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The labeling of stem cells by superparamagnetic iron oxide nanoparticles modified with PEG/PVP or PEG/PEI

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ABSTRACT

Poly(ethylene glycol) (PEG) and poly(vinyl pyrrolidone) (PVP) co-modified superparamagnetic iron oxide nanoparticles (SPIONs) (PEG/PVP-SPIONs), and PEG and poly(ethylene imine) (PEI) co-modified SPIONs (PEG/PEI-SPIONs) synthesized by thermal decomposition have been used as magnetic resonance imaging (MRI) contrast agents to label adipose-derived stem cells (ADSCs). Efficient cell labeling was achieved after incubation with PEG/PVP-SPIONs and PEG/PEI-SPIONs for 12 h, and the MRI of labeled cells was evaluated. The cell viability tests showed the low cytotoxicity of PEG/PVP-SPIONs and PEG/PEI-SPIONs. The cellular iron content incubated with PEG/PVP-SPIONs at a concentration of 25 μg/ml was 6.96 pg/cell, the cellular iron contents incubated with PEG/PEI-SPIONs at concentrations of 12 and 25 μg/ml were 20.16, 35.4 pg/cell, respectively. The SPIONs were located predominantly in the intracellular vesicles. The cellular iron oxide uptake was significantly high after incubation with PEG/PEI-SPIONs as compared with the commercial iron oxide agents (Feridex, Feridex@PLL, Resovist and Resovist@PLL) reported. This work demonstrates that PEG/PEI-SPIONs are the competent agents for the labeling of ADSCs.

1. Introduction

Adipose-derived stem cells (ADSCs) have attracted much attention because of their advantages such as easy acquisition from sufficient adipose tissue with a little harm to patients and easier induction of differentiation and neurogenesis [1]. An important approach leading to successful stem cell therapy is cell tracking, where a nontoxic, biocompatible, efficient and highly sensitive exogenous cell marker is required [2]. Magnetic resonance imaging (MRI) is considered as an emerging detection technique for cell tracking experiments to evaluate the fate of transplanted stem cells [3]. MRI tracking of stem cells requires labeling of the cells with contrast agents which allow them to be distinguished from the background. Various commercial contrast agents are usually designed for clinical diagnosis of diseases, not specifically for stem cell tracking [4]. To label cells efficiently surface engineering of contrast agents is needed [5]. Superparamagnetic iron oxide nanoparticles (SPIONs) have been widely used as T_2 contrast agents for MRI. Surface modification of SPIONs by polymers, proteins or other molecules is usually performed to offer these magnetic nanoparticles physiological stability and biocompatibility [5–7]. Coupling nonviral transfection agents with iron oxide nanoparticles such as poly(ethylene imine) (PEI) with a high density of positive charges facilitate high transfection efficiency [8–9]. PEI is a typical water-soluble cationic polymer and has been shown to be able to interact with the negatively charged cell membranes and internalize itself into the cell through endocytosis [10–11]. However, the large positive charge that makes PEI so efficient is also highly toxic to cells through disruption of the cellular membrane. Combining poly(ethylene glycol) (PEG) with PEI to create a PEI-PEG copolymer has been shown to lessen PEI's toxicity [12]. Furthermore, PEG is a water soluble polymer and has been widely used to enhance the water solubility of hydrophobic inorganic nanoparticles [13–14]. PEG was used as the solvent, reducing agent, and modifying agent in the synthesis of SPIONs by the thermal decomposition of $Fe(acac)_3$ [15]. Combining poly(vinyl pyrrolidone) (PVP) with PEG offers the SPIONs high steric hindrance, therefore high colloidal stability [16]. Although iron oxide nanoparticles have been used as contrast agents to track transplanted stem cells using noninvasive MRI in recent years, however, stem cell uptake of iron oxide nanoparticles is inherently low in the absence of chemical/biological uptake enhancing strategies such as cell targeting peptides and transfection agents, a high internalization efficiency of the particles is required without compromising cell

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function. The use of poly-L-lysine complexed with iron oxide nanoparticles reported a high labeling efficiency of stem cells (80%) [17], but the use of transfection agents to label cells might have a harmful side effect. The study to label human neural stem cells with commercial iron oxide nanoparticles coated with dextran with core diameters of 50, 100 and 250 nm in the absence of transfection agents showed that iron oxide nanoparticles used did not affect cell viability, cell morphology, cell differentiation or cell cycle dynamics. Both the size of the particle and the presence of the cationic agent poly-L-lysine significantly influence the time needed to label the cells, but the presence of poly-L-lysine also produced a significant decrease in cell viability. Iron oxide particles with smaller sizes were the most appropriate to label stem cells at short time [18].

As stem cell uptake of iron oxide nanoparticles is greatly influenced by cell targeting transfection agents. In this work we investigated the labeling efficiency of stem cells by SPIONs modified with or without the transfection agent PEI. The SPIONs were synthesized with a facile one-pot reaction strategy via the thermal decomposition of $Fe(acac)_3$ in PEG containing PVP or PEI [15–16,19–21]. ADSCs were labeled through co-culture with PEG/PVP-SPIONs and PEG/PEI-SPIONs to evaluate the effect of PEG/PVP-SPIONs and PEG/PEI-SPIONs on the ADSCs labeling efficiency, the viability of ADSCs labeled was investigated as well.

