Mesoporous-Silica-Coated Upconversion Nanoparticles Loaded with Vitamin B₁₂ for Near-Infrared-Light Mediated Photodynamic Therapy

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ABSTRACT

Near-infrared (NIR)-to-visible upconversion nanotechnology has potential to be used for photodynamic therapy (PDT) because the use of NIR-irradiation provides deep tissue penetration and avoids photodamage to normal cells, thus overcoming a major hurdle in the treatment of deep-seated tumours. In this study, a novel nano-platform of mesoporous-silica-coated NaYF₄:Yb/Er upconverting nanoparticles with a large surface area of 770 m²/g and an average pore size of 2 nm, was fabricated and used for NIR-mediated PDT. Vitamin B₁₂ (VB₁₂), a nontoxic, easy-to-obtain and biocompatible photosensitizer, was incorporated into the mesoporous silica and used as PDT drug. There is a good overlap (545 nm) between the fluorescence emission of NaYF₄:Yb/Er nanoparticles and the UV-visible absorption of VB₁₂. Under NIR-irradiation, the visible fluorescent emission from the upconverting nanoparticles will be absorbed by the VB₁₂ coated on their surfaces. Then, the VB₁₂ molecules under excited-state interact with surrounding ground-state molecular oxygen, generating reactive oxygen species, leading to oxidative damage of cancer cells.

Keywords: Upconversion, Vitamin B₁₂, Photodynamic therapy, Nanoparticles, Nanocomposite, Photosensitizers

1. Introduction

Photodynamic therapy (PDT) has emerged as a promising and noninvasive treatment for various types of cancer [1]. Modern PDT applications involve three key components: photosensitizer (also called PDT drug), light source and tissue oxygen. The combination of these three components leads to the generation of reactive oxygen species (ROS) through energy transfer or electron transfer processes. ROS can cause oxidative damage to biological substrates and ultimately result in cancer cell death [2]. Compared with other conventional methods, PDT has several advantages, such as spatial and temporal control as well as the possibility of repeated doses. One of the key components of PDT in clinical use is the photosensitizer, which is also the research focus in the PDT-related field. In the past decades, PDT drugs including porphyrins and porphyrin derivatives have attracted much attention due to their excellent cellular affinity and low dark toxicity [3-11]. Features desired for an ideal PDT drug, including: (1) have a high photochemical reactivity, that is, it can effectively produce ROS when exposed to appropriate irradiation; (2) exhibit low dark toxicity; and (3) can be excited at a wavelength in the region where tissue penetration of irradiation is at a maximum [2].

Vitamin B₁₂ (VB₁₂), also called cobalamin, is a kind of important water-soluble vitamins and widely present in-vivo and natural environments [12,13]. It contains a unique porphyrin-like structure and a biochemically rare element cobalt sitting in the center of planar tetra-pyrrole ring called a corrin ring (Fig. 1A). The corrin rings are a class of 18 π -electron aromatic tetrapyrroles formally derived from porphyrins by saturation of a peripheral double bond, serving as an excellent light-harvesting chromophore [13]. VB₁₂ has been recognized as an excellent photosensitizer for generating radical species (hydroxyl radical or methyl radical) under cellular conditions [12-15], which inspires us to probe the possibility of VB₁₂ used as a novel PDT drug.

Conventional PDT drug including porphyrin-like structure can be activated using high-energy UV or visible irradiation, which may bring some severe photodamage to normal cells [2]. In particular, the application of PDT can be significantly expanded if NIR irradiation is used, because it is able to take advantage of the optical window of tissue (600-1000 nm) and provide deep tissue penetration, enabling the treatment of deep-seated tumours [16,17]. However, the design and synthesis of photosensitizers that absorb NIR irradiation are restricted since (1) stable NIR absorbing photosensitizers are notoriously difficult to synthesize and (2) the triplet electronic energy level of the photosensitizer must be higher than the singlet energy level of the oxygen molecule [18]. To address this issue, the NIR-to-visible upconversion nanoparticle (UCNP) is a potential approach to overcome these limitations [17, 18]. These materials convert lower-energy photons to higher-energy photons through excitation with multiple photons process, which have been widely used in bioimaging, cancer therapy and so on [19-23].

In this work, mesoporous-silica-coated NaYF₄:Yb/Er nanoparticles with a core/shell structure were fabricated and used for PDT of cancer cells. VB₁₂, a nontoxic, easy-to-obtain and biocompatible photosensitizer, was used as PDT drug and incorporated into the mesoporous silica shell. As shown in Fig. 1, the visible fluorescent emission from the UCNPs will be absorbed by the VB₁₂ coated on their surfaces under NIR irradiation. Subsequently, the excited VB₁₂ molecules will interact with surrounding ground-state molecular oxygen, generating ROS, leading to oxidative damage of cancer cells.

