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Uniformly sized iron oxide nanoparticles for efficient gene delivery to mesenchymal stem cells

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ABSTRACT

Recent advances in nanomaterials have made iron oxide nanoparticles (IONPs) as an innovative approach for the delivery of genes. However, the effectiveness of IONPs-assisted gene delivery is currently suffering from their poor uniformity, which not only exhibits detrimental effect on the magnetic property, but also leads to the poor reproducibility to maintain the optimal gene delivery. To this end, the present study developed extremely uniform 15 nm-sized IONPs with a good monodispersity in water phase. These ultra-uniform IONPs exhibit notable potentials for an efficient gene delivery to human mesenchymal stem cells (hMSCs) with the assistance of magnetic force, which partly owes to their abilities to facilitate the cellular uptake, as well as to induce the rapid DNA release and the following nuclear transport. Besides, no significant detrimental effects of these IONPs are observed either on the proliferation or the multi-lineage differentiation potential of hMSCs. The current study highlights the potential advantages of designing extremely uniform magnetic nanomaterials for an efficient and safe delivery of genes to stem cells.

1. Introduction

Despite the breakthroughs achieved in stem cell-based therapy during the past decades, the application of this promising strategy is still restricted by the current inability to genetically modify stem cells effectively with low risks (Pack et al., 2005). Viral-based methods have demonstrated significant advantages in the high efficiency for delivering genes to stem cells (Ma et al., 2003). Nonetheless, several harmful side effects including potential cytotoxicity, mutagenesis, and immunogenicity are putting the clinical application of this approach at risks (Hacein-Bey-Abina et al., 2003; Luo and Saltzman, 2000; Pack et al., 2005). On the other hand, non-viral delivery methods that rely on biomaterials, including cationic polymers, lipids, and liposomes, have recently attracted considerable interests as a promising alternative option. These biomaterials-relied methods possess advantages in low cost, easy to scale up, and less immunogenic (Godbey and Mikos, 2001; Morille et al., 2008). Yet, their significant lower efficacy in the delivery of genes to primary cells than that of the viral-based methods so far, leading them still far from the medical application (Douglas, 2008; Zhang et al., 2015).

Tremendous advances in magnetic iron oxide nanomaterials over the past decade have provided another potential option for the efficient delivery of genes to stem cells (Park et al., 2014; Pickard et al., 2011; Shah et al., 2013). Thanks to their unique nanoscale magnetic properties (Ling et al., 2015), genes containing in these magnetic nanoparticles will be rapidly pulled toward the target cells by the external magnetic force, thus concentrates the DNA to the cells and results in a highly efficient gene delivery (Ma et al., 2011; Scherer et al., 2002). Such unique mechanism makes the magnetic property of iron oxide nanoparticles (IONPs) essential for an efficient gene delivery. Currently, superparamagnetic iron oxide nanoparticles (SPIONs), which are smaller than 20 nm in the particle size, have been intensively investigated as the potential magnetic materials for gene delivery, owing to their easy synthesis and good stability (Barrow et al., 2015; Zhang et al., 2017). It has been noted that the particle size of SPIONs has significant impacts on the saturation magnetisation, which in turn

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affects the efficiency of gene delivery (Wang et al., 2009). Likewise, the particle size of nanoparticles also exhibits important impacts on cellular uptake (Verma and Stellacci, 2010). An optimized size of SPIONs not only benefits the gene delivery, but also minimizes the cytotoxicity (Adjei et al., 2014). Consequently, synthesis of uniformly sized SPIONs becomes critical for their applications as an efficient and biocompatible material for gene delivery. Nonetheless, most current SPIONs-based agents for magnetofection, such as dextran-coated SPIONs (e.g., Feridex), are suffering from their poor uniformity (Park et al., 2007). The large size variation of SPIONs exhibits significant detrimental effects on the size-dependent magnetic properties (Kwon and Hyeon, 2011; Lee et al., 2015). Besides, the broad size distribution also brings the poor reproducibility, which puts up an obstacle for the scaled-up utilization in clinic (Kwon et al., 2018; Ling and Hyeon, 2013). A typical example is the Feridex, which is usually used as a T2 contrast in magnetic resonance imaging (MRI) and widely investigated in gene delivery. Their withdrawal from American and European markets is believed partly because of their nonuniform size distribution (Li, 2014). In fact, researchers have explored several ways to synthesise SPIONs with uniform sizes over the past two decades (Park et al., 2005; Sun and Zeng, 2002). However, these chemical synthetic methods are typically processing in organic media, preventing the produced nanoparticles from being dispersed in aqueous and biological media (Ling et al., 2015). In addition, the hydrophobic ligands capped on these SPIONs result in a poor biocompatibility, setting another barrier to their applications in gene delivery.

Herein, the present study aims to overcome this sticky situation as mentioned above through a designed synthesis of extremely uniform SPIONs with a good stability in aqueous solution. A “heat-up” method was applied for the size-controlled synthesis of highly uniform SPIONs (Hyeon et al., 2001). To stabilise these hydrophobic nanoparticles in aqueous solution, an ingenious surface modification was then performed to form a corona-like encapsulation on each nanoparticle. This polymer encapsulation further makes the SPIONs in positive charge, which enables the possibility of carrying negative charged genes. In addition, the benefits of highly uniform SPIONs for the optimal gene delivery to human mesenchymal stem cells (hMSCs) and the potential risks of these magnetic nanoparticles were also figured out in the present study. It is expected that this study would highlight the potential advantages of designing extremely uniform magnetic nanomaterials for a powerful and safe delivery of genes to stem cells.