2. Materials and methods

2.1. ADSCs

The ADSCs were isolated from fat in the groins of SD-rats within four weeks of age, then were cultured and passaged in vitro. Animal procedures were in agreement with the guidelines of the Institutional Animal Care and Use Committee. The cells were passaged routinely into 3rd generation, developing into homogeneous ADSCs, which were capable of undergoing neuronal differentiation. ADSCs were identified and characterized by positive staining for CD29 and CD44, but negative for CD45 and CD106 by flow cytometry (BD FASCanto TM, America).

2.2. Prussian blue staining

ADSCs were seeded in poly-D-lysine coated 6 well plates and were incubated in cell-culture medium (CCM) with PEG/PVP-SPIONs and PEG/PEI-SPIONs for 6 h, 12 h, 24 h and 48 h at 37 °C in a humidity atmosphere with 5% CO_2, the concentrations of these SPIONs in CCM were 0, 6, 12, 25, 50, and 100 μg/ml of Fe. After the incubation the ADSCs were fixed with 4% paraformaldehyde for 10 min, washed with phosphate buffer solution (PBS, pH 7.2) and then incubated with Pearls reagent (3% potassium ferrocyanide and 2% HCl) for 20 min at room temperature under agitation. The cells were rinsed thoroughly with PBS, and counterstained with neutral red for 40 s, finally, after being rinsed twice with PBS, the cells were detected by an inverted light microscope (Leica-DMLRB, Germany).

2.3. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay

ADSCs were seeded in poly-D-lysine coated 6 well plates and incubated with an increasing concentration of SPIONs (0–50 μg/ml) for 12 h and 24 h at 37 °C in a humidity atmosphere with 5% CO_2. The cells were rinsed with PBS (pH 7.2). MTT-reagent (1 mg/ml) was added for 3 h incubation at 37 °C. The medium was removed again and the cells were lysed with dimethyl sulfoxide (DMSO). Absorption was measured at 570 nm wavelength with a Bio-tek microplate reader. The viability was calculated with respect to the control cells which were assumed to have 100% viability.

2.4. Trypan blue counting

ADSCs were seeded into 6 well plates and incubated with SPIONs (0–50 μg/ml) for 6 h, 12 h, 24 h and 48 h at 37 °C in a humidified atmosphere with 5% CO_2. After incubation the medium was removed and the cells were washed with PBS (pH 7.2). Cell counting was carried out using a Countess™ automated cell counter. The cells were detached using trypsin and resuspended in 1 ml fresh medium. Trypan blue was added to 100 μl in equal volume and incubated at room temperature for 5 min. The viable cells were counted using a hemocytometer. The cell viability with respect to the control well was calculated whereby the control well was assumed to have 100% viability.

2.5. Magnetic resonance imaging (MRI) of labeled cells

T_2-weighted turbo spin echo MRI was performed in a 3.0 T MR system (Magneto Trio Tim, Siemens, Germany) and sequence was acquired using the following image parameters: TR: 20,000 ms; TE: 85 ms; matrix: 318 × 448; field of view: 220 × 220 mm; slice thickness: 1.5 mm.

2.6. Cellular iron contents

The iron contents of labeling cells were assessed by Inductively Coupled Plasma-Atomic Emission Spectrometry (ICP-AES, ThermoFisher Scientific). Before the ICP-AES measurements of iron contents of the cells, the ADSCs were rinsed three times with PBS (pH 7.2), 500 μl concentrated nitric acid (65–68%) was added to the 10^6 cells in a glass tube, and heated at 120 °C for 3 h in an autoclave for cell lysis, then the solution was diluted to 6 ml for ICP-AES test.

2.7. Transmission electron microscopy (TEM) of ADSCs

The ADSCs were collected and then fixed with 2.5% glutaraldehyde and 0.1 M sodium cacodylate for 1 h. The sample was then rinsed with sodium cacodylate, followed by a second fixing with 1% osmium tetroxide for 1 h. The sample was dehydrated by rinsing with 50%, 70%, 95%, and 100% ethanol. The samples were impregnated overnight in a 1:1 mixture of acetone and Epon 812 at room temperature. Then the samples were embedded in Epon 812 resin at 60 °C. The embedded samples were cut in sections of 50–70 nm, using a Leica EM UC6 microtome (Leica, Groot Bijgaarden, Belgium). Sections were then transferred to a copper grid coated with a carbon film. The samples were lightly stained with 0.5% uranyl acetate and lead citrate solution. TEM analysis was performed with a JEM-1230.

3. Results and discussion

3.1. TEM and XRD characterization of the SPIONs

Fig. 1 shows the TEM images with size distributions of PEG/PVP-SPIONs and PEG/PEI-SPIONs synthesized by thermal decomposition of $Fe(acac)_3$ in PEG containing PVP or PEI at 260 °C [15–16,19–21]. More details about synthesis procedure and characterization results are given in the Supporting Information. The average sizes of PEG/PVP-SPIONs and PEG/PEI-SPIONs are 9.2 ± 1.4 nm and 11.0 ± 1.6 nm, respectively. The XRD patterns of the sample inserted in Fig. 1a and b are matching well with the standard XRD pattern for magnetite (JCPDS File no. 01-088-0315).

3.2. Hydrodynamic sizes, zeta potential and the colloidal stability of the SPIONs

The hydrodynamic sizes and zeta potential of PEG/PVP-SPIONs and PEG/PEI-SPIONs in deionized water (pH ~7.4) were 19.6 and 22.1 nm (Fig. S1a), 0 and 24.6 mV (Fig. S1b), respectively. PEG/PVP-SPIONs and