2. Material and methods

NaYF₄:Yb/Er nanocrystals with uniform size distribution were synthesized via an efficient and user-friendly method [17]. In a typical procedure, igeal CO-520 (0.8 mL), cyclohexane (50 mL) and

NaYF₄:Yb/Er nanocrystal solution in cyclohexane (50 mL, 0.04 M) were added into a 250 mL three-neck round-bottom flask and stirred for 30 min, respectively. Then, 1.0 mL ammonia (28 wt%) was added into the previous solution and sonicated for 30 min until a transparent emulsion was formed. The mixture solution of 200 μ L TEOS and 80 μ L octadecyltrimethoxysilane (C₁₈TMS) was then added into the previous solution. Finally, the solution was stirred for 2 d at a speed of 800 rpm at ambient temperature of 25°C. Silica-coated NaYF₄ nanoparticles were collected by centrifugation after precipitation by adding acetone, washed with ethanol three times and finally dried at 50°C for 12 h. The as-prepared sample was calcinated at 500°C in static air with a heating rate of 1.0°C/min and a dwelling time of 2.0 h.

Mesoporous-silica-coated NaYF₄:Yb/Er nanoparticles (100 mg) were soaked in VB₁₂ aqueous solution (10 mL, 10 mg/mL) for 24 h at ambient temperature of 25°C. The nanoparticles were then collected by centrifugation (5000 g, 5 min) and washed with PBS solution. Initial and residual VB₁₂ concentrations were analyzed by a high performance liquid chromatography system (HPLC-1100, Agilent Inc., USA) equipped with a VWD detector and a Hypersil ODS column.

MDA-MB-231 cells were used to evaluate the effect of this NIR-initiated PDT and cultured in RPMI 1640 medium, supplemented with 10% fetal bovine serum and 2 mM L-glutamine at 37°C using a humidified 5% CO₂ incubator. The MDA-MB-231 cells were seeded onto 96-well plates at 2.0×10^3 cells per well and further incubated for 24 h. The culture medium was then replaced with 100 μ L of freshly prepared culture medium containing either free VB₁₂ or VB₁₂/UCNP composites at different VB₁₂ concentrations. Cells were incubated for 24 h and then exposed to visible or NIR irradiation. All cells were then incubated for another 48 h at 37°C and evaluated by MTT viability assay.

3. Results and Discussion

NaYF₄:Yb/Er nanoparticles have been recognized as one of the most efficient UCNPs and were used in this study [17-20]. Fig. 2A shows the typical transmission electron microscopy (TEM) image of the NaYF₄:Yb/Er nanocrystals. The general overview of the TEM image indicated that the products were uniform sphere-like nanoparticles with an average diameter of 25 nm. Mesoporous silica-coated NaYF₄:Yb/Er nanoparticles, with a large surface area of 770 m²/g and an average pore size of 2 nm, were further achieved successfully by one pot facile synthetic process [22]. Octadecyltrimethoxysilane (C₁₈TMS) was used as template, which has been used for preparing hydrophobic coatings and self-assembled monolayers. Fig. 2B shows that a thin and uniform silica shell was coated on the single nanoparticle and the thickness of the silica shell was about 20 nm.

As shown in Fig. 2C, there is a good overlap (545 nm) between the fluorescence emission of NaYF₄:Yb/Er nanoparticles and the UV-visible absorption of VB₁₂, which indicates that VB₁₂ can be absorbed and activated by the UCNPs to produce ROS and kill cancer cells when exposed to the NIR light. VB₁₂ has been reported to be photolabile under UV irradiation [14]. The photostability of VB₁₂ under visible or NIR irradiation was investigated in this study. Fig. 2D illustrates the evolution of UV-visible spectra of the solution as a function of visible irradiation time and similar results have been obtained under NIR irradiation (Fig. S1). VB₁₂ was found to be photostable under our experimental conditions, which was then loaded into the pores of the mesoporous silica. VB₁₂ was determined using HPLC and the amount of adsorbed into the porous silica was about 1.0 wt%.

In-vitro cytotoxicity assays were further performed using the human breast cancer cell line MDA-MB-231 from the American Type Culture Collection (ATCC). Cells with free VB₁₂ were exposed to a 50-W Xe-arc lamp (XD-300, Nanjing Yanan special light factory, China) equipped with an UV-cutoff (>400 nm) for 10 minutes. Effect of VB₁₂ concentration on the cell viability was first evaluated.

