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Intranuclear biophotonics by smart design of nuclear-targeting photo-/radio-sensitizers co-loaded upconversion nanoparticles

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ABSTRACT

Biophotonic technology that uses light and ionizing radiation for positioned cancer therapy is a holy grail in the field of biomedicine because it can overcome the systemic toxicity and adverse side effects of conventional chemotherapy. However, the existing biophotonic techniques fail to achieve the satisfactory treatment efficacy, which remains a big challenge for clinical implementation. Herein, we develop a novel theranostic technique of "intranuclear biophotonics" by the smart design of a nuclear-targeting biophotonic system based on photo-/radio-sensitizers covalently co-loaded upconversion nanoparticles. These nuclear-targeting biophotonic agents can not only generate a great deal of multiple cytotoxic reactive oxygen species in the nucleus by making full use of NIR/X-ray irradiation, but also produce greatly enhanced intranuclear synergetic radio-/photodynamic therapeutic effects under the magnetic/luminescent bimodal imaging guidance, which may achieve the optimal efficacy in treating radio-resistant tumors. We anticipate that the highly effective intranuclear biophotonics will contribute significantly to the development of biophotonic techniques and open new perspectives for a variety of cancer theranostic applications.

1. Introduction

With the rapid development of nanomaterials and nanotechnology, there emerges a new research field-biophotonics [1], which deals with the biological effects of light/radiation on physical matters and refers to new modalities of light/radiation-guided/activated therapies. As two representative biophotonic techniques, near-infrared (NIR) light-triggered photodynamic therapy (PDT) [2–4] and X-ray-induced radiotherapy (RT) [5–7] have been widely used for treating deep-seated malignant tumors by generating a great deal of reactive oxygen species (ROS) to damage the DNA of cancerous cells. Considering that the principal cell death mechanism of both PDT and RT is ROS-mediated DNA breakage, the combinational use of PDT/RT will lead to the synergetic therapeutic effects, which can reduce the laser power/radiation dose and lower the potential side effects [8–11]. Therefore, it is of great importance to design a powerful biophotonic system to integrate PDT and RT in a single platform, which is especially desirable for treating fast-growing radio-resistant tumors, such as human fibrosarcoma (HT-1080) tumor [6].

Thanks to the emerging of upconversion nanoparticles (UCNPs) that can convert NIR light into visible light, most photosensitizers can be activated to produce toxic singlet oxygen ($^1O_2$) to induce apoptotic/necrotic cell death upon NIR light irradiation [12–15] based on the fluorescence resonance energy transfer (FRET) [16–18]. Among various photosensitizers, phthalocyanine-based derivatives (e.g. silicon phthalocyanine dihydroxide (SPCD), etc) [19,20] and porphyrin-based compounds (e.g. protoporphyrin IX (PpIX), etc) [21–23] may be potentially more ideal for PDT due to their unique advantages (e.g., intense absorption in the visible region, low dark toxicity, easy structural modification, etc). More importantly, the absorption spectra of SPCD and PpIX overlap with

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the emission spectra of UCNPs in some visible regions, so UCNPs can simultaneously activate these two photosensitizers using a single NIR light source [24,25]. Furthermore, PpIX can also serve as a radiosensitizer by the radiolysis of water into hydroxyl radical ($OH^{\bullet}$) and superoxide radical ($O_2^{\bullet-}$) under X-ray irradiation [26,27], which can significantly enhance the RT efficacy in killing those radio-resistant cells. Consequently, the integration of UCNPs and SPCD/PpIX into a single system will be expected to result in the substantially enhanced synergetic PDT/RT effects upon NIR/X-ray irradiation.

How to simultaneously achieve the high loading of photo-/radio-sensitizers while avoiding their adverse interactions still remains a big challenge. The traditional physical encapsulation method inevitably causes the low loading capacity and premature leakage [28–31]. Fortunately, the selective covalent grafting may provide an advanced strategy for solving this problem [13,19]. Another important issue is the short lifetime (less than 3.5 μs) and limited diffuse distance (up to 0.02 μm) of ROS [32], which seriously lowers the therapeutic effects because much ROS generated in the cytoplasm may vanish before acting on the DNA in the nucleus. Therefore, it is crucial to realize the intranuclear delivery [33,34] of photo-/radio-sensitizers, which can produce substantial ROS in the nucleus to directly damage the DNA in a very short time.

Herein, in order to address the above issues, we develop a novel theranostic technique of "intranuclear biophotonics" (Fig. 1) aimed at directly transporting photo-/radio-sensitizers into the cell nucleus for generating substantial intranuclear multiple ROS ($^1O_2$, $OH^{\bullet}$, $O_2^{\bullet-}$) on efficiently damaging the DNA upon simultaneous NIR/X-ray irradiation, which can be expected to achieve the optimal treatment efficacy. To realize this goal, novel multifunctional biophotonic agents were successfully designed by engineering UCNPs with covalently co-loaded photo-/radio-sensitizers (SPCD/PpIX). After conjugation with a biocompatible polymer PEG and a nuclear localization signal (NLS) peptide TAT, the nuclear-targeting biophotonic agents can achieve much higher oncolytic efficacy based on the greatly elevated NIR/X-ray-triggered intranuclear PDT/RT effects. To the best of our knowledge, this is the first introduction of intranuclear biophotonics into the biomedical field *via* the smart design of nuclear-targeting biophotonic agents, which may reveal new insights into the theranostic techniques and hold highly valuable potential for future clinical applications.

