Biomaterials 155 (2018) 135–144

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

# $SnWO_4$-based nanohybrids with full energy transfer for largely enhanced photodynamic therapy and radiotherapy

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ARTICLE INFO

*Article history:*
Received 10 June 2017
Received in revised form
10 November 2017
Accepted 13 November 2017
Available online 14 November 2017

*Keywords:*
Tin tungstate
Photodynamic therapy
Efficiency
Radiotherapy
Tumor

ABSTRACT

The “partial matching” between upconversion nanoparticle (UCNP) emission and absorption by photosensitizers (PSs) often leads to a theoretically reduced therapeutic efficiency in UC-based photodynamic therapy (PDT) strategies in which the chosen PSs have limited capabilities and are unable to utilize all the near-infrared-upconverted light. In this study, needle-like $SnWO_4$ nanocrystals (SWs) with a broad UV−vis absorption region were synthesized to solve the problem. After covalent conjugation with UCNPs, all the UCNP-emitted light was effectively absorbed by SWs, triggering the type-I PDT process to activate ROS maxima. The unique nanostructure of the as-formed UCNP-$SnWO_4$ nanohybrids (USWs) also enhanced the receiving light intensities of SW, which further boosted the antitumor efficacy. Meanwhile, the strong X-ray attenuation capacity of both tungsten and tin elements qualified the USWs as excellent radio-sensitizers for radiotherapy (RT) enhancement, which played a complementary role with PDT treatment because PDT-mediated induction arrested the cells in the G0-G1 cell cycle phase, and RT was more damaging toward cells in the G2/M phase. The remarkably enhanced UC-PDT/RT efficiency of USWs was next validated in vitro and in vivo, and the combined NIR light and ionizing irradiation treatment completely suppressed tumor growth, revealing its great potential as an efficient anticancer therapeutic agent against solid tumors.

## 1. Introduction

As a promising complement to conventional cancer treatment in recent years, photodynamic therapy (PDT) is known for its minimally invasive and highly tumor-localized features [1]. Generally, PDT involves the generation of reactive oxygen species (ROS) (mostly $^1O_2$, $\cdot OH$, and $H_2O_2$) activated by light-excited photosensitizers (PSs), inducing cell death and tumor blood vascular damage [2]. But in fact, most solid tumors are located several millimeters under human skin, while almost all PSs can but be effectively excited by ultraviolet (UV) or visible light, which suffers greatly from limited penetration depth in tissues, resulting in undesirable tumor-killing efficiency [3]. Considering this conflict, upconversion nanoparticles (UCNPs), an “intermediary agent” that can convert deeper penetrated near-infrared (NIR) light into UV or visible light, are get noticed and incorporated into PDT systems in which the UCNPs and PSs are designed as an integrated nanostructure that activates PSs at a much deeper site under human tissues [4]. Good matching of the PS absorption and emission by the UCNPs is a basic requirement for efficient ROS generation in this strategy. For the perfect situation, however, the absorption region should comprise the entire emission light so that all the upconverted photon energy

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[1] http://people.ucas.ac.cn/~0003699?language=en

https://doi.org/10.1016/j.biomaterials.2017.11.013
0142-9612/

can be effectively utilized by PSs, producing ROS maxima at the same time. Unfortunately, previously reported NIR-induced PDT strategies, in which the selected PSs have limited capabilities that only absorb parts of the UCNP-converted emission light (for example, UV light), have always overlooked the importance of "the perfect matching and the best efficiency" principle, which substantially decreases the PDT efficiency [5–7]. Moreover, those elaborated nanostructures suffer greatly from the intricate preparation procedures and compromised reproducibility of PDT results, which are not favored for clinical use [8,9]. Therefore, it is essential to develop a desirable PS that make "the best matching" with UCNP and redesign an easy-to-get nanoconstruct for optimal and repeatable efficiency in NIR-induced PDT treatment.

Compared to traditional molecular photosensitizers whose therapeutic efficacy may be limited by the availability of surrounding molecular oxygen [10,11], inorganic semiconductor nanoparticles (such as $TiO_2$ and ZnO) take advantage of the type I PDT process that directly activates free radicals from substrate molecules, such as water, oxygen or the cell membrane [12], making them the primary choice as PSs in PDT. Among various semiconductor materials, tin tungstate ($SnWO_4$) caught our attention because its unique band structure has remarkable visible-light-active photocatalytic performance, which is not detected in any other tungsten-based binary or ternary compounds [13,14]. With the broad UV–vis absorption region, which can utilize all emission light of UC-converters, and the stable light-activated-ROS capability for inducing cell death, nano-$SnWO_4$ was hypothesized as a competitive candidate solving the above "best matching" problem. Interestingly, β-$SnWO_4$ nanoparticles were recently systematically evaluated in vitro and in vivo by Seidl et al., and they were validated and suggested as a new photosensitizer with high biocompatibility, lower side effects as well as significant antitumor effect under blue-light LED illumination in PDT [15,16]. Besides, based on our previous studies [10,17,18], with the presence of high-Z elements tungsten ($Z = 74$) and tin ($Z = 50$) in the lattice of $SnWO_4$ nanocrystals, nano-$SnWO_4$ could analogously serve as a potential radiosensitizer toward cancer radiotherapy (RT), and the additional tin element would presumably result in a more enhanced radio-therapeutic efficiency than for tungsten-based binary compounds ($W_{18}O_{49}$ nanowires, $WO_{3-x}$ nanodots, $WS_2$ quantum dots and so on) [19–21], which is rooted in the concept that high-Z elements contribute to the efficacy of radiation-mediated cellular damage [22].

