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Synthesis of carboxyl superparamagnetic ultrasmall iron oxide (USPIO) nanoparticles by a novel flocculation–redispersion process

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ABSTRACT

We report a novel flocculation–redispersion method to synthesize and purify the biocompatible superparamagnetic ultrasmall iron oxide (USPIO) nanoparticles coated with carboxyl dextran derivative. First, USPIO nanoparticles were synthesized and flocculated to form the large clusters through bridging effect of polyvinyl alcohol (PVA) during coprecipitation process. Then the flocculated USPIO was separated and purified from the solution conveniently through magnetic sedimentation. Finally, USPIO in the clusters were released again and well dispersed through electrostatic repelling effect of citric acid with the aid of ultrasonic. The dispersed carboxyl-functionalized USPIO was conjugated with the monoclonal antibodies. And it has been proved that the antibodies anchored on USPIO still retained their bioactivity after the conjugation. These results implied that the USPIO synthesized have good potential as active targeting molecular probe in biomedical application.

1. Introduction

Ultrasmall superparamagnetic iron oxide (USPIO) has been used in magnetic resonance imaging (MRI) as contrast enhancement agent for clinical diagnosis, because it can accelerate the proton dephasing process and significantly enhance T2 relaxation signal contrast between the target tissue and the background, which leads to the better detection of diseases [1–3].

Coupled with targeting biomolecules like monoclonal antibody (Mab), USPIO can act as the active targeting probe for cellular imaging due to its specific targeting and long half-life in blood circulation [4–8]. As active targeting probe, the USPIO must be small enough so as to avoid the phagocytosis of reticuloendothelial system (RES). And its surface must be biocompatible and modified with functional groups, such as carboxyl or amino groups, in order to couple the targeting molecules.

Currently, chemical methods have been widely used to synthesize the magnetic nanoparticles due to their straightforward nature and ability of producing products in large quantity in one pot [9–12]. Among those methods, coprecipitation method was regarded as a conventional soft synthesis method which required less strict experimental conditions and had been used to synthesize commercial superparamagnetic contrast agent in MRI [13–15]. However, after coprecipitation reaction, it was difficult to separate the resulting USPIO from the reaction solution and purification due to its highly watersoluble property and low magnetism. Therefore, the products have to go through many cycles of ultrafiltration and classification to eliminate various impurities instead of being purified simply by magnetic sedimentation [15,16]. Obviously the existing process of manufacturing USPIO seems not only time consuming but low yielding [17].

Here, we report a novel strategy to synthesize and purify USPIO through a two-stage process: flocculation and redispersion. In flocculation stage, the carboxyl dextran-coated USPIO nanoparticles were synthesized and flocculated to form large clusters through the bridging effect of polyvinyl alcohol (PVA) during the coprecipitation, which resulted in the faster and more efficient separation of USPIO from the solution by using magnetic sedimentation. Then, USPIO nanoparticles trapped in the clusters were redispersed in aqueous solution through the electrostatic repelling effect of citric acid with the aid of ultrasonic. The carboxyl groups on the surface of USPIO not only make USPIO more stable in aqueous solution due to the double effects of polymer and electrostatic stabilization, but also facilitate USPIO to couple to bioactive molecules such as monoclonal antibody. In order to test the effectiveness of the synthesized USPIO as molecular probe, the monoclonal antibody (rhuMabHER2) was conjugated with USPIO and its bioactivity was measured.

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2. Experimental

2.1. Materials

Ferric chloride hydrate ($FeCl_3 \cdot 6H_2O$), ferrous sulfate hydrate ($FeSO_4 \cdot 7H_2O$), citric acid, ammonia (25%) and polyvinyl alcohol (M.W. 1750 ± 50) were purchased from Shanghai Reagent Company of China, carbodiimide and N-Hydroxysuccinimide (NHS) were purchased from Shanghai Medpep Inc, and all the reagents were used as received. The carboxyl dextran was synthesized as the previously reported procedure [13]. Both the D2F2/E2 cell line overexpressing HER2 antigen and the monoclonal antibody against HER2 antigen, rhuMabHER2, were provided by the Second Military Medical University of China.