2. Experimental

2.1. Materials

Polycaprolactone (PCL, average Mw = 14,000), oleic acid (OA, 90%), eicosane (99%), branched polyethylenimine (PEI, Mw = 25,000), and nuclear fast red were purchased from Sigma-Aldrich Chemical Co. (St. Louis, USA). Potassium ferrocyanide (99%) was purchased from Aladdin Co. (Shanghai, China). Chloroform, ethanol, concentrated nitric acid and tetrahydrofuran (THF) in analysis pure were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 4% paraformaldehyde was obtained from Wuhan Boster Biological Technology Co., Ltd. (Wuhan, China). 0.25 wt% trypsin with 0.02 wt% ethylenediaminetetraacetic acid (EDTA) was purchased from Gibco BRL (Gaithersberg, USA). FITC-labelled DNA (FITC-DNA) was prepared by Sangon Biotechnology Co. Ltd. (Shanghai, China). Plasmid DNA coding for enhanced green fluorescence protein (eGFP) was purchased from OriGene Co. (Beijing, China). And plasmid DNA coding for luciferase (pGL-3) was kindly gifted by Prof. Yasuhiko Tabata in Kyoto University.

2.2. Cells

Human placental mesenchymal stem cells (hMSCs) are generously gifted from Professor Hongcui Cao (State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, The First Affiliated Hospital, Medical College, Zhejiang University, Hangzhou, China). Cells were incubated in special medium consisted of Human Basal Medium with 10% of Stimulatory Supplements (MesenCult™, Stemcell Technologies Inc., Vancouver, CA) at a humid atmosphere of 37 °C and 5% CO_2.

2.3. Synthesis of uniformly sized SPIONs

For synthesising the uniformly sized SPIONs, the iron oleate complexes were firstly prepared according to the previous study (Park et al., 2004). The acquired iron oleate complexes (e.g. 0.95 g) were then mixed with eicosane (e.g. 5.0 g) and oleic acid (e.g. 0.20 g). After being degassed at 100 °C for 2 h, the solution was heated to 343 °C at the heating rate of 3.3 °C·min^{-1} with vigorous stirring under argon atmosphere. The reaction mixture was kept at this temperature for extra 30 min, followed with a rapid cooling with acetone. Until the mixture solution was cooled down to 60 °C, ethanol was added to wash the solution. The mixture solution was washed thrice with acetone to precipitate the particles with the assistance of centrifugation at 11,000*g* for 10 min. The separated precipitates of SPIONs were re-dispersed in chloroform, and their concentration was determined by inductively coupled plasma mass spectrometry (ICP-MS; XSeries 2, Thermo Fisher Scientific, Bremen, Germany). Additionally, the uniformity of the prepared SPIONs was observed by both transmission electron microscopy (TEM; JEM-2010, Tokyo, Japan) and scanning electron microscopy (SEM; Sirion-100, FEI, Hillsboro, USA).

2.4. Preparation of water-dispersible uniformly sized SPIONs

To convert the above hydrophobic SPIONs into aqueous solution, a hydrophilic surface modification was performed following the method proposed by Kim et al (Kim et al., 2017) with a certain modification. In a typical experiment, SPIONs (11.2 mg) were dried under vacuum overnight before being re-dispersed in a mixture solution (4 mL) composed of chloroform and THF (v/v = 1) with a constant mechanical stirring. Afterwards, PCL (2 mg) and OA (1 mg) were added to the solution. The reaction was carried out at room temperature for 2 h. Then, excessive branched PEI (MW = 25 k) dissolved in another chloroform/THF mixture with same volume (4 mL) was dropped into the reaction mixture with vigorous mechanical stirring. This reaction was further carried out for another 2 h at room temperature. At last, double distilled water (DDW, 20 mL) was added to the mixture to form a milky brownish emulsion with the assistance of sonication at 600 W for 5 min. Rotary evaporation was then applied to remove the organic solvent, thereby obtaining an aqueous solution of surface modified SPIONs. The unused PEI was removed by centrifugation with DDW for three times. To determine the component of the polymer coating layers, a Fourier transform infrared (FTIR) spectra was performed at 20 °C (FT/IR-4100 spectrometer, JASCO, Tokyo, Japan). The dried samples of hydrophilic SPIONs (SPION-W) and hydrophobic SPIONs (SPION-O) were grounded into fine powder with a mortar and pestle prior for measurement. Spectra were determined with a resolution of 1 cm^{-1} and the wavenumber range was from 500 cm^{-1} to 3500 cm^{-1}. In addition, to observe the influence of polymer shell layers on the magnetisation, M-H curves of both SPION-O and SPION-W were performed by a superconducting quantum interference device (SQUID; MPMS-XL, Quantum Design, San Diego, USA).

2.5. Preparation of SPIONs-contained gene complexes

Gene complexes that contained the hydrophilic SPIONs were prepared freshly before their application. Briefly, sterilised SPIONs were diluted by 5% sucrose aqueous solution to an appropriate concentration. The SPIONs-contained solution was then incubated with same volume of pDNA (100 μg·mL^{-1}) for 15 min at room temperature to self-