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Research paper

Biodegradable magnetic calcium phosphate nanoformulation for cancer therapy

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ABSTRACT

We fabricated a magnetic calcium phosphate nanoformulation by the biomineralization of calcium phosphate on the surface of magnetic nanoparticles with abundant amino groups, and thus the inorganic layer of calcium phosphate can improve the biocompatibility and simultaneously the magnetic iron oxide can maintain the magnetic targeting function. Two types of anticancer drug models, doxorubicin hydrochloride and DNA, were entrapped in these nanocarriers, respectively. This delivery system displayed high pH sensitivity in drug-controlled release profile as the dissolution of CaP under acid pH condition. Magnetofection was performed to investigate the intracellular uptake and the anti-proliferative effect of tumor cells in the presence of an external magnet. The transfection of the DNA-loaded magnetic system in A549 and HepG2 tumor cells demonstrated that the magnetic nanoformulation could enhance the transfection efficiency to 30% with an applied external magnetic field.

1. Introduction

The application of nanoparticles in cancer chemotherapy has attracted a great deal of interest because of their small size, which facilitates drug delivery in cancerous tissues by the enhanced permeability and retention (EPR) effect [1,2]. On the basis of the nanoparticle system, the delivery of gene to the targeting cells offers a new strategy to effectively treat the solid tumors. Organic nanoparticles have been widely used for the transfer of DNA into living cells through the electrostatic attraction between the negatively charged DNA and cationic polyelectrolytes, such as polylysine [3,4] and polyethylenimine (PEI) [5–8] or cationic liposomes/micelles modified with cationic surfactants. At the same time, the varieties of inorganic nanocarriers have also been reported for the application in DNA delivery, such as calcium phosphate, carbon nanotubes, silica, gold, magnetite, quantum dots, strontium phosphate, magnesium phosphate, manganese phosphate and double hydroxides [9]. Among the cationic polymers, PEI is often considered as the gold standard of gene transfection [10]. High molecular weight PEI has high gene transfection efficiency but high cytotoxicity. So it is necessary to reduce the cytotoxicity of PEI and improve gene expression in cancer gene therapy.

In addition, how to deliver the small amount of gene effectively to specific cells and thus improve the gene expression is a hot topic in the field of gene delivery. Among the targeting nanocarriers, superparamagnetic iron oxide nanoparticles (SPIONs) generally applied in targeted drug delivery can be guided through the use of an applied magnetic field to tissues or cells, resulting in a decrease in the amount of DNA injected and the time necessary to reach the desired target cells [11].

Currently the surface modification of the magnetic nanoparticles is essential and challenging for endowing them more functions and consequently widening their applications in nanomedicine. In contrast to the bare magnetic nanoparticles, the magnetic nanoparticles with a suitable surface coating show an enhanced performance in biomedical applications [12]. One important approach of modifying the surface is by coating with inert inorganic materials such as silica and Au [13–15]. Here, we choose calcium phosphate as the coating material, for which is a well-known biomaterial constituent of the human bone and teeth [16]. Additionally, calcium phosphate based nanocarriers have also been extensively used in drug, gene or siRNA delivery due to their excellent biocompatibility and non-inflammatory properties [17–19]. The first formulation of calcium phosphate loaded with DNA was reported by Graham and van der Eb in 1973 [20], and they found that there was a strong affinity of calcium phosphate to the phosphate groups of DNA. In our previous study, we developed a multifunctional nanocomposite

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including Fe_3O_4 nanoparticles entrapped with hydroxyapatites through biomineralization [21]. Most of these modifications are referred to the magnetic nanoparticles with negative surface charges [22–24], however, attempts to design a positively charged magnetic calcium phosphate based nanocarriers for multifunctional applications have rarely been studied. As it is well known, it is very important for the vectors with positive charges in binding and compacting DNA with negative charges to enhance the loading efficiency for DNA [25]. The application of the magnetic calcium phosphate nanoformulation is an emerging filed in nanomedicine [26].

In this study, we fabricated a positively charged magnetic nanoformulation through the biomineralization of calcium phosphate on the surface of the superparamagnetic iron oxide nanoparticles with abundant amino groups. The nanocarriers were also employed to load two types of therapeutic agents separately for the investigation of their potential applications in cancer therapy. On the one hand, DNA with negative charges was electrostatically compressed into the magnetic calcium phosphate nanoparticles, on the other hand, the anticancer drug, doxorubicin (DOX·HCl) was loaded with the nanoparticles by a physical adsorption. Such a drug-loaded magnetic CaP nanoparticles showed a pH-dependent drug release behavior and had an equivalent tumor growth inhibition effect under an external magnetic field compared to the free drug. Besides, the flow cytometry showed that this magnetic nanocarrier as DNA delivery system had a high transfection efficiency in A549 and HepG2 cell lines.

