Biomaterials 63 (2015) 115–127

Contents lists available at ScienceDirect

Biomaterials

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

An imaging-guided platform for synergistic photodynamic/photothermal/chemo-therapy with pH/temperature-responsive drug release

Ruichan Lv [a], Piaoping Yang [a, *], Fei He [a], Shili Gai [a], Guixin Yang [a], Yunlu Dai [a], Zhiyao Hou [b], Jun Lin [b, *]

[a] Key Laboratory of Superlight Materials and Surface Technology, Ministry of Education, College of Material Sciences and Chemical Engineering, Harbin Engineering University, Harbin 150001, PR China
[b] State Key Laboratory of Rare Earth Resource Utilization, Changchun Institute of Applied Chemistry, Chinese Academy of Sciences, Changchun 130022, PR China

ARTICLE INFO

Article history:
Received 31 January 2015
Received in revised form
1 May 2015
Accepted 14 May 2015
Available online 23 May 2015

Keywords:
Up-conversion
Photodynamic
Photothermal
pH/temperature-responsive

ABSTRACT

To integrate biological imaging and multimodal therapies into one platform for enhanced anti-cancer efficacy, we have designed a novel core/shell structured nano-theranostic by conjugating photosensitive $Au_{25}(SR)_{18}$ − (SR refers to thiolate) clusters, pH/temperature-responsive polymer P(NIPAm-MAA), and anti-cancer drug (**doxorubicin, DOX**) onto the surface of mesoporous silica coated core−shell up-conversion nanoparticles (UCNPs). It is found that the photodynamic therapy (PDT) derived from the generated reactive oxygen species and the photothermal therapy (PTT) arising from the photothermal effect can be simultaneously triggered by a single 980 nm near infrared (NIR) light. Furthermore, the thermal effect can also stimulate the pH/temperature sensitive polymer in the cancer sites, thus realizing the targeted and controllable DOX release. The combined PDT, PTT and pH/temperature responsive chemo-therapy can markedly improve the therapeutic efficacy, which has been confirmed by both *in intro* and *in vivo* assays. Moreover, the doped rare earths endow the platform with dual-modal up-conversion luminescent (UCL) and computer tomography (CT) imaging properties, thus achieving the target of imaging-guided synergistic therapy under by a single NIR light.

1. Introduction

For the drug delivery systems (DDSs), if referred to imaging-guided photodynamic therapy (PDT) and photothermal therapy (PTT), two irrelevant lights were usually utilized to achieve the diagnosis and therapy which made it hard for real-time assessment. Meanwhile, the loaded drug molecules are confronted with the leak problem before arriving at the tumor focus, which may introduce serious side damage to the normal organs and body [1–7]. The up-conversion luminescent (UCL) emission generated upon NIR excitation is potential to track the treatment, and the UCL emissions in visible regions could be used as the donors for transferring energy to photosensitizers [8–11]. Moreover, NIR light located in the optical transmission window of biological specimens has the merits of high penetration depth, high detection sensitivity, increased image contrast, and low damage to cells [12–16].

The traditional organic dyes as photosensitizers have some disadvantages, such as poor water solubility, high skin toxicity, low selectivity, instable physical/chemical property, and other ambiguous problems [17–19]. Thus, developing novel inorganic materials as the photosensitizers is of great importance. Recent research progress on the solution-phase atomically precise thiolate-protected $Au_n(SR)_m^-$ clusters have taken up part of photosensitizers field [20–22]. Especially, $Au_{25}(SR)_{18}^-$ clusters with higher body clearance and lower kidney toxicity have been proposed to produce $^1O_2$ production owing to its high stability, long lifetime of the electronic excited states, presence of triplet excited states with which the adsorbed $^3O_2$ could be transferred to $^1O_2$ [23,24]. Meanwhile, $Au_{25}(SR)_{18}^-$ clusters may also generate the photothermal effect owing to the strong electromagnetic fields inside particles caused by their closely positioned and coupled sharp features [25,26]. When the PDT agent is used for *in vivo* clinical

* Corresponding authors.
E-mail addresses: yangpiaoping@hrbeu.edu.cn (P. Yang), jlin@ciac.ac.cn (J. Lin).

