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808 nm Light-triggered and hyaluronic acid-targeted dual-photosensitizers nanoplatform by fully utilizing Nd^{3+}-sensitized upconversion emission with enhanced anti-tumor efficacy

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ABSTRACT

The current near-infrared (NIR) light-induced photodynamic therapy (PDT) can enhance the tissue penetration depth to trigger photosensitizers (PSs) far from the surface. NIR-mediated PDT is still challenged by overheating effect on normal tissues, limited tumor selectivity and low reactive oxygen species (ROS) yields. Here we construct a dual-agent photosensitizing nanoplatform by combining UV-blue upconversion emitting $NaYF_4$:Yb/Tm@$NaYF_4$:Yb@$NaNdF_4$:Yb@$NaYF_4$ (labeled as UCNPs) multi-shell nanocrystals with titanium dioxide (TiO_2, UV-light-excited PS) and hypocrellin A (HA, blue-light-excited PS), which can induce cancer cell apoptosis by 808 nm light-triggered and hyaluronic acid (Hyal)-targeted PDT. In this construction strategy, the crystallized TiO_2 shells on the surface of UCNPs can play dual roles as UV-light excited PS and conjugation site for Hyal, and then Hyal is served as targeting-ligand as well as the carrier of HA simultaneously. The step-by-step reactive mode of loading PSs and modifying targeting-ligands is a controllable and ordered design based on the use of one intermediate product as the reaction site for the next component. The Nd^{3+}-sensitized UCNPs with quenching reduction layer can efficiently convert 808 nm NIR light to UV-blue emission for simultaneous activation of two PSs with enhanced intracellular ROS generation. Through the *in vitro* and *in vivo* experiment results, the dual-photosensitizers nanoplatform presents enhanced anti-tumor efficacy by effective targeting cellular uptake and taking full advantage of upconversion emission, which may make a major step toward next generation of NIR-mediated PDT.

1. Introduction

Photodynamic therapy (PDT) is a minimally-invasive treatment for cancers, which relies on the reactive oxygen species (ROS) due to the excitation of photosensitizers (PSs) by light [1–12]. Near-infrared (NIR) light mediated PDT can extend the treatments from 1 to 2 millimeters with UV/visible light irradiation to a few centimeters [13–17]. Lanthanide-doped upconversion nanoparticles (UCNPs) [18–32], which can convert NIR light into UV/visible light for activating the attached PSs to generate ROS [33–35], offer the excellent prospects in developing strategies for the next generation of PDT on deeper tissues. To sum up the previous NIR light induced PDT based on upconversion process [36–39], further progress has been largely hindered by several drawbacks, containing the selection of irradiation source, the mode of PSs loading, the full utility of upconversion (UC) luminescence and the targeting delivery of PSs.

UCNPs are typically sensitized by Yb^{3+} ions that only respond to narrow band NIR excitation centered at around 980 nm [40–48], however, long-duration or high-power-density irradiation to trigger UC process would cause overheating issues due to the overlap of absorption between Yb^{3+} ions and water molecules, resulting in non-negligible risk of *in vivo* applications [49–53]. As the excitation source, an 808 nm light may both overcome the overheating issues and improve the penetration depth in tissue

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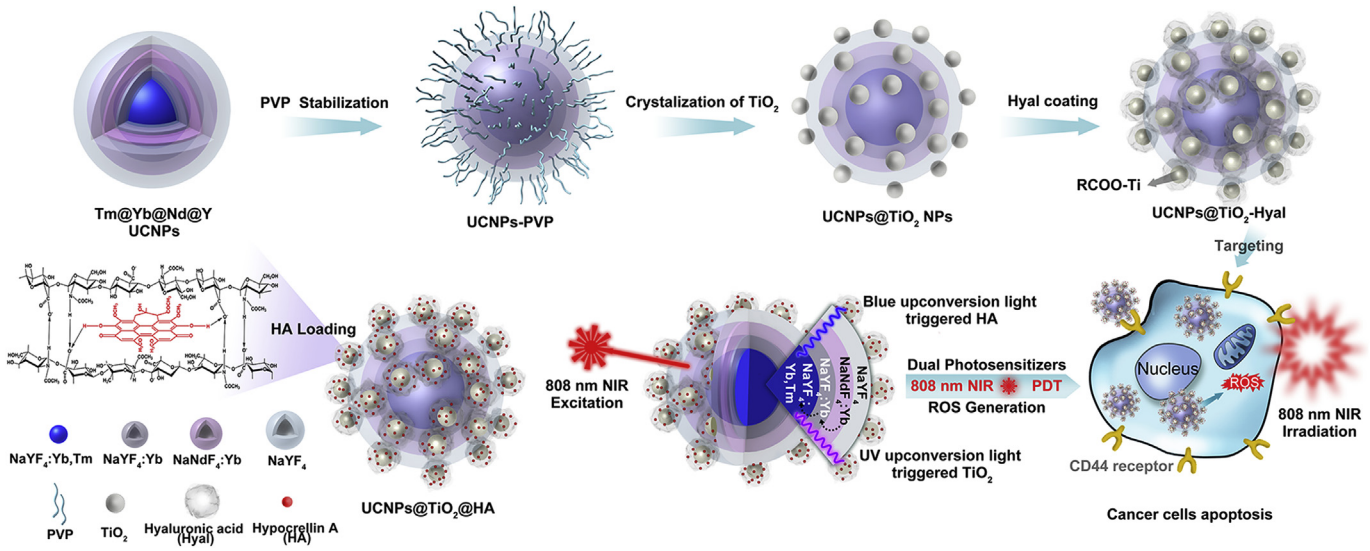
[54–56], since the NIR region around 700–900 nm reaches a minimum absorbance for all biomolecules [57–59]. With the intense absorption cross-section at around 800 nm, Nd^{3+} ion can be served as a sensitizer for the UC process through the energy migration process such as Nd^{3+}-Yb^{3+}-activators [60–63]. It is believed that the Nd^{3+} sensitized UCNPs offer new opportunities for the researches of 808 nm NIR light mediated PDT [64–66]. These well-established 808 nm light triggered UCNPs PDT systems can only attach one kind of PS without *in vivo* PDT efficacies demonstrated. Although these active-targeting PDT systems presented high tumor selectivity and anti-tumor efficacy, the loading mode of PSs and targeting-ligands is the traditional design with using the amine-groups on the surface of UCNPs as the common covalent conjugation site to modify UCNPs with two different components (PS and targeting-ligand) [67–69]. Additionally, in some previous reported PDT systems, the hydrophobic PSs were adsorbed in the mesoporous or rattle structure shell coated UCNPs [70,71]. The outer shells are too thick (more than 10 nm), and the longer distance between PSs and UCNPs may result in inefficient energy transfer and low ROS yields. Meanwhile, due to the short lifetimes (<40 ns) and action radius (<20 nm) in biological systems, ROS are difficult to release completely out of the system and acted on cells, thus diminishing the PDT efficiency. Titanium dioxide (TiO_2) NPs, which could serve as a PDT agent due to UV-irradiation induced phototoxicity [72–74], and could crystallize directly on the surface of 980 nm light triggered UCNPs with the upconverting UV-blue emission in our former research [75]. This strategy can ensure the maximum of energy transfer from donors to acceptors so as to facilitate the generation and release of ROS. However, the blue emission is wasted because the attached TiO_2 NPs only absorb the upconverted UV light. This situation also occurs in the other UC-based single-PS systems [38]. Therefore, it is desirable to develop a single 808 nm NIR light mediated upconverting system by taking full advantage of UC luminescence for enhanced PDT.