2. Experimental section

2.1. Materials

Ferrous chloride tetra-hydrate ($FeCl_2{\cdot}4H_2O$), ferric chloride hexahydrate ($FeCl_3{\cdot}6H_2O$) iron salts, aqueous ammonium hydroxide (25–28%), sodium citrate (CA) and *N*-hydroxysulfosuccinimide (sulfo-NHS) were purchased from Chengdu KeLong Chemical Reagent Company (Sichuan, China). Poly-ethylenimine branched (PEI, M_n = 25,000) was purchased from Aldrich (USA). *N*-(3-dimethylaminopropyl)-*N*′-eth-ylcarbodiimide hydrochloride (EDC) and MES buffer were purchased from Pierce (Rockford, IL, USA). Doxorubicin hydrochloride (DOX·HCl) was used as the model drug obtained from Sigma. The plasmid DNA and the plasmid of green fluorescent protein (GFP) were presented by Sichuan University (China).

2.2. Preparation of SPIONs@PEI-CPs

The SPIONs@PEI nanoparticles were prepared by an amide reaction of SPIONs@CA and PEI solution in 30 mL distilled water upon addition of NHS and EDC at room temperature for 48 h under argon protection, according to the literature [23]. The resultant mixture was collected with a permanent magnet and washed with distilled water at least three times, and finally lyophilized. SPIONs@PEI nanoparticles were immersed in 40 mL simulated body fluid (SBF) with the concentration of 2 times at 37 °C for predesigned days. The products were collected after 3, 5 and 10 days incubation. The resultant samples were collected with a permanent magnet and washed several times with ethanol and distilled water, respectively. Finally, they were freeze-dried and stored at 4 °C for further use.

2.3. Characterization of the nanoformulation

Fourier transform infrared (FT-IR) spectra were performed to identify the chemical structure using a Nicolet Magna 5700 Series II spectrometer. The modified nanoparticles were analyzed for phase composition using X-ray powder diffraction (XRD, Philips, X'Pert PRO, Netherlands) over the 2θ range from 10° to 90° at the rate of 2.5°/min, using Cu Kα radiation (λ = 1.54060 Å) at room temperature. The energy dispersive X-ray (EDX) and the absorption spectroscopy (AAS, HITACHI, Z-5000) were used to determine the composition of the modified particles. The magnetic properties of the resultant Fe_3O_4 nanoparticles were measured with a VSM (Quantum Design) at room temperature. Particle size analysis and distribution were measured by dynamic laser light scattering using a Particle Size Analyzer (ZETA-SIZER, MALVERN Nano-ZS90). The maximum loading amount of DOX in the magnetic nanoparticles was determined by Fluoromax spectrometer (F-7000, HITACH, Japan).

2.4. Stability of SPIONs@PEI-CPs

The stability of the SPIONs@PEI-CPs nanoparticles immersed for 10 days was studied in specific environments by the UV–visible spectrophotometry (UV-2550, Shimadzu, Japan). Measurements were performed in NaCl solutions with different concentrations, at different temperatures from 25 to 41 °C and for various stored days, respectively. Particle size and the concentration of Ca^{2+} were also employed to investigate the stability of the nanoparticles at pH 7.4 and 5.5.

2.5. DOX·HCl loading

The maximum loading amount of DOX·HCl was determined by serial addition of the SPIONs@PEI-CPs nanoparticles (10 days) to DOX·HCl solution using Fluoromax spectrometer (F-7000, HITACH, Japan) at excitation 370 nm and emission recorded in the interval of 500–700 nm. An increasing mass weight of SPIONs@PEI-CPs (0.200, 0.532, 0.870, 1.232, 2.150, 3.843, 5.112, 6.023, 6.513, 7.140 mg) was added into the DOX·HCl solution (0.1 mg in 3 mL of PBS solution). The fluorescence of DOX·HCl quenched gradually when the drug was adsorbed to the surface of the CaP layer.

2.6. Zeta potential of SPIONs@PEI-CPs/DNA composites

Zeta potential of SPIONs@PEI-CPs/DNA composites was evaluated using a Particle Size Analyzer (ZETA-SIZER, MALVERN, Nano-ZS90). The aqueous solutions of SPIONs@PEI-CPs/DNA composites at various N/P ratios (mass ratio of PEI to DNA) were prepared. Before measurement, the composites were incubated at room temperature for 15 min.

2.7. DNA binding assay

The binding ability of DNA with SPIONs@PEI-CPs was determined by agarose gel electrophoresis. A series of composites at predetermined N/P ratios were formed by mixing the plasmid DNA (0.1 mg mL^{-1}) with the SPIONs@PEI-CPs. The mixture was vortexed for 15 s and then kept still at room temperature for 30 min, after which 10 μL mixture solution was loaded onto 1% agarose gel to conduct the agarose gel electrophoresis under 90 V for 30 min. And the naked DNA was used as control.

2.8. DNase I protection assay

SPIONs@PEI-CPs/DNA nanoparticles at various N/P ratios and naked DNA (0.3 μg) were incubated for 15 min at 37 °C with DNase I (1 unit) in DNase/Mg^{2+} digestion buffer consisting of 50 mM Tri–Cl, pH 7.6 and 10 mM $MgCl_2$. The DNase I was then inactivated by adding 4 μL of 250 mM ethylenediaminetetraacetic acid (EDTA) (pH = 8) and incubated for 15 min at room temperature. 2 μL of 1 mg mL^{-1} of sodium heparin was added to release the DNA from the complex nanoparticles for 2 h. The DNA integrity was assessed