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Near-infrared light activated photodynamic therapy of THP-1 macrophages based on core-shell structured upconversion nanoparticles

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ABSTRACT

Upconversion nanoparticles (UCNPs) with fascinating properties hold great potential as nanotransducers for solving the light penetration problem that traditional photodynamic therapy (PDT) is facing. In this report, the synthesis and utility of UCNPs/silica core-shell structured nanoparticles for upconversion (UC) PDT and imaging of THP-1 macrophages were described. The UCNPs ($NaYF_4$:Yb, Er) with a uniform diameter of 28 ± 1 nm were synthesized as core and 10 nm thick biocompatible mesoporous silica was coated as shell. Photosensitizer (PS) was covalently grafted inside mesoporous silica shell. Upon excitation, NIR light is converted into visible one by UCNPs for the absorption of PS to generate singlet oxygen for killing THP-1 macrophages and inhibiting the development of atherosclerosis. The fluorescence images showed the resulting nanoparticles are readily taken up by macrophages and TEM results indicated that they are highly phototoxic. The statistical *in vitro* results revealed PDT could cause the apoptosis of THP-1 macrophages with a remarkable therapeutic efficacy of cell inhibition ratio of 40%.

1. Introduction

Atherosclerosis (AS) is one of the most common chronic inflammatory vascular diseases in clinical patients, which poses a severe threat to human health [1]. The main pathological change in atherosclerosis is the formation of the plaques, which is characterized by heterogeneous composition. Macrophages, constituting up to 20% of the cells within atherosclerotic lesions, are known to play a key role both in the development of atherosclerosis and in the formation of unstable plaques vulnerable to rupture [2].

Some mechanical endovascular interventions, such as endarterectomy, balloon angioplasty and stenting, have significantly decreased the mortality of atherosclerotic diseases. However, these techniques are correlated with restenosis resulting from intimal hyperplasia or constrictive remodeling [3–5]. It is therefore urgent to develop alternative methods to pacify and identify vulnerable plaques.

Photodynamic therapy (PDT), based on the use of photosensitizers (PS) and light irradiation, has been known as a tissue-specific light-activated cytotoxic therapy for the treatment of many diseases [6]. Under proper light excitation, PS can interact with molecular oxygen and generate cytotoxic singlet oxygen ($^1O_2$, type II) for killing target cell [7,8]; The inherent photoluminescence from PS can also be used for fluorescence imaging and localizing the target tissues [9–12]. The ability to target nanoparticles to specific cells is one of the most important factors for prospective application of PS in bioimaging and PDT treatment [6]. The effectiveness of PDT for the treatment of atherosclerotic plaque has been proved previously [6,13]. In the early stage of such studies, Hematoporphyrin derivative (HpD), the first of generation of photosensitizers, was used to selectively accumulate within atherosclerotic plaque for PDT treatment [13]. Subsequent studies have underscored the affinity and PDT efficiency of porphyrin derivatives for diseased arterial wall in rabbits and miniswine [14,15] and human plaque [16]. Further, biotechnology has developed a new generation of photosensitizers and catheter-based technological advances in light delivery have allowed the more feasible introduction of PDT into the vasculature. Motoya Hayase et al. [17] used the photosensitizer Motexafin lutetium, which could accumulate in

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atherosclerotic plaque and activate by red light for PDT treatment. It is demonstrated that the PDT treatment could result in a significant decrease of macrophage and a small decrease in atheroma burden without damage to the normal vessel wall.

One major challenge for employment of the traditional PDT, however, is the limited tissue penetration depth of excitation light because most traditional PDT agents were activated at wavelengths below 700 nm. At those wavelengths, blood and tissue may substantially attenuate the delivery of light to target cells [18]. In particular, the plaque can be formed in the deep parts of the body. Tissue optics therefore suggests that near-infrared (NIR) light with higher wavelengths of absorption band in the range of either 700–800 or 950–1100 nm is desired for PDT given its deep tissue penetration [19].

Upconversion nanoparticles (UCNPs) with fascinating properties of acting as nanotransducer to upconvert near-infrared (NIR) light into UV or visible light for activating PS hold great potential for solving the penetration problem [20,21]. The fabrication of various hierarchically structured nanoparticles integrating UCNPs with photosensitizers have been reported for upconversion (UC) PDT treatment of diseases i.e., cancer [22–25]. Among these fabricated nanoparticles, the UCNPs/silica core-shell structured nanoparticles are particularly popular because silica is outstanding biocompatible material with versatile structure and functionalization abilities for loading PS for PDT application [26–28]. Although such core-shell structured nanoparticles with multimodal theranostic functions has been well documented for UC PDT as well as imaging of cancer cell in recent years, to the best of our knowledge relatively little has been reported on UC PDT treatment of atherosclerosis so far.

In this study, UCNPs/silica core-shell structured nanoparticles were synthesized and used for UC PDT treatment of THP-1 macrophages. As shown in Fig. 1, the UCNPs core is prepared as a nanotransducer for UC emissions. The UCNPs core is coated with a mesoporous silica shell where photosensitizer Chlorin e6 (Ce6) were chemically grafted through hydrolysis of condensation of PS-conjugated silica coupling agent. Upon 980 nm NIR light irradiation, the UC core emitted 660 nm red emissions to activate PS to generate singlet oxygen species. These singlet oxygen species can cause either apoptosis or necrosis of these THP-1 macrophages to inhibit the development of atherosclerosis. On the other hand, PS is also used as an optical nanoprobe for cell imaging. Upon 405 nm laser irradiation, the Ce6 incorporated in the silica shell could be downconversionly excited to produce red luminescence for labeling and locating the taken-up nanoparticles. Hereby, bi-functions of UC PDT and cell imaging are integrated together using a core-shell structured nanoplatform for promising atherosclerotic theranostics.

