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A direct surface modification of iron oxide nanoparticles with various poly(amino acid)s for use as magnetic resonance probes

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ABSTRACT

Water soluble and biocompatible iron oxide nanoparticles coated with poly(aspartic acid) (PAsp), poly(asparagines) (PAsn), poly(2-hydroxy-ethyl L-aspartamide) (PHEA), and poly-α,β-(*N*-2-dimethylaminoethyl L-aspartamide) (PDMAEA) were prepared by hydrophobic interaction between hydrophobic iron oxide nanoparticles and each amphiphilic poly(amino acid)s graft polymer. The octadecyl side chain grafted poly(succinimide)(PSI-*g*-C_{18}), used as a precursor polymer, was easily aminolyzed with nucleophilic compounds to form various poly(amino acid)s graft polymer (PAsp-*g*-C_{18}, PAsn-*g*-C_{18}, PHEA-*g*-C_{18}, PDMAEA-*g*-C_{18},) and simultaneously stabilize the dispersion of iron oxide nanoparticles in aqueous solution. The diameters of the poly(amino acid)s coated iron oxide nanoparticles (PAIONs) were smaller than 30 nm in aqueous solution, extremely stable in aqueous solutions with a wide range of pH and salt concentrations. Further, all the PAIONs showed excellent MR signal intensities (high r_2 values) and the cellular uptake property of the PAIONs was also evaluated.

1. Introduction

Superparamagnetic iron oxide nanoparticles have received attention in the biomedical field for medical imaging, drug delivery, cell and protein separation, and magnetic cellular labeling [1–3]. In particular, iron oxide nanoparticles (Fe_3O_4 and γ-Fe_2O_3) synthesized by thermal decomposition methods have been widely used because of their high crystallinity and monodispersed size distribution [4–6]. However, the stabilized iron oxide nanoparticles are only soluble in organic solvents due to the hydrophobic alkyl ligands on the nanoparticles surface [7,8]. Surface modification of these nanopartilces is essential to render their surface hydrophilic and maintain the dispersion stable under physiological conditions [9,10]. The size and surface properties of iron oxide nanoparticles are important parameter for an in vivo magnetic resonance imaging (MRI) application. In general, nanoparticles of a hydrodynamic size smaller than 40 nm can escape from the non-specific uptake by a reticular-endothelial system (RES) more easily than those of larger than 40 nm [2,7]. In addition, positively charged nanoparticles would be cleared in the blood circulation in a body by the absorption of plasma proteins (opsonization) [11,12], but negatively charged nanoparticles have a high resistance against opsonization [13] However, positively charged nanoparticles have a higher adsorption affinity at negatively charged cell membranes than negatively charged nanoparticles do [14].

Recently, various methods have been reported for the surface modification of hydrophobically stabilized iron oxide nanoparticles [15–25]. Poly(ethylene glycol)(PEG) are widely used as shell materials because of their attractive advantages such as biocompatibility, diminished non-specific uptake by RES and prolonged blood circulation time [17–23]. However, some studies reported that PEG may possess antigenic, immunogenic properties and accelerated blood clearance phenomenon [26–28].

Biocompatible and biodegradable poly(α-amino acid)s and their derivatives synthesized from poly(succinimide) can be excellent alternative materials and are already widely investigated as drug delivery carriers [29–34] because of their biodegradability by proteloytic enzymes [35,36]. Poly(α-amino acid)s have many attractive properties such as absence of toxicity, antigenicity, and immunogenicity [37]. In particular, carboxylic group of PAsp can attach the small molecules such as drug and targeting moiety. PHEA coated liposome results in significantly prolonged blood circulation times over those of non-coated and PEG coated liposomes [38]. Dimethylaminoethyl group of PDMAEA is widely used as a functional moiety in many polymethacrylate-based drug delivery

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carriers for gene delivery and stimuli-sensitive drug delivery. Recently, our group reported the synthesis and application of poly(amino acid)s derivatives, PHEA, coated iron oxide nanoparticles with hydrodynamic size smaller than 40 nm through dual interaction [39,40]. However, the surface modification method was multistep procedure and could not control the polymer shell of nanoparticles because only PHEA was soluble and succeeded in coating the nanoparticles in the reaction mixture. Thus, a more simplified and shell controllable coating procedure is still desired.

The present study investigates simplified surface modification of iron oxide nanoparticles with different poly(amino acid)s, namely, PAsp-, PAsn-, PHEA-, and PDMAEA. Different poly(amino acid) shell were synthesized from a same precursor polymer, octedecyl side chain grafted poly(succinimide), by reaction with different nucleophilic compounds in aqueous solutions and simultaneously coated the hydrophobic nanoparticles with each amphipihlic poly(amino acid)s via hydrophobic interaction for phase transfer of nanoparticles from organic to aqueous phases. This is a very simple and convenient way to synthesize water soluble and biocompatible iron oxide nanoparticels having different surface properties and a hydrodynamic size smaller than 30 nm.

2. Materials and methods

2.1. Materials

Iron (III) acetylacetonate, benzyl ether, oleic acid (90%), oleylamine (>70%), 1,2-hexadecanediol (90%), L-aspartic acid, mesitylene, sulfolane, aminoethanol, octadecylamine, tetrahydrofuran, sodium hydroxide, ammonium hydroxide were purchased from Sigma–Aldrich and were used as received. Phosphoric acid, dimethyl sulfoxide, and *N*,*N*-dimethylformamide were purchased from Junsei. Dimethyl sulfoxide-d_6 (DMSO-d_6) used in NMR experiments were Sigma–Aldrich products.