2. Materials and methods

2.1. Materials

$YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$, $ErCl_3 \cdot 6H_2O$, $GdCl_3 \cdot 6H_2O$, $TmCl_3$, Ammonium fluoride (NH_4F), 1-Octadecene (90%), Igepal CO-520 (NP-5), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), N-hydroxysuccinimide (NHS), Silicon phthalocyanine dihydroxide (SPCD; 75%, Aldrich), Protoporphyrin IX (PpIX; 95%), 3-aminopropyltrimethoxysilane (APTES, ≥98%), Methylene blue (MB) and 1,3-Diphenylisobenzofuran (DPBF) were purchased from Sigma–Aldrich. TAT: YGRKKRRQRRR was purchased from Chinese Peptide Company. Tetraethyl orthosilicate (TEOS), Sodium chloride (NaCl) and Sodium hydroxide (NaOH) were obtained from Shanghai Lingfeng Chemical Reagent Co., LTD. Oleic acid (OA), Acetonitrile, Methanol and Ammonia solution (30%) were obtained from Sinopharm Chemical Reagent Co., LTD. All reagents were of analytical grade and used without any purification.

2.2. Synthesis of UCNPs

$NaYF_4$:Yb(18%)/Gd(15%)/Er(2%)/Tm(1%) was prepared according to a thermal decomposition method. $YCl_3 \cdot 6H_2O$ (388.3 mg, 1.28 mmol), $YbCl_3 \cdot 6H_2O$ (139.50 mg, 0.36 mmol), $GdCl_3 \cdot 6H_2O$ (79.08 mg, 0.3 mmol), $ErCl_3 \cdot 6H_2O$ (15.51 mg, 0.04 mmol), $TmCl_3$ (5.5087 mg, 0.02 mmol) in 4 mL deionized water were added to a 100 mL flask containing 15 mL oleic acid and 30 mL 1-octadecene. The solution was stirred at room temperature for 1 h. Then the mixture was slowly heated and kept at 120 °C for 1 h and 156 °C for another 1 h under argon atmosphere to remove water, and then cooled down to the room temperature. Subsequently, 10 mL methanol solution of NaOH (200 mg, 5 mmol) and NH_4F (296.3 mg, 8 mmol) was added and the system was stirred at room temperature for 2 h. After the evaporation of methanol, the solution was slowly heated to 280 °C and maintained for 1.5 h, and then cooled down to the room temperature. The resulting products were washed with cyclohexane and ethanol several times, and finally dispersed in 20 mL cyclohexane.

2.3. Synthesis of UCSs

According to an O/W reverse microemulsion method, 1 mL of Igepal CO-520 (NP-5) was dispersed in 20 mL cyclohexane. Afterward, oleic acid capped UCNPs in cyclohexane solution (1.5 mL, 100 mM) was added into the cyclohexane/NP-5 mixture. After magnetic stirring for 3 h, 140 μL ammonia (30%) was added dropwise, and the system was stirred for 2 h, followed by adding 1 mL cyclohexane solution of SPCD (2.5 mg/mL). Then 175 μL tetraethyl orthosilicate (TEOS) was injected into the system through a syringe pump (WZS-50F6) at a rate of 175 μL/h. The mixture was sealed and kept stirring for 24 h before adding methanol to terminate reaction. The resulting UCSs products were washed with ethanol and cyclohexane several times to remove excess NP-5, and finally dispersed in 5 mL deionized water.

2.4. Amino-silanization of PpIX

In order to make PpIX encapsulated into the dense silica framework, PpIX was first let to react with APTES to form the amino-functionalized PpIX silane precursor. PpIX (15 mg), EDC (25.6 mg) and NHS (17.6 mg) were dissolved 6 mL deionized water, followed by addition of 45 μL APTES. The system was stirred for 24 h at room temperature to produce PpIX-NH_2 silane precursor for future use.

2.5. Synthesis of UCSPs

According to a water-phase regrowth method, UCSs in 5 mL deionized water was diluted with 20 mL ethanol. 5 mL ammonia

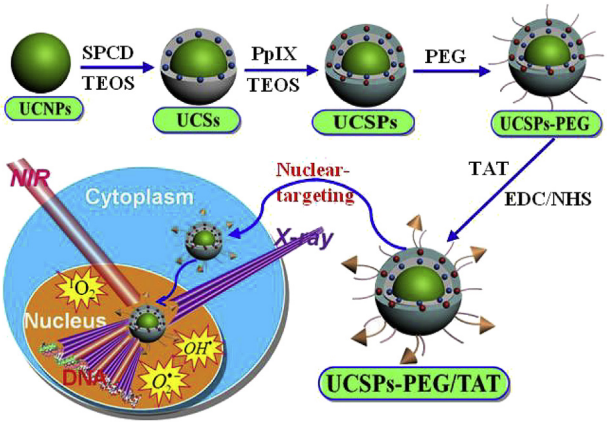

Fig. 1. Schematic illustration of the theranostic technique of intranulear biophotonics and the synthetic procedure of nuclear-targeting biophotonic agents (UCSPs-PEG/TAT).