The cell viability with different initial VB₁₂ concentrations (0.3125, 0.625, 1.25, 2.5, 5 and 10 µg/mL) is illustrated in Fig. 3A. The cell viability decreased with an increase in initial VB₁₂ concentration. The control-1 cells incubated without visible irradiation showed that the photosensitizer of VB₁₂ is nontoxic and biocompatible. The control-2 cells were incubated under visible irradiation without adding VB₁₂. The results of the control tests show that VB₁₂ and visible irradiation were both required. Effect of exposure time to visible irradiation on the cell viability was also evaluated. As shown in Fig. 3B, the results indicated that the cell viability decreased with the increase of exposure time under visible irradiation, suggesting an increased amount of ROS produced by the photosensitizer of VB₁₂. For comparison, cells with VB₁₂/UCNP were exposed to a 980 nm-NIR-laser with an output power of 1000 mW for 10 minutes. Fig. 3C showed that the viability of cells incubated with the amount of nanoparticles loaded with VB₁₂ and exposed to NIR laser for 10-minute was significantly lower as compared to the nanoparticles without VB₁₂ or without IR irradiation. Fig. 3D showed the effect of exposure time to NIR irradiation on the cell viability. The cell viability decreased with an increase of exposure time to NIR irradiation. Control experiments with NIR irradiation but without adding nanoparticles also showed that NIR irradiation alone did not initiate cell death. Silica-coated NaYF₄ upconversion nanocrystals have also been approved to be good biocompatibility and did not cause cell death.

4. Conclusion

In summary, a novel nanocomposite of mesoporous-silica-coated NaYF₄:Yb/Er composite nanoparticles incorporated with photosensitizers (VB₁₂) has been successfully fabricated; which exhibited a better performance for the PDT of cancer cells under NIR-irradiation. Compared to other photosensitizers used in PDT process, VB₁₂ is not only nontoxic, easy-to-obtain, and biocompatible, but also known as an anti anemia drug. These results demonstrate that photosensitizer of the proposed design has great potential in clinical applications. However, the VB₁₂-initiated ROS generation, which cause cancer cell death, is a very complex process that has not been well recognized to date. Thus, in-depth further investigations are warranted.

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Supporting Information

Supplementary data associated with this article can be found in the online version. Synthesis of silica-coated NaYF₄:Yb/Er nanoparticles, Analytical methods (TEM, UV-visible Analysis, Fluorescence Analysis, HPLC Analysis). Photodegradation of vitamin B₁₂ (VB₁₂).

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Figure Captions

Fig.1. Schematic illustration of the VB₁₂/UCNP nanoparticles for PDT and Proposed NIR-mediated ROS generation leading to oxidative damage of cancer cells. (A) Structural formula of the photosensitizer (VB₁₂).

Fig.2. (A) TEM image of NaYF₄:Yb/Er nanoparticles; (B) TEM image of mesoporous silica-coated NaYF₄:Yb/Er nanoparticles; (C) Emission fluorescence spectrum of UCNP (NaYF₄:Yb/Er) excited at 980nm NIR and UV-visible spectrum of the photosensitizer of VB₁₂; and (D) UV-visible spectra of the VB₁₂ solution as a function of visible irradiation time.

Fig.3. MDA-MB-231 cancer cell viability: (A) Different free VB₁₂ concentration after 10-minute visible irradiation; (B) 1.25 µg/mL free VB₁₂ after different visible irradiation time; (C) Different VB₁₂/UCNP concentration after 10-minute NIR irradiation; and (D) 5 mg/mL VB₁₂/UCNP after different NIR irradiation time. The exposure time of NIR-irradiation was divided into 5 section (5*2 min) to avoid the over-heating possibility. Further cultured 48 hours after irradiation, cell death was measured and bar graphs represent means of triplicates±standard deviations.

Fig.1. Schematic illustration of the VB₁₂/UCNP nanoparticles for PDT and Proposed NIR-mediated ROS generation leading to oxidative damage of cancer cells. (A) Structural formula of the photosensitizer (VB₁₂).

Fig.2. (A) TEM image of $NaYF_4:Yb/Er$ nanoparticles; (B) TEM image of mesoporous silica-coated $NaYF_4:Yb/Er$ nanoparticles; (C) Emission fluorescence spectrum of UCNP ($NaYF_4:Yb/Er$) excited at 980nm NIR and UV- visible spectrum of the photosensitizer of VB_{12} ; and (D) UV-visible spectra of the VB_{12} solution as a function of visible irradiation time.

Fig.3. MDA-MB-231 cancer cell viability: (A) Different free VB_{12} concentration after 10-minute visible irradiation; (B) 1.25 $\mu g/mL$ free VB_{12} after different visible irradiation time; (C) Different $VB_{12}/UCNP$ concentration after 10-minute NIR irradiation; and (D) 5 mg/mL $VB_{12}/UCNP$ after different NIR irradiation time. The exposure time of NIR-irradiation was divided into 5 section (5*2 min) to avoid the over-heating possibility. Further cultured 48 hours after irradiation, cell death was measured and bar graphs represent means of triplicates $\pm$ standard deviations.

Graphical Abstract

A new approach for photodynamic therapy has been developed, in which a novel photosensitizer, vitamin B₁₂, was excited under near-infrared (NIR) irradiation based on the upconversion nanotechnology. The use of NIR irradiation provides deep tissue penetration and avoids photodamage to normal cells, thus overcoming a major hurdle in treatment.