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Upconversion luminescence tracking of gene delivery *via* multifunctional nanocapsules

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ABSTRACT

The real-time fluorescence tracking of gene delivery is very important as it helps to figure out how a vector enters a cell and also to follow its fate within the cell interior. Lanthanide-doped upconversion nanoparticles (UCNPs) have shown great potential in biomedical applications in virtue of their unique optical and biological properties. Herein, we report a simple and versatile strategy to fabricate a multifunctional nanocapsule for effective gene delivery and real-time luminescence tracking. The hydrophobic UCNPs were modified by positively charged amphiphilic polymer together with polyethylene glycol-poly(lactic-co-glycolic acid) (PEG–PLGA) polymer, affording biocompatible nanocapsules with high gene loading capacity and good stability. Red UC luminescence of UCNPs are able to track the delivery of nanocapsules in cells without background fluorescence interference, in the meantime, the green fluorescence of green fluorescence protein (GFP) expressed by the pDNA could subtly monitor the gene transfection efficacy. The results demonstrated that our nanocapsule has ideal biocompatibility, satisfactory gene loading capacity and great bioimaging ability, which is promising for imaging guided cell therapy and gene engineering.

1. Instruction

Gene therapy, as one of the greatest discovery in the 21st century, has attracted and is still drawing wide attention [11,17–19,25,48,49]. One of the key requirements for gene therapy is a safe and effective strategy to deliver nucleic acids into the targeted cells, tissues or organs [30,42]. In the meantime, real-time tracking of gene delivery is appealing, since it allows for monitoring detail gene delivery process, which is of crucial importance in *in vivo* gene therapy. Up to now, various types of siRNA-carrier complexes have been explored for effective gene delivery and therapy and could be categorized into two types: viral and non-viral vectors [2,23,29]. Serious immunological problems that might bring about by viral-based vectors greatly limited their applications in clinical therapy [10], while non-viral delivery systems, which are typically based on cationic lipids or polymers, have shown great potentials in terms of their simplicity of surface modification and less induction of immune responses [26,4,47,52].

Polymer-based nanovectors [6] hold many advantages including high structural stability, high encapsulation efficiency, and most importantly, easy modification to achieve multifunctional and targeted delivery, so-called “smart” delivery vehicles. Since most of the therapeutic genes are either non-fluorescent or not able to express fluorescent proteins, traditional fluorescent dyes [22], gold nanostructures [44], and luminescent QDs [50,51] have been employed for construction of multifunctional luminescent nanocarriers. For example, CdSe quantum dots (QDs) were employed by Gao's group for efficiently intracellular delivering siRNA [28]. With regard to *in vivo* biomedical applications, lanthanide-doped upconversion luminescence nanoparticles (UCNPs) have been regarded as promising candidates for simultaneous gene delivery and real-time tracking due to unique and excellent optical properties [31,33], such as long-wavelength excitation and emission, superior chemical stability, and good photostability [14,20,21,3,34,40,46,9], deep tissue penetration, low toxicity for biomedical applications upon NIR (980 nm, low energy) excitation and low autofluorescence [39,41,5,7,9]. Considering all the advantages mentioned above, many groups including ours have explored UCNPs as novel optical nanoprobes in biomedical imaging [32,37,38,5,7,39,41]. With respect to imaging-guided gene delivery, though there are several examples have been reported [12], it remains a vast of possibilities to construct UCNP-based nanocarriers with low cytotoxicity and high gene loading capacity.

Herein we presented a new and facile strategy to fabricate a biofunctional nanocarrier, which was designed for gene delivery and noninvasive UC luminescence imaging tracking (Scheme 1).

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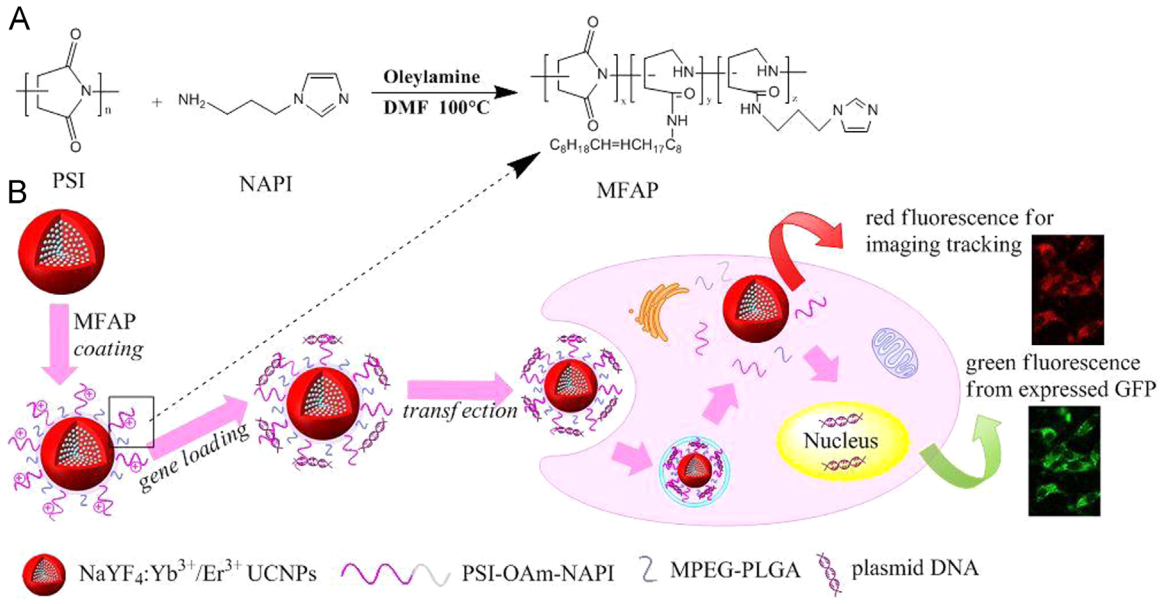

Scheme1. Schematic illustration for the synthesis of amphiphilic polymer (MFAP) (A) and the fabrication of gene nanocapsules, real-time luminescence tracking of gene delivery and monitoring transfection efficiency (B).