http://dx.doi.org/10.1016/j.biomaterials.2015.05.016
0142-9612/

application, short irradiation time, high $^1O_2$ quantum yield and low laser pump power are required. The luminescent spectral overlap between the donor (phosphor) and acceptor (photosensitizer), the distance between the two counterparts, and the quantity of loaded photosensitizers all play important role in the $^1O_2$ yield. Thus, the structure design of the UCL host/dopant and material structure is essential [27–31]. The silica coated functional materials have been especially used as effective drug carriers to reduce the toxicity of the inorganic and organic materials [32–36]. By controlling the shell thickness of mesoporous silica, the BET surface which decides the quantity of loaded nanoparticles and the distance between donors and acceptors could be adjusted. Particularly, the large surface area, porous structure, adjustable pore channel, and easily modified surface of mesoporous silica make it possible to store more drug molecules.

For the conventionally synthesized multi-functional nanoparticles with silica or hollow structure for the DDSs, the anticancer drug molecules in these systems always cannot distinguish the normal cells from the cancer ones. Besides, they may show an early leak and uncontrollable release under the normal body fluid environment (37 °C, neutral) [37,38]. To resolve this formidable challenge, the gatekeepers are needed to regulate the release and prevent the early leak before entering the tumor cells [39,40]. In recent years, pore blocking caps have been proposed such as polymer brushes and macromolecule chains which could be self-changed under various conditions. Poly(*N*-isopropyl acrylamide) (P(NIPAm)), as one of the most important temperature-sensitive polymer, could translate its phase in aqueous environment under changed temperature which is suitable for blocking pores as a gatekeeper. Especially, by conjugating co-monomer or tuning the environment pH value, the low critical solution temperature (LCST) of the P(NIPAm) polymer can be changed which is above normal body temperature (even higher than 50 °C under pH value of 7.4) [41–44]. In the tumor-bear body, the tumor focus always has higher temperature and lower pH value than the normal ones due to the inflammation and cancer cell immortalization [45]. Thus, the positive pH/temperature-sensitive "on-off" drug release is attractive to apply in the anti-cancer therapy DDSs because photothermal effect could be generated due to the photosensitive agent irradiated by NIR laser and they can intelligently distinguish the normal cells from the tumor ones due to the characteristic property of cancer focus.

In this study, we proposed a facile and mass-production method to construct core/shell structured Y_2O_3:Yb,Er@Y_2O_3:Yb@mSiO_2-Au_{25}-P(NIPAm-MAA) (YSAP) up-conversion nanoparticles with adjustable shell thickness. An inert Y_2O_3:Yb shell was employed to enhance to the UC emission intensity of Y_2O_3:Yb,Er core. The silica shell thickness is controlled by adjusting the amount of added TEOS. Meanwhile, $Au_{25}(SR)_{18}^-$ clusters with size of 2.5 nm were conjugated and well dispersed in the silica mesopores to produce PDT and PTT effect by receiving energy from the UCNPs. Meanwhile, the pH/temperature-responsive polymer NIPAm-MAA is introduced to realize the targeted and controllable release triggered by the high temperature arising from the system under NIR irradiation and low pH condition in the cancer cells. The biocompatibility, dual-modal imaging (CT and UCL), especially *in vitro* and *in vivo* toxicity of Y_2O_3:Yb,Er@Y_2O_3:Yb@mSiO_2-Au_{25}-P(NIPAm-MAA) have also been investigated in detail.

