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Photosensitizer-conjugated silica-coated gold nanoclusters for fluorescence imaging-guided photodynamic therapy

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ABSTRACT

Multifunctional theranostics have recently been intensively explored to optimize the efficacy and safety of therapeutic regimens. In this work, a photo-theranostic agent based on chlorin e6 (Ce6) photosensitizer-conjugated silica-coated gold nanoclusters (AuNCs@SiO_2–Ce6) is strategically designed and prepared for fluorescence imaging-guided photodynamic therapy (PDT). The AuNCs@SiO_2–Ce6 shows the following features: i) high Ce6 photosensitizer loading; ii) no non-specific release of Ce6 during its circulation; iii) significantly enhanced cellular uptake efficiency of Ce6, offering a remarkably improved photodynamic therapeutic efficacy compared to free Ce6; iv) subcellular characterization of the nanoformula via both the fluorescence of Ce6 and plasmon luminescence of AuNCs; v) fluorescence imaging-guided photodynamic therapy (PDT). This photo-theranostics owns good stability, high water dispersibility and solubility, non-cytotoxicity, and good biocompatibility, thus facilitating its biomedical applications, particularly for multi-modal optical, CT and photoacoustic (PA) imaging-guided PDT or sonodynamic therapy.

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1. Introduction

The development of activatable theranostics with the capacity to respond to a given stimulus has attracted extensive interest owing to their dramatically enhanced selectivity and specificity for disease destruction with highly localized cytotoxicity and little collateral damage [1–7]. So far, both exogenous and endogenous stimuli (such as temperature, pH, applied magnetic/electrical field, ultrasound, light, and enzymatic action, etc.) have been developed to control the activation of theranostics [8–16]. Light stimulus, as a particularly attractive and convenient option, owing to its specific spatial and temporal controllable ability, has been extensively applied for photodynamic therapy (PDT), photothermal therapy (PTT) and photo-triggered chemotherapy [17–29].

PDT is an emerging external light activatable therapy for various diseases involving three key components: the photosensitizer (PS), light (typically a laser), and tissue oxygen [30–32]. After systemic, local, or topical administration of PS with sufficient incubation period, selective illumination is conducted at the region of interest with appropriate wavelength and power of light [33]. Upon illumination, PS is able to transfer the absorbed photon energy to surrounding oxygen molecules, generating reactive oxygen species (ROS) such as singlet oxygen (SO) or free radicals to induce cell death and tissue destruction without damage to the adjacent healthy tissue in the dark [34]. However, most PSs are limited by prolonged cutaneous photosensitivity, poor water-solubility and inadequate selectivity [12,35–37]. Hydrophobic nature of most PSs results in strong self-aggregation in aqueous media, which significantly reduces their photodynamic efficacy because only monomeric species are appreciably photoactive [21]. To address these issues, various nanocarriers, such as liposomes, polymeric nanoparticles, proteins, ceramic nanoparticles, carbon nanomaterials, gold nanoparticles, quantum dots (QDs), magnetic nanoparticles (MNPs), upconversion nanoparticles (UCNPs) and so on, have been developed to fabricate a stable dispersion of PDT pharmaceutical formulations in aqueous systems for effective delivery of PSs [35–44].

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To date, two main strategies have been employed for the delivery of PSs: (1) Encapsulation, in which PSs are loaded in the core of core–shell structured nanocarriers or mixed in the matrix of nanocarriers [3,36,37,45]. (2) Conjugation, in which PSs are conjugated with oligonucleotides, monoclonal antibodies, carrier proteins, lipids, carbohydrates, hydrophilic polymers, or organic coupling agent on the surface of inorganic nanocarriers [3,36,37,46–48]. It is understood that PDT does not require the release of PSs to directly interact with the lesion cells/tissues, but the ROS generated by PSs upon illumination need to interact with diseased cells to achieve the photodynamic efficacy [49,50]. If suitably formulated, it will be able to avoid the leakage of PSs in the blood circulation without compromising the photodynamic efficacy of PSs [51]. Comparing encapsulation with conjugation, the encapsulation strategy has the following disadvantages: (1) Most of them suffer from poor drug loading and increased self-aggregation of the drug in the entrapped state [21,45]. (2) The shell layer materials or the matrix will impair the absorbed photon energy of PSs [33,49,50]. (3) In the process of PDT, the dispersion of ROS will react with the shell layer materials or the matrix, especially polymeric materials, resulting in lower photodynamic efficacy than free PSs [12].

