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Multifunctional hetero-nanostructures of hydroxyl-rich polycation wrapped cellulose-gold hybrids for combined cancer therapy

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ABSTRACT

The development of new hetero-nanostructures for multifunctional applications in cancer therapy has attracted widespread attention. In this work, we put forward a facile approach to synthesize multifunctional hetero-nanostructures of cellulose nanocrystal (CNC)-gold nanoparticle hybrids wrapped with low-toxic hydroxyl-rich polycations to integrate versatile functions for effective cancer therapy. Biocompatible CNCs with the superior rod-like morphology for high cellular uptake were employed as substrates to flexibly load spherical gold nanoparticles (Au NPs) or gold nanorods (Au NRs) through gold-thiolate bonds, producing hetero-layered nanohybrids of CNC-Au NPs or CNC-Au NRs. Profound hydroxyl-rich cationic gene carrier, CD-PGEA (comprising β-cyclodextrin cores and ethanolamine-functionalized poly(glycidyl methacrylate) arms), was then assembled onto the surface of CNC-Au nanohybrids through host-guest interaction and gold-thiolate bonds, where PEG was employed as the intermediate and spacer. The resultant CNC-Au-PGEA hetero-nanostructures exhibited excellent performances as gene carriers. Furthermore, CNC-Au NR-PGEA comprising Au NRs demonstrated favorable optical absorption properties and were validated for photoacoustic imaging and combined photothermal/gene therapy with considerable antitumor effects. The present work provided a flexible strategy for the construction of new multifunctional hetero-nanostructures with high antitumor efficacy.

1. Introduction

Traditional methods for cancer treatment such as surgical excision, chemotherapy and radiation therapy face great challenges due to low efficiency and safety issues [1,2]. Gene therapy offers a promising approach in treating genetic diseases including cancers [3–5]. Recently, the conversion of near-infrared (NIR) light energy to thermal energy (photothermal therapy, PTT) with minimal invasion has been received considerable attention [6,7]. On the other hand, the demand for imaging techniques such as X-ray computed tomography (CT), magnetic resonance (MR) and photoacoustic (PA) imaging is pressing for cancer treatment [8]. PA imaging as an emerging diagnostic method is particularly attractive because of high resolution and penetration depth [9,10]. It will be desirable to develop a multifunctional platform enabling imaging and combined gene/PTT therapy within one nanostructure and achieving optimal antitumor efficacy, which still remains a great challenge.

Utilization of nanostructured system for biomedical application has been generally paid attention [11,12]. Hetero-nanostructures with controlled morphology comprising discrete domains of two or more components have been extensively exploited owing to the integration of multi-functions, which allows enhanced imaging and multimodal therapeutic applications [13–17]. Gold nanoparticles with many virtues demonstrate diverse applications in biomedical areas, such as CT imaging and CT-guided therapy [18,19]. Gold nanorods (Au NRs) stand as the appealing representative for applications in PA imaging and PTT [20–23]. Compared with the spherical nanoparticles, rod-shaped nanoparticles are more likely to be swallowed by cells [24,25]. Cellulose as a natural polysaccharide is biodegradable, non-toxic and renewable [26]. Recent work indicates that biocompatible cellulose nanocrystals (CNCs) are promising in constructing gene carriers due to their rod-shaped morphology and rich hydroxyl groups on the surface for functionalization [27,28]. Hybrids of CNCs and gold nanoparticles could integrate their individual advantages for multifunctional applications in cancer therapy. Few work focuses on hybrids of CNC-spherical gold nanoparticles (Au NPs), where polymer brushes on the surface of

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CNCs were employed as capping agents and/or reductants [28,29]. However, to the best of our knowledge, hybrids of CNC and Au NRs have not been reported yet. Thus, it would be desirable to develop flexible strategies to straightforward fabricate hybrids of CNC and gold nanoparticles with different morphologies. Moreover, more functionality integrated into CNC-Au hybrids would be appealing for the design of new multifunctional hetero-nanostructures.

