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Preparation of immunomagnetic nanoparticles and their application in the separation of mouse CD34$^+$ hematopoietic stem cells

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ABSTRACT

The magnetic nanoparticles with a diameter of about 60 nm were synthesized by coprecipitation from ferrous and ferric iron solutions and coated with silica. Then the nanoparticles were modified with *N*-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AEAPS) in order to immobilize anti-CD34$^+$ monoclonal antibodies to the surface of modified magnetic particles. The results of transmission electron microscope (TEM) and Fourier transformed infrared (FT-IR) indicated that the nanoparticles were successfully prepared. Scanning electron microscope (SEM) photo confirmed that the mouse CD34$^+$ cells (cells expressing CD34) were separated by the immunomagnetic nanoparticles. The viability of the separated cells was studied by hematopoietic colony-forming assay, the result of which showed that the target cells still had an ability of proliferation and differentiation. The application of the separated CD34$^+$ cells was in testing the pharmacological effect of three samples isolated from enzyme-digested traditional Chinese medicine Colla corii asini.

1. Introduction

In the last few decades, nanobiotechnology has developed to such an extent that it has become possible to fabricate, characterize, and specially tailor the functional properties of nanoparticles for biomedical applications and diagnostics [1–4]. The application of small iron oxide particles *in vitro* experiments and diagnostics has been practised for nearly 40 years [5]. Recently, increased investigations with several types of iron oxides have been carried out in the field of nanosized magnetic particles among which magnetite is a very promising candidate since its biocompatibility has already proven [6]. The unique feature of magnetic nanoparticles is their response to a remote magnetic field [7]. They become magnetized only in a magnetic field and lose their magnetization when the field is removed. Based on their unique physical, chemical, thermal, and mechanical properties, magnetic nanoparticles have taken precedence in several fields, including biotechnology, material sciences [8], and offered a high potential for biomedical applications, such as cell separation [9], RNA and DNA purifications [10,11], drug delivery, and magnetic resonance imaging (MRI) [12].

Hematopoietic stem cells (HSCs) are defined as cells that are capable of both self-renewal multilineage reconstitution of the hematopoietic system [13]. HSCs have the ability to differentiate into progenitor cells and mature blood cells of all hematopoietic lineages [14]. CD34 was the first differentiation marker to be recognized on primitive human hematopoietic cells [15] and is the most common marker used to obtain enriched populations of human HSCs and progenitors for research or clinical use. CD34 is expressed on approximately 1–4% of the nucleated cells in normal human bone marrow (BM) and on $<0.1\%$ of the nucleated cells in steady-state human peripheral blood [16–18]. Therefore, CD34$^+$ is the most widely used marker protein of HSCs. Because of homology between human and mouse CD34, it is significant to isolate mouse CD34$^+$ cells for experimental study. Much progress has been made in both isolating these cells and in developing assays to characterize them.

Nowadays, there are several methods used for isolation of cells, including antibody panning selection, high-speed fluorescence-activated cell sorting, and immunoadsorption column and immunomagnetic selection [15]. Among these methods, immunomagnetic separation has several advantages in comparison with other techniques. It permits the target cells to be isolated directly from crude samples and is relatively simple, cheap, and fast and in a way may be considered a sample enrichment step for further analysis [19]. Herein, we report a new immunomagnetic nanoparticle used efficiently to isolate mouse CD34$^+$ cells.

2. Experimental materials and procedure

Ferric chloride hexahydrate ($FeCl_3$–$6H_2O$), ferrous chloride heptahydrate ($FeCl_2$–$7H_2O$), ammonium hydroxide (28%, w/w), sodium hydrate, and tetraethyl orthosilicate (TEOS) were purchased

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from the Sino-Pharm chemical reagent Ltd. (Shanghai, China). N-(2-aminoethyl)-3-aminopropyltrimethoxysilane (AEAPS) and glutaraldehyde were purchased from J&K Chemical Ltd. (Beijing, China). RPMI-1640 and IMDM medium were purchased from Invitrogen Corporation (USA). The rat anti-mouse monoclonal antibody against $CD34^+$ cell surface antigen was obtained from Biomeda Corporation (USA). Pepsin and trypsin were purchased from Sigma-Aldrich Corporation (USA). All the chemicals, used in the experiment, were commercial available and were of analytical reagent grade.

The immunomagnetic nanoparticles were synthesized through 3 steps. All of the chemical reagents used in the experiment were analytical grade without further purification. The first step is synthesis of silica-coated magnetic nanoparticles. The silica-coated γ-Fe_2O_3 magnetic nanoparticles were prepared using the reverse microemulsions method as previously described in Ref. [20]. In particular, we adopted the water-in-oil reverse microemulsions of water/Triton X-100/*n*-hexylalcohol/cyclo-hexane. 0.1 g γ-Fe_2O_3 was dispersed in the 60 ml above microemulsion with 3-min ultrasonication, then the solution was poured into three-necked flask with vigorous stirring at 22 °C. Concentrated ammonia and tetraethoxysilane (TEOS) were added dropwise into the system in turn. The reaction ended after 10 h. The nanoparticles were aged overnight and then washed with ethanol three times. At last, the silica-coated γ-Fe_2O_3 nanoparticles were got after sintered at 650 °C for 2 h. The second procedure is modification of silica-coated superparamagnetic nanoparticles with AEAPS. γ-Fe_2O_3 nanoparticles were dispersed in the mixture of methanol and glycerol with 30-min ultrasonication. Then AEAPS was dispersed in the above mixture by vigorous stirring. The above two reactions were carried out for 6 h at 60 °C. The modified nanoparticles were washed with methanol and double-distilled water for three times and finally dried in the vacuum oven. The final step is with connection of silica-coated superparamagnetic nanoparticles with antibody. The typical procedure is that, we activated the nanoparticles by the glutaraldehyde method in a reported way [21,22]. Then the activated nanoparticles were dispersed in the phosphate buffered saline (PBS, 0.1 M, pH 7.4). The rat anti-mouse monoclonal antibody against $CD34^+$ cell surface antigen was added into the above PBS solution. The antigen–antibody reaction was incubated at 4 °C for 2 h. The final antibody-immobilized maghemite nanoparticles were washed with PBS three times and redispersed in it with concentration of 0.5 mg/ml.