Herein, we report a dual-agent photosensitizing nanoplatform, which combines UV-blue emitting UCNPs with hypocrellin A (HA, blue-light excited PS) and titanium dioxide NPs (TiO_2, UV-light excited PS) for 808 nm light triggered PDT on tumors (Scheme 1). $NaYF_4$:Yb/Tm@$NaYF_4$:Yb@$NaNdF_4$:Yb@$NaYF_4$ (labeled as Tm@Yb@Nd@Y or UCNPs) multi-shell nanocrystals can produce strong UV and blue luminescence under 808 nm light excitation. Crystallized shells of TiO_2 NPs were coated on the Tm@Yb@Nd@Y cores to form UCNPs@TiO_2 core/shell nanocomposites (NCs). As a polycarboxylate polymer, hyaluronic acid (Hyal) is able to chemisorb on the surface of TiO_2 NPs by the formation of a RCOO-Ti bond [76–78]. HA, a type of non-porphyrin PSs isolated from the fungus Hypocrella bambuase [79,80], could be introduced into Hyal layer through the linking of one HA molecule with two Hyal molecules by hydrophobic interaction and hydrogen bonds to form the UCNPs@TiO_2@HA NCs [81]. Hyal is also known to be an attractive targeting-ligand that binds cluster determinant 44 (CD44) receptors, which are overexpressed in many kinds of tumor cells [82]. In this construction strategy, the crystallized TiO_2 shells could play dual roles of UV-light excited PS and conjugation site for Hyal, and then Hyal was served as targeting-ligand as well as the carrier of HA simultaneously. Such designing of 808 nm light-triggered and Hyal-targeted dual-PSs nanoplatform offers a potential route to induce remarkable cancer cell apoptosis and enhance anti-tumor efficacy.

2. Experimental section

2.1. Materials and reagents

All the chemical reagents were directly used without further purification. The rare earth oxides Y_2O_3, Yb_2O_3, Nd_2O_3, Tm_2O_3 and Er_2O_3 were purchased from Science and Technology Parent Company of Changchun Institute of Applied Chemistry. Sodium oleate ($C_{15}H_{33}NaO_2$), Sodium fluoride (NaF), Ammonium fluoride (NH_4F), Sodium hydroxide (NaOH), Anhydrous ethanol, n-hexane and Cyclohexane were all purchased from Beijing Chemical Reagent Company. Trifluoroacetic acid (CF_3COOH), Oleic acid (OA), Octadecene (ODE), Dimethyl sulfloxide (DMSO), polyvinylpyrrolidone (PVP, M_w = 40,000) and fluorescein isothiocyanate (FITC) were purchased from Aldrich. TiF_4 (99%) was purchased from Acros. Hyaluronic acid sodium (Hyal-Na, 95%) was obtained from Aladdin. Chitosan (CS, degree of deacetylation ≥90%) were purchased from Jinan Haidebei Marine Bioengineering Co., Ltd. Hypocrellin A (HA, 98%) was purchased from Shanghai Yuanye Bio-Technology Co., Ltd. The rare-earth trifluoroacetates were prepared by dissolving the corresponding rare-earth oxides in trifluoroacetic acid. The rare earth oleate complexes were synthesized according to a literature with a little modification [83].



Scheme 1. Illustration of 808 nm laser-induced dual-agent photosensitizing nanoplatform by combining UV-blue UC emitting multi-shell UCNPs with TiO_2 (UV-light-excited PS) and HA (blue-light-excited PS).