Host-guest assembly based on β-cyclodextrin (CD) and adamantine (Ad) offers a facile and efficient strategy to introduce new functionalities to cancer therapy systems, such as drug/gene therapy, targeting and fluorescent imaging [30–33]. In this work, two kinds of organic-inorganic hetero-nanostructures were synthesized through gold-thiolate bonds and CD-Ad host-guest interaction for effective biomedical applications. The rigid part is CNC-Au (CNC-Au NP or CNC-Au NR) hetero-layered hybrid composed of CNC and Au NP or Au NR, while the flexible part is cationic CD-PGEA, a type of low-toxic hydroxyl-rich cationic gene carrier comprising β-CD cores and BUCT-PGEA arms (poly (glycidyl methacrylate) (PGMA) functionalized with ethanolamine (EA)) [34]. This rational design ensures the integration of the favorable properties of CNCs, CD-PGEA and Au NRs as well as the corresponding functions of gene therapy, PTT and PA imaging. CNC-Au hybrids were first synthesized through the interactions between dithiolane rings from lipoic acid (LA) modified CNCs and Au NPs or NRs through multiple gold-thiolate bonds [35–37]. For the construction of CNC-Au-PGEA hetero-nanostructures (CNC-Au NP-PGEA or CNC-Au NR-PGEA), polyethylene glycol (PEG) with one end conjugated with Ad and the other end with LA was prepared as intermediate (Ad-PEG-LA). CNC-Au NRs (or CNC-Au NPs) were conjugated with Ad-PEG-LA through multiple gold-thiolate bonds, followed by the functionalization with CD-PGEA via CD-Ad host-guest assembly (Fig. 1). Ad-PEG-LA as the spacer, could not only increase the connecting sites for CD-PGEA onto the surface of CNC-Au hybrids, but also enhance the flexibility of grafted polycations to benefit the condensation of plasmid DNA (pDNA). The gene transfection mediated by CNC-Au NP-PGEA and CNC-Au NR-PGEA hetero-nanostructures was compared and the morphology of CNCs was proved to dominate. Furthermore, the PA imaging and gene/PTT combined therapy efficacy of the as-fabricated CNC-Au NR-PGEA systems were investigated in details.

2. Materials and methods

2.1. Materials

PEG (Mw ~4000 Da), 2-bromoisobutyryl bromide (98%), 1,1′-carbonyldiimidazole (CDI, 97%), ethylenediamine (ED, > 98%), adamantanecarboxylic acid chloride (Ad-COCl), lipoic acid (LA), 4-dimethylaminopyridine (DMAP), and dicyclohexylcarbodiimide (DCC) were obtained from Sigma-Aldrich Chemical (St. Louis, MO. USA).

2.2. Preparation of CNC-Au nanoparticles

Rod-like CNCs were first prepared following methods reported previously [27]. For surface functionalization, 200 mg of CNC and 100 mg of CDI were dissolved in 20 mL of anhydrous DMSO, and the reaction mixture was stirred at room temperature for 4 h. Then 2 mL of ED was added and the reaction was degassed by bubbling nitrogen for 10 min and stirred at room temperature for 24 h. The product (CNC-NH_2) was precipitated with diethyl ether. CNC-LA was synthesized as follows. CNC-NH_2 (200 mg), LA (123.6 mg), NHS (60 mg) and EDAC (105 mg) were dissolved in 15 mL of anhydrous DMSO, and the reaction was degassed by bubbling nitrogen for 10 min and stirred at 30 °C for 48 h. Au NPs and Au NRs were synthesized following the previous procedures [38].

For the fabrication of CNC-Au NPs or CNC-Au NRs, the as-prepared 0.25 mg/mL of Au NPs or Au NRs were dissolved in 8 mL of deionized water, and 5 mg of CNC-LA dissolved in 2 mL of water was added under sonication. The resultant products were collected by centrifugation and washed with deionized water for two times to remove CTAB and free Au NPs or Au NRs.

