ASA-coated nanoparticles, was prepared by one-shot precipitation and modification of the primary γ-Fe_2O_3 colloid (Run I/1) synthesized according to the following protocol. $FeCl_3 \cdot 6H_2O$ (40 mmol) and $FeCl_2 \cdot 4H_2O$ (20 mmol) were dissolved in water (470 mL) and charged in a 500 mL glass reactor equipped with a turbine impeller and the mixture was heated at 70 °C with stirring (600 rpm). 25% NH_4OH (30 mL) was added at once to the mixture, black magnetite (Fe_3O_4) was precipitated and the reaction mixture was heated at 90 °C for 1 h with stirring (600 rpm). After cooling to room temperature (RT), 35% HCl was added to adjust pH to 5–6. Resulting Fe_3O_4 was oxidized to maghemite (γ-Fe_2O_3) by addition of 30% hydrogen peroxide (5 mL) under slow heating from 20 to 90 °C for 1 h with stirring (600 rpm). Finally, the γ-Fe_2O_3 particles were magnetically separated, washed with water (100 mL), and redispersed using a Branson S-450D Sonicator (Danbury, USA; 10 mm sonotrode) at 10% output for 5 min. After triple washing with water, a dispersion (100 mL) containing 45 mg of the particles per mL was obtained. Consequently, aqueous γ-Fe_2O_3 colloid (containing 100 mg of dry γ-Fe_2O_3) was diluted with water to 15 mL, charged in a 25-mL glass reactor equipped with a turbine impeller, and the mixture was heated at 40 °C with stirring (700 rpm). After dropwise addition of aqueous saccharide (40 mg; 5 mL), the reaction continued at RT for 2 h with stirring (500 rpm) under purging with argon. Resulting γ-Fe_2O_3@SC, γ-Fe_2O_3@GA, and γ-Fe_2O_3@ASA nanoparticles were washed with water (30 mL), magnetically separated, and redispersed in water (20 mL) under sonication at 10% output for 3 min to concentration of 4.4 mg of γ-Fe_2O_3 per mL.

The second set of surface-modified γ-Fe_2O_3 nanoparticles (Run II/2-4; Table 1) was prepared from primary γ-Fe_2O_3 colloid (Run II/1) by two-step precipitation method according to the following protocol. Aqueous 0.2 M $FeCl_3$ (100 mL) was mixed with 0.5 M NH_4OH (95 mL; less than an equimolar amount) with sonication (12.7 mm sonotrode) at RT for 2 min to form $Fe(OH)_3$ colloid. Aqueous 0.2 M $FeCl_2$ (50 mL) was then added with sonication and the mixture poured into aqueous 0.5 M NH_4OH (350 mL). The resulting Fe_3O_4 coagulate was left to grow for 15 min, magnetically separated and repeatedly (7–10×) washed (peptized) with water to remove all impurities (including NH_4Cl) remaining after the synthesis. Finally, 0.1 M trisodium citrate (12.5 mL) was added with sonication, and Fe_3O_4 was oxidized by slow addition of 5% sodium hypochlorite solution (10 mL). The above-described washing procedure was repeated to yield the primary γ-Fe_2O_3 colloid that was filtered *via* syringe with mixed cellulose ester membrane (0.45 μm pores). To prepare γ-Fe_2O_3@MAN particles (Table 1), aqueous D-mannose (2 mL; 128 mg mL^{-1}) was added dropwise to the primary γ-Fe_2O_3 colloid (8 mL; 44 mg of iron oxide) with sonication (1 mm sonotrode) for 5 min. Similarly, aqueous PLL (0.2 mL; 1 mg mL^{-1}) and aqueous PDM (5 mL; 8.8 mg mL^{-1}) were added to the same primary colloid (9.8 or 5 mL) to yield γ-Fe_2O_3@PLL and γ-Fe_2O_3@PDM, respectively.