Recently, we developed a multifunctional drug delivery system based on photosensitizer-conjugated MNPs that was strategically designed and prepared for simultaneous targeting PDT and dual-modal near-infrared (NIR) fluorescence imaging and MRI of gastric cancer tissue *in vivo* [33]. We found that the covalently incorporated Ce6 molecules retained their spectroscopic and functional properties for NIR fluorescence imaging and PDT, and the core MNPs offered the functions of magnetically guided drug delivery and magnetic resonance imaging (MRI). Furthermore, we developed a theranostic platform based on Ce6-conjugated carbon dots (C-dots-Ce6). Our results indicated that C-dots-Ce6 is a good candidate with excellent imaging and tumor-homing ability for NIR fluorescence imaging-guided PDT treatment [34]. Ce6-conjugated hexagonal phase $NaYF_4$:Yb,Er/$NaGdF_4$ core–shell upconversion nanoparticle (UCNP) platform was also developed for *in vivo* dual-modal luminescence imaging and MRI, and PDT treatment [52]. By taking the above-mentioned facts into consideration, the PS conjugation strategy is a better choice than PS encapsulation strategy in PDT, since the conjugation strategy owns high drug loading, does not affect the absorbance of the PSs, effectively avoids self-aggregation and leakage of the PSs, and blocks the direct interaction between PSs and matrices [34,38].

Many types of fluorescent materials, such as QDs, and UCNPs have been applied for optical imaging and as platforms for drug/gene delivery. However, most traditional QDs contain heavy metal elements (such as Cd^{2+}, Pb^{2+}, etc.). The cytotoxicity of the released heavy metal ions in biological systems and potential environmental hazard of these ions limit further applications of QDs in theranostics [53–55]. For UCNPs, the lanthanide complexes as a typical delegate have disadvantages in thermal stability and mechanical stability which limit further applications [56]. On the other hand, noble metal nanoclusters such as gold nanoclusters (AuNCs) are highly attractive because of their high fluorescence, good photostability, non-toxicity, excellent biocompatibility and water-solubility [57]. Herein, we constructed a photo-theranostics based on Ce6-conjugated silica-coated gold nanoclusters (AuNCs@SiO_2–Ce6) for fluorescence imaging-guided PDT.

2. Materials and methods

2.1. Synthesis of AuNCs

Gold nanoclusters (AuNCs) were prepared by a previously reported method [58]. Briefly, 10 ml of $HAuCl_4$ solution (10 mM) was added into 10 ml of bovine serum albumin (BSA) solution (50 mg/ml) under vigorous magnetic stirring for over 2 min at 37 °C, then 250 ul of L-ascorbic acid (0.35 mg/ml) was added by dropwise. After 5 min, 1 ml of NaOH solution (1 M) was introduced, and the resulting mixed solution was incubated at 37 °C for 9 h. The color of the solution turned into light brown and finally changed into dark brown. The reaction mixture was kept in the refrigerator (4 °C) under the dark for further use.

2.2. Synthesis of AuNCs@SiO_2

Silica coating was conducted to obtain a core–shell structure fluorescence nanoparticle via a modified Stöber method. In a typical experiment, 200 μl of AuNCs was added into 20 ml of alcoholic solution containing 800 μl of ammonia (ca. 28 wt %) under sonication for 5 min. Then, 200 μl of tetraethylorthosilicate (TEOS, 99.9%) was added under vigorous magnetic stirring for 1 h. Afterwards, another 200 μl of TEOS was added under vigorous stirring. The entire system was vigorously stirred for 24 h. AuNCs@SiO_2 were collected by centrifugation at a speed of 9000 rpm and washed with ethanol and deionized (DI) water for several times. The obtained purified AuNCs@SiO_2 samples were redispersed into DI water for further characterization and application.