2.3. Preparation of CNC-Au NP-PGEA and CNC-Au NR-PGEA hetero-nanostructures

CD-PGEAs of two different molecular weights (CD-PGEA1 from low-molecular-weight CD-PGMA1 and CD-PGEA2 from high-molecular-weight CD-PGMA2) were synthesized and collected following our previously reported methods [34]. Ad-PEG-LA was prepared as follows. 1 g of PEG was dissolved in 20 mL of anhydrous CH_2Cl_2 and kept in an ice bath for 0.5 h. Then, 136 mg of Ad-COCl in 3 mL of CH_2Cl_2 was added dropwise and the reaction mixture was stirred at room temperature for 24 h. The resultant PEG-Ad was precipitated with excess diethyl ether, prior to being dried under reduced pressure. Afterwards, 1 g of Ad-PEG, 190 mg of LA, 94 mg of DCC and 20 mg of DMAP were dissolved in 25 mL of CH_2Cl_2, degassed by bubbling nitrogen for 10 min and stirred at room temperature for 24 h. Ad-PEG-LA was acquired with excess diethyl ether, prior to being dried under reduced pressure.

For the synthesis of hetero-nanostructures, 5 mg of CNC-Au NRs (or CNC-Au NPs) were dissolved in 5 mL of deionized water, and then 15 mg of Ad-PEG-LA was added and stirred at 40 °C for 48 h. The products was collected by centrifugation and washed with deionized water for three times to remove excess Ad-PEG-LA. Thereafter, the precipitate was dissolved in 3 mL of deionized water, followed by the addition of 24 mg of CD-PGEA1 (or 48 mg of CD-PGEA2). The solution was stirred at room temperature for 24 h. The resultant CNC-Au NR-PGEA (or CNC-Au NP-PGEA) was purified and collected by centrifugation and lyophilization.

Details on methods and procedures for the characterization of CNC-Au-PGEA, in vitro cytotoxicity assay, in vitro transfection assay, and determination of cellular internalization are included in Supporting information.

2.4. Photothermal effect of CNC-Au NR-PGEA

To test the photothermal property, CNC-Au NR-PGEA2 aqueous solutions with various concentrations were put into quartz cuvettes, and irradiated under an 808 nm laser (Daheng New Epoch Technology, Inc., Beijing, China) at a power density of 2.0 W/cm^2 for 5 min. A thermal camera (FLIR Systems Inc., Ohio, USA) was used to record the real-time temperature and thermal images. To verify the photothermal effect, 5×10^4 C6 cells were plated in 24-well microtiter plate and incubated for 24 h. Then 12.5 μL of 5 mg/mL CNC-Au NR-PGEA2 was added and incubated for 30 min. Thereafter, the cells were irradiated by 808 nm laser at a power density of 2.0 W/cm^2 for 5 min. The cells after photothermal treatment were stained with FDA and PI for 10 min in dark and imaged using a Leica fluorescence microscope. Control experiments were done in the same way employing C6 cells without CNC-Au NR-PGEA2 as control.

Female BALB/c nude mice (6 weeks old, weight 18–20 g) were obtained from Beijing HFK Bioscience Co., LTD (Beijing, China). Animal studies were approved by Ethical Committee of Chinese Academy of Medical Sciences (CAMS) and Peking Union Medical College and performed under legal protocols. The preparation of C6 tumor-bearing mice and in vivo photothermal experiments followed the previous procedures [39]. Typically, 75 μL of PBS or CNC-Au NR-PGEA2 solution (5 mg/mL) was intratumorally injected. The tumor region was irradiated by an 808 nm laser (2 W/cm^2) for 5 min. An infrared camera was used to record infrared thermal images at different time intervals.

2.5. PA imaging of CNC-Au NR-PGEA

CNC-Au NR-PGEA2 solutions with various concentrations (0.125, 0.25, 0.5, and 1 mg/mL) were loaded in prosthesis supplied by the