2.2. Synthesis of the precursor polymer

The precursor polymer, poly-(succinimide) (PSI), was synthesized via acid-catalyzed polycondensation of L-aspartic acid using phosphoric acid as the catalyst [29–31]. Purified PSI (0.97 g, 10 mmol succinimide unit) was dissolved in water-free DMF (7 mL), followed by aminolysis with 10 mol% of octadecylamine at 70 °C for 25 h. (PSI-*g*-C_{18}). The reaction mixture was precipitated twice in cold ether and dried in vacuo at 50 °C.

2.3. Synthesis of as-synthesized iron oxide nanoparticles

As-synthesized iron oxide nanoparticles were produced by a seed-mediated growth method using 6 nm nanoparticles synthesized using a thermal decomposition method [8] Briefly, to synthesize nanoparticles 6 nm in diameter, iron (III) acetylacetonate (2 mmol), 1,2-hexadecanediol (10 mmol), oleic acid (6 mmol), oleyl-amine (6 mmol), and benzyl ether (20 mL) were mixed in a three-neck round flask under an N_2 atmosphere. Next, the mixture was heated to 200 °C for 2 h and further heated to 300 °C for 1 h under reflux. After the mixture was cooled to room temperature, excess ethanol was used to wash the reactant. Nanoparticles were collected by centrifugation.

2.4. Preparation of poly(amino acid)-coated iron oxide nanoparticles (PAION)

First, 18 mg of PSI graft copolymer (PSI-*g*-C_{18}) and 18 mg iron oxide nanoparticles were dissolved in 2.2 mL of a mixed solvent (THF [tetrahydrofuran]: DMF [*N*,*N*-dimethylformamide] = 4:1,v/v). (the molar ratio between the amounts of PSI-g-C_{18} and nanoparticles was over 300). The solution was constantly stirred for 0.5 h, and the mixture was subsequently added dropwise to 18 mL of aqueous solution of NaOH, NH_4OH, 2-aminoethanol, or *N*,*N*-dimethylethylene-diamine to synthesize the PAsp-, PAsn, PHEA-, or PDMAEA-coated iron oxide nanoparticles, respectively. After 24 h of stirring, the mixture was dialyzed against DDI water to remove mixed organic solvent (THF and DMF), and centrifuged (6000 rpm, 10 min) to remove the uncoated nanoparticles. Finally, PAIONs were selectively collected by centrifugation at 15,000 rpm for 1 h.

2.5. Characterization of poly(amino acid)s coated iron oxide nanoparticles (PAIONs)

Transmission electron microscopy (TEM) was obtained using a Philips CM-200 instrument operating at 200 kV. A solution of poly(amino acid)-coated iron oxide nanoparticles containing 0.1% (w/v) phosphotungstic acid (PTA; a negative stain) was placed on a copper grid covered with a formvar carbon membrane. The grid was exposed to air enough to evaporate the solvent. Nanoparticle sizes were measured by dynamic light scattering instrument (ELS-Z2, particle size analyzer & Zeta potential, Otsuka electronics Co., Ltd., Japan). Fourier transform infrared (FTIR) spectra were recorded using a Spectrum GX & AutoImage instrument (Perkin–Elmer) at room temperature. Spectra were recorded in the range 4000–450 cm^{-1} using KBr pellets. The TGA was carried out on a setsys 16/18 (Setaram, France). We analyzed the iron oxide nanoparticles and PAIONs at a temperature range of 30–1000 °C. The saturation of magnetization was evaluated using a vibrating-sample magnetometer (Lakeshore, model 955287(A)). The atomic weight percentage of Fe in each PAION was measured by inductively-coupled plasma atomic emission spectrometer (ICP-AES, model: Jarrell Ash IRIS-AP, Thermo) for further in vitro study.

2.6. MRI phantom study

All MR imaging experiments were performed using a 4.7 T clinical MRI instrument (Bruker BioSpec 47/40). The parameters were as follows for T_2 relaxivity coefficients: TE = 7.4 ms, TR = 8,000 ms, FOV = 5.5 cm × 5.5 cm, matrix = 128 × 128, slice thickness = 2 mm.

2.7. Cell culture and cytotoxicity test

MDA-MB 231 and KB cell lines were provided from professor Dai-Wu Seol (BioNano Research Institute, Kyungwon University, Korea), and the Korean cell bank, respectively. These cell lines were cultured in Dulbecco's modified Eagle medium (DMEM) with Glutamax-1 supplemented with 10% FBS (Gibco-BRL) and 1% antibiotics/antimycotics (100 units/mL penicillin and streptomycin) and maintained at 37 °C in a humidified atmosphere of 5% CO_2. The cells were passaged at sub-confluence and plated on culture petri dish.

The cells were plated at a concentration of 5×10^4/mL for 24 h prior to the experiment. To test cytotoxicity of the nanoparticles, 10 μL of nanoparticle solution of a predetermined concentration was added into each well and incubated for 24 h, and 48 h. After the indicated time, the 10 μL of MTT solution of 5 mg/mL was also added followed by incubation for 4 h. The formed formazan crystal was dissolved in 100 μL of lysis buffer (DMF 50 v/v%, SDS 20 w/v%, acetic acid, pH 4.7). The absorbance of the sample proportional to the cell viability was measured at 570 nm with a background absorbance at 650 nm.

2.8. Prussian blue staining

2.5×10^4 cells of MDA-MB 231 and KB cell lines were seeded on each well of 8-chamber slide and grown for 24 h. Then the cells