2. Experimental section

2.1. Reagents and materials

All the chemical reagents in this experiment are of analytical grade and used without any further purification, including urea, hydrochloric acid (HCl), nitric acid (HNO_3), $HAuCl_4 \cdot 3H_2O$, methanol, dimethyl sulfoxide (DMSO) (Beijing Chemical Corporation), Y_2O_3, Yb_2O_3, and Er_2O_3 (Sinopharm Chemical Reagent Co., Ltd.), cetyltrimethyl ammonium Bromide (CTAB), NaOH, tetraethoxysilane (TEOS), ammonium nitrate (NH_4NO_3), 1,4-dioxane, phosphate buffered saline (PBS), potassium hydrogen phthalate (PHP), glutaraldehyde (Tianjin Kermel Chemical Co., Ltd.), diphenyl (2, 4, 6-trimethylbenzoyl)-phosphine oxide (TPO) (Energy Chemical), and aminopropyltrimethoxysilane (APTES), tetraoctylammonium bromide (TOAB), $NaBH_4$, N-isopropyl acrylamide (NIPAm), methacrylic acid (MAA), DOX, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), nhydroxysuccinimide (NHS), folic acid (FA), 3–4,5-dimethylthiazol-2-yl-2,5-diphenyl tetrazolium bromide (MTT), 4′,6-diamidino-2-phenylindole (DAPI), calcein AM and propidium iodide (PI), trypan blue (Sigma–Aldrich).

2.2. Synthesis

2.2.1. Synthesis of Y(OH)CO_3:Yb,Er

Briefly, by dissolving corresponding Ln_2O_3 (Ln = Y, Yb, and Er) into HNO_3 under heating, 1 mmol of $Ln(NO_3)_3$ solutions were obtained. In the co-precipitation process, 1 mL of $Ln(NO_3)_3$ (94%Y, 5% Yb, and 1%Er) were mixed with 3 g of urea in a beaker containing 50 mL of deionized water. The solution was heated to 90 °C and kept for 3 h. After washed several times, Y(OH)CO_3:Yb,Er precursor was prepared.

2.2.2. Synthesis of Y(OH)CO_3:Yb,Er@Y(OH)CO_3:Yb

The coating process of the Y(OH)CO_3:Yb was similar to the precursor. Firstly, the as-synthesized Y(OH)CO_3:Yb,Er was dispersed into 50 ml deionized water and ultrasonic treated, and then 1 mmol of $Ln(NO_3)_3$ (95%Y and 5%Yb) and 3 g of urea were mixed and dissolved into the solution. After stirring for another 5 min, the mixture was kept stable with water bath at 90 °C for 3 h. The resulting precipitate was centrifuged and dried at 60 °C for 12 h, and the Y(OH)CO_3:Yb,Er@Y(OH)CO_3:Yb spheres were obtained.

2.2.3. Synthesis of core/shell Y_2O_3:Yb,Er@Y_2O_3:Yb@mSiO_2

Before the coating process, the Y(OH)CO_3:Yb,Er@Y(OH)CO_3:Yb spheres were firstly calcinated at 600 °C for 3 h in order to obtain Y_2O_3:Yb,Er@Y_2O_3:Yb spheres. Here, NaOH solution instead of conventional $NH_3.H_2O$ solution was used to give rise to the facile and fast coating process. Typically, 0.15 g of Y_2O_3:Yb,Er@Y_2O_3:Yb and 0.1 g of CTAB was mixed with 10 mL ethanol and 40 mL of deionized water with continuous stirring, and then 3 mL of NaOH (1 mol L^{-1}) was added. Then, the mixture was heated up to 70 °C, and then 150 μL of TEOS was added slowly. After kept for 10 min, the mixture was centrifuged and washed with ethanol and water for several times. To remove CTAB surfactant, the as-synthesized silica-coated spheres were mixed with 100 mL of ethanol with 1 g of NH_4NO_3, and then kept at 70 °C for 2 h. After that, the mixture was centrifuged and washed with ethanol several times. Finally, the Y_2O_3:Yb,Er@Y_2O_3:Yb@mSiO_2 composite was obtained after drying at 60 °C for 12 h. The samples with different silica shell thickness were synthesized by adjusting the amount of added TEOS.

2.2.4. Synthesis of $Au_{25}(SR)_{18}^-$ clusters

In a typical process, 78.7 mg of $HAuCl_4 \cdot 3H_2O$ and 126.8 mg of TOAB were dissolved in 10 mL of methanol solvent and stirred for 20 min vigorously. After that, 1 mmol of captopril dissolved in 5 mL of methanol was rapidly added into the solution, which was further stirred for another 30 min. Then, 2 mmol of $NaBH_4$ dissolved in 5 mL of ice-cold deionized water was rapidly injected in the