We first synthesized a new positively charged amphiphilic polymer by aminolyzing polysuccinimide (PSI) with *N*-(3-aminopropyl) imidazole (NAPI) and oleylamine (OAm). Then newly synthesized multifunctional amphiphilic polymer(MFAP), together with polyethylene glycol-poly (lactic-co-glycolic acid) (PEG–PLGA), was further employed to coat hydrophobic $NaYF_4:Yb^{3+}/Er^{3+}$UCNPs, affording UCNPs@MFAP nanocapsules with high water dispersibility, biocompatibility and low cytotoxicity due to the introduction of PEG moiety. According to previous work [1], PEG–PLGA could reduce nonspecific absorption of plasma proteins, prolong the *in vivo* circulation time and facilitate accumulation targeted tissues [27,43]. Besides, the hydrophobic moiety PLGA could facilitate the encapsulation of hydrophobic UCNPs, which made it easier to form a uniform and stable composite nanovector. As shown in Scheme1B, amphiphilic biocompatible polymer MFAP and PEG–PLGA were self-assembled onto $NaYF_4:Yb^{3+}/Er^{3+}$ UCNPs, endowing the hydrophilic UCNPs@MFAP nanocapsules with positive charge surface. Then negatively charged plasmid DNA (pDNA) was absorbed on the surface of the nanocapsules through electrostatic interaction. The red luminescence of UCNPs would be used as tracking signals for real-time monitoring the location of nanovectors. Meanwhile, the model plasmid DNA encoding green fluorescent protein would serve as an indicator for the efficient gene delivery and successful gene transfection.

2. Material and methods

2.1. Chemicals and materials

N-(3-Aminopropyl) imidazole was purchased from Alfa Aesar Chemical Co. Polysuccinimide (PSI) (*Mw*=6000) was obtained from Shijiazhuang Desai Chemical Company. All lanthanides were purchased from Beijing Ouhe Chemical Reagent Co. Oleylamine (OAm), dimethylsulfoxide (DMSO), oleic acid, sodium stearate, 1-octadecene were obtained from Sigma-Aldrich. Methyl thiazoly tetrazolium (MTT) was also supplied by Sigma-Aldrich and used for cytotoxicity test. NaOH, $Na_2EDTA\cdot 2H_2O$, acetic acid, cyclohexane, chloroform, ethanol, and methanol were purchased from Beijing Chemical Reagent Company. 2-Amino-2-(hydroxymethyl)-1, 3-propanediol (Tris, $(HOCH_2)_3CNH_2$) and Ethidium bromide (EB) were obtained from Amresco. Agarose was obtained from Spain BIOWEST. PEG_{1000}-$PLGA_{5600}$ (50/50) was supplied by Jianan Daigang Biomaterial Co. LTD. The plasmid DNA used in this work was pDNA encoding enhanced green fluorescence protein (pEGFP) and was kindly provided by professor Fujian Xu (College of Materials Science and Engineering, Beijing University of Chemical Technology). All the reagents and chemicals were of analytical grade and used as received without further purification. Ultrapure water was obtained with a Milli-Q filtration system.

2.2. Characterization

The photoluminescence measurements were carried out on an F-4600 spectrophotometer (Hitachi) equipped with a plotter unit, a quartz cell (1 cm × 1 cm) and 980 nm diode laser as the irradiation source. The shape and size of the UCNPs@MFAP nanocapsules were examined *via* the H-800 transmission electron microscope (TEM, JEOL) with a tungsten filament at an accelerating voltage of 100 kV. Dynamic light scattering (DLS) particle size analysis and zeta potentials of the UCNPs@MFAP/ pDNA complexes were measured using a Zetasizer Nano-ZS90 (Malvern) zeta and size analyzer according to previous protocols [13]. Gene loading ability of the nanocapsules was examined on the agarose gel electrophoresis (DYY-6C, Beijing Liuyi Instrument Factory). The cytotoxicity tests were carried out by an ELISA plate reader (F50, TECAN). Confocal laser scanning microscopy (CLSM) observation was performed using TCS SP5 two-photon confocal microscopes (Leica) equipped with a Mai Tai NIR diode laser. The FTIR was performed on Nexus 670 Fourier-transform infrared (FT-IR) spectrophotometer (Nicolet, USA).

2.3. Synthesis of $NaYF_4:Yb^{3+}/Er^{3+}$ UCNPs

The luminescent β- $NaYF_4:Yb^{3+}/Er^{3+}$ UCNPs were prepared following previous reported procedures [7]. In brief, a mixture of 8 mL of oleic acid, 7 mL of octadecene and 0.35 g of sodium stearate were added into a three-necked flask and heated to 80 °C under vigorous stirring. Then 4 mL of the as-prepared rare-earth metal oleate solution and 1.05 mL of the HF-OAm solution were quickly injected into the flask with stirring for another 20 min. After that the mixture solution was heated to 180 °C for 10 min