Considering these issues together, we reported a well-designed UCNP-$SnWO_4$ nanohybrid (USW) with the maximal external NIR light-to-ROS productivity in PDT as well as increased radiosensitivity of RT. The synthesized $SnWO_4$ nanocrystals (SW), which can be activated by both UV and visible light (wavelength below 600 nm), were considered the ideal medium to absorb all UV and blue light emitted by the core-shell $NaYF_4$:Yb,Tm@$NaGdF_4$ nanoparticles (UCNP) under 980 nm NIR laser irradiation, realizing the perfect energy transfer, which was not achieved in previous NIR-triggered PDT systems. Meanwhile, while W and Sn as high-Z metals are able to emit photo/compton/auger electrons to produce free radicals under X-ray irradiation, SW could also be used to sensitize the RT-mediated cell killing. The combination of the two treatments would significantly improve its therapeutic efficiency, as the cells in the latter part of the S phase of the cell cycle that are not sensitive to ionizing radiation become more fragile and vulnerable with PDT [23–25], and PDT-induced irreversible arrest of the cells in the G0-G1 phase could further inhibit cell division and proliferation [26], achieving a complementary effect between PDT and RT. After surface modification, SWs were covalently conjugated with water-stable UCNPs, a controllable, repeatable yet easy-to-handle PS-loading strategy to form the final USWs. Unlike the traditional PS loading method that amount of molecular PS attached to a single UCNP core, which is doubtful whether all PSs are effectively activated, the needle-like SWs with a large length-diameter ratio have enough space to immobilize plenty of UCNPs on one core, so that each SW nanoparticle could be activated by sufficient intensity of light upconverted through numerous tiny UCNPs, producing ROS in a "full-loaded" way. The design of the USW is illustrated in Fig. 1, and its capacity to suppress tumor growth was then tested in vitro and in vivo to demonstrate its feasibility as a photosensitizer and radiosensitizer for tumor treatment.

## 2. Experimental section

### 2.1. Materials

$YCl_3 \cdot 6H_2O$, $YbCl_3 \cdot 6H_2O$, $GdCl_3 \cdot 6H_2O$, $TmCl_3$, $SnCl_2 \cdot 2H_2O$, $Na_2WO_4 \cdot 2H_2O$, 1-octadecene, oleic acid, oleylamine, ammonium fluoride ($NH_4F$) were purchased from Sigma-Aldrich. EDC, NHS, 1,3-diphenylisobenzofuran (DPBF), PEG-$NH_2$ were obtained from Aladdin. NaOH, methanol, cyclohexane, ethanol, ammonium hydroxide were gained from Sinopharm Chemical Reagent Co. All reagents were used without any further purification. The 2-well chamber slide system were gained from Thermo Fisher Scientific, and the Anti-Histone H2A.X (phospho S139) antibody (Alexa Fluor® 488) were purchased from Abcam Co.

### 2.2. Characterization

Transmission electron microscopy (TEM) images were acquired on the electron microscope (JEM-2100F) working at 200 kV. Scanning electron microscope (SEM) images were performed on a Hitachi SU8200 UHR FE-SEM, Japan. X-ray diffraction patterns were obtained on a Rigaku D/MAX-2250 V diffractometer. Fourier transform infrared (FT-IR) spectra were recorded on a Varian 3100 FTIR spectrometer with the aid of KBr pellets. The hydrodynamic size distribution was measured on a Microtrac Nanotrac Wave II (Microtrac Inc, America). Ultraviolet–Visible–Near infrared spectra was conducted on a UV-3101PC Shimadzu spectroscope. Fluorescence spectra measurement were carried out using a Fluorolog-3 Spectrofluorometer (Jobin Yvon, France), with an external tunable 980 nm laser. Confocal laser scanning microscopic (CLSM) images were collected on the Olympus FluoView FV1000 Confocal Microscope, Japan. Cell apoptosis were assessed on a guava easycyte 6HL-2L flow cytometry, Germany.

### 2.3. Synthesis of the core-shell upconversion nanoparticles (UCNPs)

The $NaYF_4$: 20%Yb, 0.5% Tm core was synthesized by a pyrolysis process. Briefly, 1.59 mmol $YCl_3 \cdot 6H_2O$, 0.4 mmol $YbCl_3 \cdot 6H_2O$ and 0.01 mmol $TmCl_3$ were mixed into a 100-mL flask with 30 mL of 1-octadecene and 15 mL of oleic acid, which was stirred for 1 h before it was slowly heated to remove water under an argon atmosphere. After cooling the solution to room temperature, 8 mL of methanol solution was dissolved with 5 mmol NaOH, and 8 mmol $NH_4F$ was quickly added into the system before continuous stirring for 2 h. Subsequently, the solution was heated again to remove methanol; it was heated to 280 °C and kept for 90 min under argon atmosphere. Then, the solution was cooled to room temperature. The cores were obtained by washing three times with cyclohexane/ethanol.

For coating a thin $NaGdF_4$ shell on the aforesaid core, a similar thermal decomposition method was used. First, 0.4 mmol $GdCl_3 \cdot 6H_2O$ in deionized water was mixed with 30 mL of 1-octadecene and 15 mL of oleic acid. Then, water was removed to