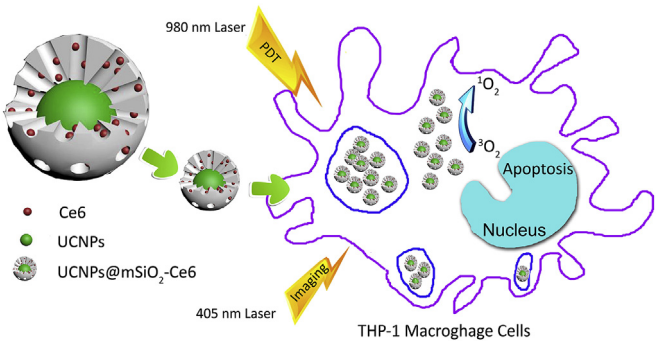

Fig. 1. Schematic illustration of NIR activated upconversion photodynamic therapy and photosensitizer-based downconversion imaging for THP-1 macrophage using UCNPs@mSiO$_2$-Ce6 nanoparticles.

2. Materials and experiments

2.1. Materials

Oleic acid, sodium hydroxide (NaOH), methanol, absolute ethyl alcohol (EtOH), cyclohexane, cetyltrimethylammonium bromide (CTAB), and tetraethyl orthosilicate (TEOS) were obtained from Sinopharm Chemical Reagent Co. Ltd. $YCl_3 \cdot 6H_2O$, $YCl_3 \cdot 6H_2O$ and $YCl_3 \cdot 6H_2O$ were purchased from the CongHua City JianFeng Rare Earth Company (China). Ammonium fluoride (NH_4F), 3-aminopropyltriethoxysilane (APTES), Chlorin e6 (Ce6), and dicyclohexylcarbodiimide (DCC) were purchased from Alfa Aesar. 1-octadecene (ODE) and 9,10-anthracenediylbis(methylene)dimalonic acid (ABDA) were purchased from Acros. All reagents were used as received without further purification. DMF was dried under CaH_2 and distillated. Fetal bovine serum and RPMI 1640 were obtained from Hyclone Laboratories (Logan, UT, USA). Penicillin-streptomycin was obtained from Sigma-Aldrich (St Louis, MO, USA). Phorbol-12-myristate-13-acetate was sourced from EMD Biosciences (La Jolla, CA, USA). 3-[4, 5-dimethylthiazol-2-yl]-2, and 5-diphenyltetrazolium bromide (MTT) were purchased from Beyotime Biotechnology (Beijing, China).

2.2. Preparation of UCNPs@mSiO$_2$-Ce6

Preparation of UCNPs: In a typical synthesis of monodisperse UCNPs of $NaYF_4$:Yb:Er (78:20:2 mol%), $YCl_3 \cdot 6H_2O$ (0.237 mg, 0.78 mmol), $YbCl_3 \cdot 6H_2O$ (0.0775 mg, 0.2 mmol), and $ErCl_3 \cdot 6H_2O$(0.0077 mg, 0.02 mmol) were added into a 250 mL three-neck round-bottom flask containing oleic acid (6 mL) and 1-octadecene (5 mL). In the presence of argon, the mixture was slowly heated to 160 °C and kept at this temperature for 30 min under vigorous magnetic stirring. Then 1-octadecene (10 mL) was added and the solution was stirred at 160 °C for another 60 min. After the reaction temperature was cooled down to 50 °C, the solutions of ammonium fluoride (148.16 mg, 4 mmol) and sodium hydroxide (100 mg, 2.5 mmol) dissolved in methanol (10 mL) were added and the system was stirred at 50 °C for 30 min. After methanol evaporated, the solution was heated to 300 °C and kept at this temperature for 60 min. The mixture was then cooled down to room temperature before the excess ethanol was added. Product was isolated by centrifugation at 10000 rpm and washed 3 times by ethanol. The final product was dispersed in 10 mL cyclohexane.

Preparation of the Ce6 molecule precursor for grafting inside the silica shell: 5.6 mg of DCC, 16 mg of Ce6 and 50 mL of APTES were sequentially added into a 10 mL dried N,N-dimethylformamide (DMF) solution and the solution was stirred for 24 h at room temperature. In this process the carboxylic group of Ce6 could react with amino groups of APTES to form an amide connecting to siloxane groups, which could hydrolyze with TEOS to form Si–O–Si covalent bonds in the next process.

Synthesis of the Ce6-grafted core–shell nanoparticles UCNPs@mSiO$_2$-Ce6: 200 mg CTAB was dissolved in 8 mL deionized water, and 800 μL $NaYF_4$:Yb, Er NPs in cyclohexane was added in the solution. The mixture was ultrasonicated for 10 min, and stirred vigorously at room temperature for another 30 min. Then, the mixture was heated at 80 °C for 30 min to evaporate the cyclohexane. The as-prepared CTAB-stabilized $NaYF_4$:Yb, Er NPs were diluted with water (20 mL), followed by adjusting the pH value of the mixture to 9 with 0.1 M NaOH solution. After that, a mixture of TEOS (200 μL) and ethanol (1 mL) was added 50 μL in the above system at 30 min intervals (50 μL at a time). 1 mL of the as-prepared Ce6 molecule precursor solution was added into the above mixture and stirred for 12 h at room temperature. Subsequently, the