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Synthesis, characterization and MRI application of dextran-coated Fe_3O_4 magnetic nanoparticles

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ABSTRACT

Biocompatible ferrofluid based on dextran-coated Fe_3O_4 magnetic nanoparticles (MNPs) was prepared through a one-step method. In contrast to the conventional co-precipitation method, hydrazine hydrate was added as reducing agent and precipitator in the present investigation. The effects of hydrazine hydrate, the weight ratio of dextran to MNPs and the molecular weight of dextran on the dispersibility of MNPs in water were investigated. Also, the particles size of modified MNP and coating efficiency of dextran on MNPs were measured. In addition, biocompatible ferrofluid was intravenously injected into rabbits, the iron content in blood and organs at different times were measured by atomic absorption spectrometer, and the bio-distribution and the bio-transportation of ferrofluid in organs was examined. Then, the magnetic resonance (MR) images of liver, marrow and lymph were acquired by MRI experiments before and after intravenous injection of ferrofluid. Image analysis revealed that the MR signal intensity of these organs notably decreased after intensified by ferrofluid. However, when there existed tumors in organs, the signal intensity of tumor did not change after injection. From that the tumor can easily be identified, which indicated a potential application of the as-prepared MNP in functional molecular imaging for biomedical research and clinical diagnosis.

1. Introduction

The nano-scale iron oxide such as magnetic nanoparticles (MNPs), mostly magnetite, Fe_3O_4 or maghemite, γ-Fe_2O_3, with diameter of 5–20 nm have attracted much attentions [1–2]. It is possible to fabricate, to characterize and especially to tailor the functional properties of nanoparticles for diagnostic applications in the last decade [3–6]. The Fe_3O_4 MNPs, which are superparamagnetic, non-toxic and biocompatible, have been intensively investigated [7]. Compared to atomic or bulky counterparts [8], nano-sized MNPs have superior physical and chemical properties, therefore, the MNPs can be used in several biomedical applications, such as (a) cellular therapy in cell labeling, separation and purification [9–10]; (b) drug delivery [11]; (c) a contrasting agent in magnetic resonance (MR) imaging [12–14]; (d) localized therapeutic hyperthermia [15]; (e) biosensors [16].

Since the introduction of particular contrast agents in MRI application in 1987, most of the superparamagnetic iron oxide (SPIO) and ultrasmall superparamagnetic iron oxide (USPIO) agents have been modified with dextran and/or other types of polymer coatings to achieve excellent dispersion [17–20]. Fe_3O_4 MNPs possess superparamagnetism which are suitable for MR contrast enhancement by alterations of proton relaxation in the tissue microenvironment [21,22]. During the preparation, storage and application of MR contrast agent, the stability of the colloid is important. However, due to the high ratio of surface to volume and magnetization, Fe_3O_4 MNPs are prone to aggregate in water or tissue fluid which limits the application. To reduce aggregation and enhance the biocompatibility between the Fe_3O_4 MNPs and water or tissue fluid, the coating of polymer onto MNPs surface is indispensable [23–24].

The nanoparticles with narrow size distribution and good dispersion in tissue fluid are important in biomedical applications [25–26]. For the polymer-coated Fe_3O_4 MNPs with the diameter

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larger than 50 nm, the MNPs would be trapped in the liver or spleen, while those smaller than 30 nm would rapidly circulate almost the whole body. Normally, the MNPs are more likely to enter the lymph node systems and would be eliminated slowly. The properties of the coating layer are also important. Hydrophobic surface may enhance the uptake of the MNPs by the liver or spleen, while hydrophilic surface may prolong the circulation in blood and increase the chance to penetrate into marrow and enter lymph finally. Li et al. [27] synthesized biocompatible MNPs with the surface covered by the monocarboxyl-terminated-poly (ethylene glycol) via a one-pot reaction approach. The MR experimental results have indicated that the so-prepared MNPs possessed very good biocompatibility and showed a long blood circulation time. Thünemann et al. [28] prepared superparamagnetic maghemite nanoparticles via a two-step layer-by-layer technique using poly-(ethylene imine) as the first layer and poly(ethylene oxide)-block-poly(glutamic acid) as the second layer. Preliminary MR experimental results showed that the particles caused a strong MR imaging contrast and indicated that the particles were biocompatible.

The newly developed SPIO SHU 555 A (Resovist, Schering, Germany) is made-up of a colloidal sol of iron oxide nanoparticles coated with carboxydextran, which shows promising results in terms of safety [29], detection and characterization of focal liver lesions [30]. The diameters of the particles range between 45 and 60 nm: the larger particles are quickly taken up by Kupffer cells; the smaller ones remain longer in the vessels, displaying blood pool characteristics.