Bone marrow was obtained from femurs as described previously [23]. Murine $CD34^+$ hematopoietic stem cells were isolated with antibody-immobilized maghemite nanoparticles. Briefly, 200 ml of above particles were added into the cell suspension including 1×10^6 cells. The mixture was incubated at 4 °C for 30 min with continuous mixing. Then, the cells were magnetically separated by using a magnet for 5 min and resuspended in 1 ml RPMI-1640 cell culture media. Hematopoietic colony assay was referred to Ref. [23].

The sample of the silica-coated magnetic nanoparticles was examined with a Hitachi-600 transmission electron microscope (TEM) and Fourier transformed infrared (FT-IR). The FT-IR spectrum was recorded in the transmission mode on a Nicolet Avatar 370 spectrometer. The magnetic particles were dispersed in the KBr and made into the solid slice for IR characterization. The spectrum was taken from 4000 to 400 cm^{-1}. Scanning electron microscopy (SEM) was used to examine cell morphology and hematopoietic colony assay was adopted to evaluate the ability of the isolated cells to proliferate. The colony-forming data were normalised to the percentage of the control and analysed by the Student *t*-test.

The fractions A–C were obtained from enzyme-digested Colla corii asini as described previously [24]. The three samples were lyophilized and stored at −20 °C until used.

3. Results and discussion

Magnetic iron oxide particles have hydrophobic surfaces with a large surface area to volume ratio, in the absence of any surface coating. Due to hydrophobic interactions between the particles, these particles agglomerate and form large clusters, resulting in increased particle size. When two large-particle clusters approach one another, each of them comes into the magnetic field of the neighbour. Since particles are attracted magnetically, surface modification is often indispensable. In our work, the γ-Fe_2O_3 nanoparticles were coated with silica. These coatings provide not only the stability to the nanoparticles in solution but also help in binding the various biological ligands at the nanoparticle surface for various biomedical applications. The TEM method was also taken to characterize the coating result. Figs. 1b and a were the TEM micrograph of γ-Fe_2O_3 with and without silica coating. Compared with Fig. 1a, Fig. 1b showed that the coated particles were with uniform size and the average diameter of the particles was about 60 nm. Specifically, the diameter is 60 ± 5 nm.

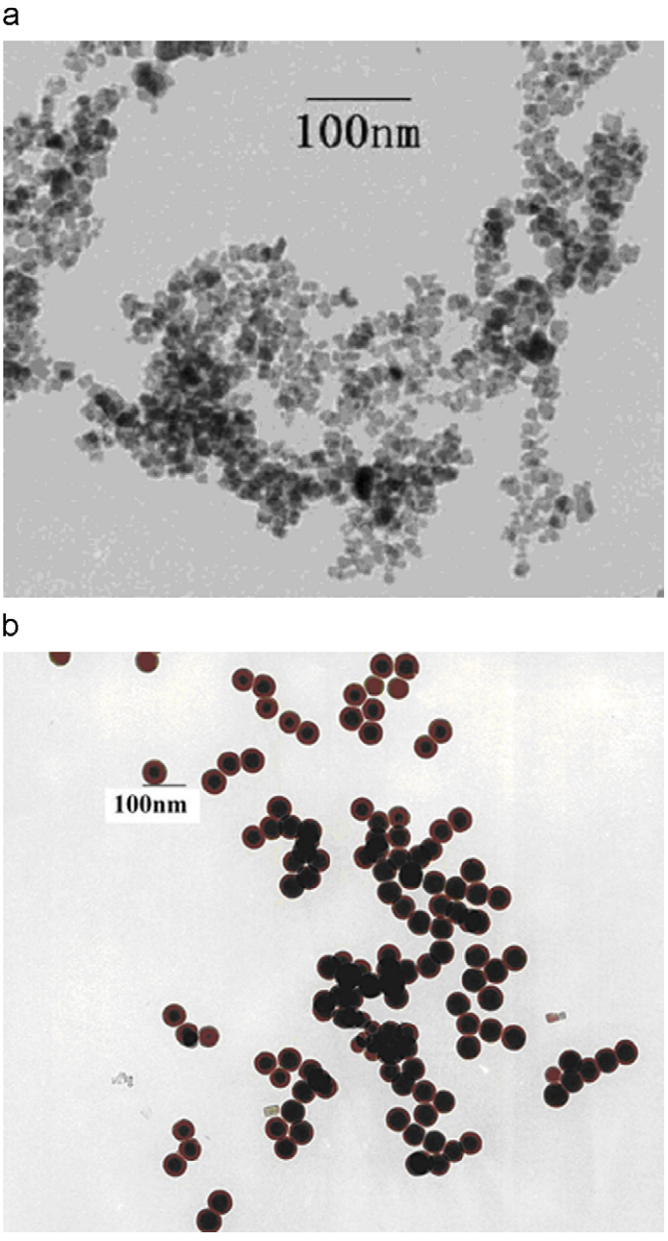

Fig. 1. TEM photos of (a) γ-Fe_2O_3 and (b) silica-coated γ-Fe_2O_3 nanoparticles.