2.2. Synthesis of USPIO coated with carboxyl dextran

The synthetic route of USPIO was divided into two stages. First, USPIO coated with carboxyl dextran was synthesized and flocculated into large clusters with the assistance of PVA in the coprecipitation process. The resulting clusters were separated from the solution and washed several times with deionized water with the help of the magnetic field (ca. 200 mT). Second, the clustered USPIO nanoparticles were dispersed again in deionized water through the electrostatic repelling effect of citric acid with the aid of ultrasonic (500 W). In a typical synthesis, 1.9 g $FeCl_3 \cdot 6H_2O$, 1.1 g $FeSO_4 \cdot 7H_2O$ and 1 g carboxyl dextran were dissolved into 15 ml deionized water and stirred under a flow of nitrogen. A 0.1 g PVA was dissolved into 10 ml deionized water and then mixed with the above solution. Under continuous stirring, the pH value of the mixed solution was gradually raised from 6 to 11 by adding 7% ammonia solution drop by drop. After the black iron oxide particles precipitated, the slurry was stirred for another 10 min and then incubated at 85 °C for 60 min. Then the product was deposited magnetically and washed several times with deionized water to eliminate impurities. Then the sediment was mixed with 20 ml 5 wt% citric acid solution and the mixture was ultrasonized for 10 min. Finally, the product was dialyzed in 1 l deionized water for 48 h to eliminate the excess citric acid and then stored at 4 °C.

2.3. Bioconjugation of the monoclonal antibody (rhuMabHER2) with USPIO

First, 30 mg carbodiimide and 18 mg NHS were dissolved in 3 ml phosphate buffer solution (PBS, pH = 6). And 1 ml USPIO aqueous solution (0.02 mol Fe/L) was added into the above solution and activated at room temperature for 30 min. The product passed through PD-10 desalting column to remove the excess salts and then was suspended again in 3 ml 10 mM phosphate buffer solution (pH = 7.4). Second, 300 μg rhuMab-HER2 dispersed in 60 μl phosphate buffer solution (pH = 7.4) was immediately added into the activated USPIO suspension and then agitated for 2 h at room temperature. Third, the product was separated by magnetic separate column (Miltenyi) and washed several times with deionized water to eliminate the uncoupled antibodies. Finally, the USPIO–Mab composites were suspended again with phosphate buffer solution (pH 7.4) and stored at 4 °C.

2.4. Flow cytometry (FACS) and laser scanning confocal microscope (confocal) analysis

D2F2/E2 cells that overexpressed HER2 antigens were incubated with the USPIO–Mab composites. Then the cells were separated by centrifuge and washed several time to remove the USPIO–Mab composites that adsorbed physically on the cell's surface. Finally, the cells were characterized by flow cytometry and laser scanning confocal microscope, respectively.

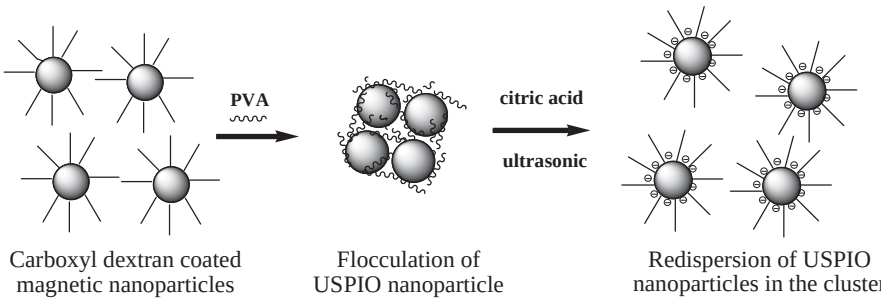
2.5. Characterization

The morphology, size and structure of the USPIO were determined by JEM 1020 transmission electron microscope (TEM) and JEM 2100F high-resolution electron microscope (HRTEM). X-ray diffraction (XRD) was recorded on a Rigaku Dmax-r C X-ray diffractometer by using CuKα radiation ($\lambda = 1.540$ Å) at 40 kV and 100 mA. Hydrodynamic size of USPIO was measured with high-performance particle sizer (HPPS, Malvern Instrument). Fourier transform infrared spectroscopy (FTIR) was collected on Bruker EQUINOX 55. The concentration of carboxyl group on the surface of USPIO was measured by conductometric titration (Shanghai S.R.D Scientific Instrument). Thermogravimetric analysis (TGA) was carried out for USPIO powder samples (~5 mg) with a heating rate of 10 °C/min by using a DTA1600 thermogravimetric analyzer (TA) under N_2 atmosphere up to 800 °C.

The bioactivity of the monoclonal antibody coupled to the USPIO was characterized by flow cytometer (BD FACS Calibur) and fluorescence confocal microscope (Leica SP2 Confocal System).

3. Results and discussion

The synthetic route of the USPIO coated with carboxyl dextran was illustrated in Scheme 1. Polyvinyl alcohol was firstly mixed with ferric, ferrous salts and carboxyl dextran solution. As an ammonia solution was added into the mixed solution gradually, the burst nucleation happened promptly and generated a large amount of iron oxide nanoparticles in the solution. Meanwhile, the carboxyl dextran molecules were covalently bonded on the surface of the nanoparticles through the interaction between carboxyl groups and iron ions, which made the nanoparticles highly watersoluble in aqueous solution. However, due to the



Scheme 1. The main process of the flocculation–redispersion precipitation method.