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Self-assembled magnetic lamellar hydroxyapatite as an efficient nano-vector for gene delivery

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ABSTRACT

Magnetic lamellar hydroxyapatite (ML-HA) nanoparticles were synthesized by a template-assisted self-assembly process. The as-prepared ML-HA nanoparticles self-assembled under different conditions were characterized by XRD, TEM, cytotoxicity assessment, and DNA-loading and transfection efficiency measurements. We found that the structure and morphology of ML-HA were controlled by self-assembly conditions. The ML-HA synthesized in this work exhibited good biocompatibility. The DNA-loading capacity and ζ-potential of ML-HA were much lower in comparison to bare lamellar HA (L-HA) without magnetic nanoparticles. Despite that, the ML-HA with good lamellar structure showed 47% higher transfection efficiency than L-HA. Results suggested that the ordered lamellar structure is a key factor in controlling transfection efficiency and magnetization is an effective way of improving the transfection efficiency of lamellar HA. Mechanisms were proposed to interpret these experimental results. It is demonstrated that the ML-HA may be a promising gene vector to deliver DNA into the cells effectively and safely.

1. Introduction

The development of efficient and safe transport vehicles (carriers) to deliver genes or drugs into cells has long been an exciting area of research. So far, various types of carriers have been developed including viral carriers, organic cationic compounds, recombinant proteins, and inorganic nanoparticles [1,2]. Among these carriers, inorganic materials, such as calcium phosphate, gold, magnetite, quantum dots, calcium phosphate, and layered materials show promise in light of their versatile advantages of easy preparation, wide availability, rich functionality, good biocompatibility, and storage stability [3]. Among these inorganic carriers, layered materials are of particular importance. Two representatives of them, montmorillonite (MMT) and layered double hydroxide (LDH), have received much attention as new types of gene vectors due to their efficient DNA storage and release, effective protection of DNA in the inter-layer, and enhanced thermal stability [4–8]. In addition to these layered materials, newly developed layered hydroxyapatite (L-HA) is of special interest as a new carrier for gene delivery due to its combined advantages in composition (similar composition to that of mammalian bone tissue), biodegradation, biocompatibility, low toxicity, and layered structure [9,10].

It is well documented that rendering nanoparticles with magnetic properties is an important strategy in improving their transfection efficiency [11]. Magnetic nanoparticles can be attracted to and maintained at a precise location by an external magnet, realizing the site-specific delivery of genes and the enhancement of transfection, enhance the action of vectors up to several hundred times and accelerate the gene transfection or transduction process [11,12]. However, magnetic vectors solely built with iron oxide (mostly Fe_3O_4) are of much concern regarding their cytotoxicity [13]. Besides the uncertainties regarding the safety profile of iron oxides, these particles have a poor binding affinity for DNA [11]. Thus, nanoparticles with magnetic coating or hybridizing with magnetic materials have been of much interest [13–15]. In our previous work, L-HA was coated with magnetic Fe_3O_4 nanoparticles using a template-assisted self-assembly process and a novel magnetic L-HA (denoted as ML-HA hereinafter) was produced [10].

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However, the control of structure and morphology of the ML-HA nanoparticles and their transfection efficiency have not been investigated.

Herein, template-assisted self-assembly was used to prepare various ML-HA nanoparticles with varying structure and morphology (size and shape) by regulating the synthesis conditions including template agent dosage and concentration of calcium and phosphate source materials. The aim of the present work was to determine the dependence of structure and morphology on synthesis conditions and preliminarily evaluate the cell cytotoxicity, DNA-loading capacity, and transfection efficiency of these self-assembled ML-HA nanoparticles, by which the synthesis conditions for improved transfection efficiency may be optimized.

2. Materials and methods

2.1. Materials

The materials for the synthesis of ML-HA were used as received without further purification. The materials used in the current work included sodium dodecyl sulphonate (SDS, $C_{12}H_{25}SO_3Na$), calcium nitrate ($Ca(NO_3)_2\cdot 4H_2O$), ammonium hydrogen phosphate ($(NH_4)_2HPO_4$), iron (III) chloride hexahydrate ($FeCl_3\cdot 6H_2O$), iron (II) chloride tetrahydrate ($FeCl_2\cdot 4H_2O$), sodium hydroxide (NaOH), ethanol, and deionized water. The ethanol was of analytical grade provided by Kewei Company of Tianjin University and all other analytical reagents were provided by Acros Organics.