In the present investigation, the dextran-coated Fe_3O_4 ferrofluid was prepared through a one-step method. The effects of hydrazine hydrate, the weight ratio of dextran to Fe_3O_4 and molecular weight of dextran on the dispersion of MNPs in tissue fluid were studied. The particles size of the modified MNPs, the coating efficiency of dextran on MNPs and magnetic properties of MNPs suspensions were investigated. In addition, biocompatible ferrofluid was intravenously injected into rabbits, bio-distribution of ferrofluid in organs and blood was investigated. The MRI of the rabbit liver, marrow and lymph were analyzed after the ferrofluid was injected.

2. Experimental

2.1. Materials

All chemicals used are analytical graded without further purification. Iron(III) chloride hexahydrate ($FeCl_3{\cdot}6H_2O$), iron(II) sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$), aqueous ammonia (25 wt.%), hydrazine hydrate ($N_2H_4{\cdot}H_2O$, 50 wt.%), dextran T3 ($Mw \approx 3000$), T10 ($Mw \approx 10000$), T20 ($Mw \approx 20000$), T40 ($Mw \approx 40000$), nitric acid and hydrogen perchloric acid were all purchased from the SCRC (Sinopharm Chemical Reagent Co., Ltd.). In all experiments, deionized water was used.

2.2. Preparation of dextran ferrofluid

A mixture of dextran and iron(III) chloride hexahydrate dissolved in 30 ml of deionized water was put into a three-neck flask equipped with a mechanical stirrer, and the 0.5 ml hydrazine hydrate was added into the flask. After the mixture was stirred and mixed completely, some sulfate heptahydrate was added. After 15 min, sulfate heptahydrate was dissolved completely, and some dosage of ammonia solution was quickly dropped into the mixture with vigorous stirring under argon protection, followed by slowly dropping additional ammonia solution until the pH of the solution reached 10. Soon afterwards the solution was stirred for an additional 3 h in argon atmosphere at the temperature of 60 °C. The black suspension was cooled and centrifugation at a speed of 7000 rpm for 20 min to separate big particles from the suspension, then the liquid at the top of the separation tube was taken out. Excess ammonia, hydrazine and its leftovers, iron cation and dextran macromolecules were removed by dialysis using a membrane bag with a 50,000 cut-off molecular weight for 24 h. The reaction sketch map is shown in Fig. 1. The MNPs were obtained by drying the as-prepared ferrofluid in vacuum at 60 °C for 24 h.

2.3. Animal model

All studies were conducted in accordance with guidelines for the use and care of laboratory animals. 4 ml of 0.25 mmol Fe/kg body weight was infused in the ear vein of rabbits within 1 min.

2.4. Induction of lymph node reactive hyperplasia model

1.0 ml of yolk latex was infused in the femoral artery of rabbits; repeat it once after 3 or 4 days. Then we got an intumescent popliteal lymph node which is about 1 cm diameter.

2.5. Induction of lymph node tumors metastasis model

VX2 tumor is the poorly differentiated squamous epithelium tumor, which grows fast and is easy to produce lymph nodes metastasis. The rabbit which has the VX2 tumor was anaesthetized with an intraperitoneal injection of sodium pentobarbital (20 mg/kg body weight) through the auricular vein. We took a fresh tissue from the edge of tumor and cut it into piece which is about 1 mm diameter in the physiology brine, then injected 0.5 ml of the suspension into unilateral muscles of rabbit. The popliteal lymph node intumesced in 2 weeks.

2.6. Analytical and characterization methods

The obtained neat and the dextran-coated Fe_3O_4 MNPs were characterized, respectively by X-ray diffraction (XRD) (D/Max-IIIC, Japan) using Cu Kα radiation ($\lambda = 1.5406$ Å). Distances between peaks were compared to the JCDPS 5-0664 of International Center for Diffraction Data to determine crystal structures.

The surface of the Fe_3O_4 and dextran-coated Fe_3O_4 MNPs was observed with a field emission scanning electron microscope (SEM, Hitachi S-570) and the inner morphology of the neat and the dextran-coated Fe_3O_4 MNPs were observed by transmission electron microscopy (TEM, Hitachi H-600-II) with an acceleration voltage of 200 kV. The SEM samples were prepared by dropping dilute suspension of powder on the glass and the surface was coated with a thin gold film under vacuum prior to the microscopy. The TEM samples for analysis were prepared by dropping the dilute suspensions of powders onto the carbon-coated copper grids and let the solvent evaporate.

Surface structure of the samples was characterized by a Nicolet Avatar 360 Fourier transform infrared (FT-IR) spectroscope. Measurements were performed with pressed pellets that were made by using KBr powder as diluent. Each sample (5 mg) was thoroughly mixed and crushed with the 500 mg of KBr using a mortar and pestle. The mixture (80 mg) was placed in a pellet former and was pressurized for 2 min to form the KBr pellet. The FT-IR spectrum was collected between the wave number of 400 and 4000 cm^{-1}.

The stability of dextran-coated MNPs in tissue fluid was characterized using a Hitachi U-2810 UV-vis spectrophotometer.

The size of the particles/aggregates suspended in tissue fluid was determined using Malvern HPPS5001 dynamic light scattering with the scanning range of 0.6–6000 nm. The samples were prepared with dilution and sonicate before measurements.