2.3. Quantification of the number of AuNCs in a single AuNCs@SiO_2

The obtained purified AuNCs@SiO_2 samples were collected by centrifugation and dried at 60 °C for 3 h in vacuum oven. Then the dry powder of AuNCs@SiO_2 was put in a quartz crucible and calcined at 600 °C for 2 h. In the process of calcination, the bovine serum albumin (BSA) molecules capping on the surface of AuNCs will decompose into CO_2 and H_2O, then all of AuNCs will fuse together into a single GNP.

2.4. Synthesis of AuNCs@SiO_2–Ce6

Excess amount of 3-aminopropyltrimethoxysilane (APTS) was added to the AuNCs@SiO_2 solution with rapid stirring for 12 h. The resultant was washed with DI water for several times. APTS modified silica-coated AuNCs (AuNCs@SiO_2–NH_2) were terminated with amine groups. Covalent binding of Ce6 to the AuNCs@SiO_2–NH_2 was performed using a standard EDC–NHS reaction. Carboxyl groups of Ce6 (2 mg) were activated by an EDC/NHS solution for 30 min. Following activation, 6 mg of AuNCs@SiO_2–NH_2 were added to form a mixed solution and allowed to react at room temperature for 12 h. The resultants were washed five times by DI water and ethanol alternatively for removing unreacted chemicals by centrifugation at 9000 rpm for 10 min. The product was dispersed in PBS buffer (pH = 7.4) for further characterization and application.

2.5. Characterization of AuNCs@SiO_2–Ce6

The morphology and size of the aqueous dispersion of the samples were characterized using transmission electron microscopy (TEM) (FEI Tecnai 12), operating at an accelerating voltage of 120 kV. UV–vis spectra were measured by a Genesys 10S UV–vis Spectrophotometer (Thermo Scientific, Waltham, MA). Fourier transform infrared (FT-IR) spectroscopy of AuNCs@SiO_2–Ce6 was recorded using a Perkin–Elmer spectrum GX spectrophotometer. Fluorescence spectra were recorded on a Hitachi F-7000 spectrofluorometer. The surface charge of the samples was measured with Zeta potential measurements in water (NICOMP 380ZLS zeta potential/particle size analyzer).

2.6. Conjugation and loading efficiency measurements

UV–vis measurements of AuNCs@SiO_2–Ce6 at different concentrations were carried out. Loading efficiency of AuNCs@SiO_2–Ce6 was also calculated by the Ce6 UV calibration curve at 663 nm (in PBS solution). Every experiment was repeated three times.

2.7. Singlet oxygen detection

Singlet oxygen sensor green (SOSG) that is highly selective for singlet oxygen, was used to evaluate the singlet oxygen generation (SOG) of AuNCs@SiO_2–Ce6. The concentration of Ce6 in AuNCs@SiO_2–Ce6 is fixed at 0.5 μM. The mixture solutions were irradiated with a 671 nm laser (200 mW/cm^2). SOSG was dissolved in water containing 2% methanol with the final concentration of 1 μM. SOSG fluorescence emission was produced using an excitation wavelength of 494 nm. The sample's SOG was evaluated by the SOSG fluorescence enhancement compared with the background or control samples.

2.8. Subcellular localization and uptake efficiency

MDA-MB-435 cell line was cultured in L-15 medium containing 10% fetal bovine serum and incubated at 37 °C in a humidified 5% CO_2 atmosphere. For subcellular colocalization, cells (1×10^4 cells per well) were seeded in 8-well lab-Tek chamber slide and incubated overnight. Then the cells were incubated with 10 μM AuNCs@SiO_2–Ce6 in the dark for 2 h. After being rinsed with PBS (pH 7.4) for three times, the cells were stained by DAPI (4′, 6-diamidino-2-phenylindole) and Alexa