2.2. Preparation of ML-HA

The preparation of ML-HA was carried out as described previously [16]. Briefly, 1.0 g of SDS was mixed with 15 mL of deionized water and 30 mL of ethanol. After the mixture was heated to 60 °C, 15 mL of 3.3 M $Ca(NO_3)_2\cdot 4H_2O$ was added, followed by the addition of 30 mL of 1 M $(NH_4)_2HPO4$ and 30 mL of ethanol. After that, 20 mL of 2.5 M NaOH and 20 mL of ethanol were added. After 0.5 h, 7.5 mL of 2.4 M $FeCl_3\cdot 6H_2O$, 7.5 mL of 1.2 M $FeCl_2\cdot 4H_2O$, 30 mL of 3 M NaOH and 45 mL of ethanol were added to the mixture. After the mixture was refluxed at 85 °C for 14 h, the precipitate was deposited at room temperature for 21 days. The precipitate was centrifuged and immersed in deionized water for 3 days, then centrifuged again and dried at 60 °C to obtain ML-HA-1. Samples ML-HA-2, ML-HA-3, ML-HA-4, and ML-HA-5 were obtained by the same procedures except that the weight of SDS and concentration of $Ca(NO_3)_2\cdot 4H_2O$ and $(NH_4)_2HPO_4$ were adjusted, as listed in Table 1. A non-magnetic bare L-HA with typical lamellar structure, used as the control, was prepared by the same procedure as previously reported [9,17] except that the SDS dosage was 1.0 g.

2.3. Characterizations

X-ray diffraction (XRD) analysis was performed using a Rigaku D/Max 2500 v/pc diffractometer (Rigaku, Japan) with Cu Kα radiation generated at 40 kV and 200 mA. The scanning rate was 4°/min over a range of 10–60° for wide-angle diffraction and scanning rate was 1°/min over a range of 2–10° for small-angle measurement. Transmission electron microscopy (TEM) analysis was conducted to determine the average mean particle size and observe the morphology of HA nanoparticles using a FEI Tecnai G2 F2 TEM operating at an accelerating voltage of 200 kV.

2.4. Cytotoxicity assay

HA nanoparticles were tested for cytotoxicity using a MDA-MB-231 cell line by cell counting kit-8 (CCK8) assay. Briefly, cells at a density of 5000 cells/well were seeded in 96-well cell culture plates and incubated in the Dulbecco's modified eagle's medium (DMEM) supplemented with 10% fetal bovine serum in a humidified atmosphere with 5% CO_2 at 37 °C for 24 h. Afterwards, cells were switched to HA-containing media with various HA concentrations (0, 100, 200, 400, and 800 μg/mL) for another 24 h and 72 h. After the culture media were removed, cells were rinsed with Hank's balanced salt solution (HBSS) twice to remove excess materials, followed by the addition of 90 μL DMEM and 10 μL CCK8 solution for further 3 h incubation. Finally, the media were transferred to a new 96-well cell culture plates and the optical density (O.D.) of each well was read at 450 nm using a microplate reader.

2.5. Synthesis of HA/DNA complexes

The synthesis of HA/DNA complexes followed the similar process reported in our previous study [9]. Briefly, 50 mg of HA was added into a conical flask with 19 mL of sterile deionized water under ultrasonic dispersion for 5 min to ensure complete dispersion of HA in water. Then, plasmid DNA (pDNA), extracted and purified from bacteria and diluted to 1 mg/mL, was added dropwise into the HA suspension under continuous oscillation. Subsequently, the mixed solution was shaken at room temperature for 18 min and followed by 60 min of static absorption. This process was repeated six times and ended with an equilibration for 24 h at room temperature. The resultant complexes were isolated by centrifugation and thoroughly washed with sterile deionized water.

2.6. DNA-loading capacity

The DNA-loading capacity of various HA samples for pDNA refers to the amount of pDNA carried by the HA nanoparticles as compared to the amount used in the loading process. It was determined by measuring the amount of pDNA that was not loaded and remained in the supernatant upon centrifugation of the nanoparticle suspension, by following the method reported by Perez et al. [18]. In a typical process, an appropriate amount of DNA stock solution (1 mg/mL) was added dropwise to diluted HA suspensions with varying concentrations. The mixture was incubated at 37 °C for different times under light agitation, and then centrifuged at 14,000 rpm for 10 min. The supernatants with varying L-HA concentrations were collected. The absorbance values of the supernatants and the initial DNA solution were measured using an ultraviolet spectrophotometer at 260 nm. Then, the concentration of DNA in the supernatants, C_T, and the DNA concentration of the

Table 1
Synthesis conditions (including SDS dosage and concentration of calcium and phosphate source materials) employed to prepare various ML-HA samples and their designation.

Samples	SDS dosage (g)	Concentration of $Ca(NO_3)_2\cdot 4H_2O$ solution (M)	Concentration of $(NH_4)_2HPO_4$ solution (M)
ML-HA-1	1.0	3.3	1
ML-HA-2	2.0	3.3	1
ML-HA-3	0.5	3.3	1
ML-HA-4	1.0	4.95	1.5
ML-HA-5	1.0	1.65	0.5