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Photothermo-chemotherapy of cancer employing drug leakage-free gold nanoshells

Lu Wang [a, c, 1], Yuanyuan Yuan [a, 1], Shudong Lin [a, 1], Jinsheng Huang [a], Jian Dai [a, c], Qing Jiang [c], Du Cheng [a, *], Xintao Shuai [a, b, **]

[a] PCFM Lab of Ministry of Education, School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, 510275, China
[b] Center of Biomedical Engineering, Zhongshan School of Medicine, Sun Yat-Sen University, Guangzhou, 510080, China
[c] Department of Biomedical Engineering, School of Engineering, Sun Yat-sen University, Guangzhou, Guangdong, 510006, China

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ABSTRACT

Combined photothermo-chemotherapy is a new cancer treatment modality that improves therapeutic outcome by synergistic actions of two different means. A reduction and pH dual sensitive polymeric vesicle encapsulating doxorubicin (DOX) was prepared and then decorated with a gold layer using a modified method of *in situ* gold seed growth. By tuning the compactness of gold layer, the gold nanoshell may possess a desirable light absorption peak for tumor photothermal therapy using near-infrared (NIR) laser irradiation, a method featuring high tissue penetrability essential for *in vivo* applications. The NIR light energy was converted into heat, which killed cancer cells in the vicinity and induced the rupture of nanoshell to release DOX inside tumor. Therefore, a combined photothermo-chemotherapy of tumor can be achieved precisely at tumor site. In addition, DOX released in the thermochemotherapeutic mode effectively penetrated tumor tissue, which is meaningful considering the intrinsic low tissue penetrability of nanomedicines. In nude mice bearing human Bel-7402 hepatoma, the photothermo-chemotherapy using DOX-loaded gold nanoshell appeared advantageous over a chemotherapy or a photothermal therapy alone.

1. Introduction

Chemotherapy as one of the three major approaches for clinic cancer treatment often fails due to the insufficient tumor killing and severe systemic side effects resulting from the inevitable drug resistance and nonspecific action on both the tumor and normal cells. Therefore, novel therapeutic means which may overcome the intrinsic shortages of traditional chemotherapy have drawn great attentions in recent years. For example, noninvasive photothermal therapy based on gold nanomaterials has been intensively investigated for improving cancer treatment [1,2].

The well developed blood vessels in normal human tissues can act as temperature-regulating system. The local blood vessels expand in normal tissues under hyperthermia, accelerating blood flow to take heat away and thus preventing tissue from hyperthermal damage. However, blood flow in tumor generally slows down due to vascular agglomeration, distortion and expansion. When the tumor tissue was heated, its temperature can be 5 to 10 °C higher than that of the adjacent normal tissue, resulting in thermal ablation of tumor without affecting normal tissues. The retarded heat flow resulting from the abnormal vasculature provides a great opportunity for tumor thermotherapy using gold nanomaterials. Various studies have shown that the light absorption peak of conventional gold nanocrystals around 520 nm can be shifted toward the absorption region (650–900 nm) of near-infrared (NIR) light by adjusting their morphology and structure [3,4]. Consequently, optical energy from NIR irradiation can be converted into thermal energy by the nanomaterials based on gold nanocrystals. More importantly, the NIR absorption by hemoglobin and water is fairly weak [5], making NIR irradiation highly penetrative but not harmful to human body. In addition, gold nanoparticles have very low cytotoxicity.

Up to now, various gold nanomaterials including gold nanorods

* Corresponding author.
** Corresponding author. PCFM Lab of Ministry of Education, School of Chemistry and Chemical Engineering, Sun Yat-sen University, Guangzhou, 510275, China.
E-mail addresses: chengdu@mail.sysu.edu.cn (D. Cheng), shuaixt@mail.sysu.edu.cn (X. Shuai).
[1] These authors contributed equally to this work.

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[6], gold nanoshells [7] and gold nanocages [8] with absorption in the NIR region have been developed to show broad application potentials in cancer treatment. In particular, gold nanoshells have strong absorption in the NIR region and good photothermal conversion efficiency [9]. They are generally fabricated using the template method of introducing preformed gold nanoparticles onto the surfaces of polymeric micelles, silicon spheres and liposomes [7,10,11]. Given that anticancer drugs were incorporated, the gold nanoshells may act as a multifunctional platform for combined photothermo-chemotherapy. However, the gold layer formed on the template surface is not microstructurally continuous and thus not drug leakage-free. Consequently, drug loss cannot be avoided when the drug-loaded gold nanoshells circulate in bloodstream before reaching the targets. In comparison, *in situ* growth of a continuous gold layer on the nanocarrier surface *via* chemical reduction of Au(III) appears more desirable since it may yield leak-tight gold nanomedicines. Unlike the former method which uses the preformed gold nanocrystals with NIR absorptions to decorate the nanomedicines, the *in situ* growth method requires a much more careful control of the gold shell microstructure (e.g. shell thickness, density) for NIR light absorption. On the other hand, effective photothermo-chemotherapy of cancer also requires that the drug is quickly released when the gold nanoshell is broken by NIR irradiation at the tumor site. Unfortunately, the preparation of gold nanomaterials with strong NIR absorption and meanwhile desired drug release profiles is highly challenging and rarely reported thus far.

In the present study, we aimed to develop a leak-tight gold nanoshell incorporating anticancer drug for NIR light irradiation-triggered photothermo-chemotherapy of cancer. To this end, we first prepared a pH and reduction dual sensitive polymeric vesicle whose inner aqueous core was loaded with the anticancer drug doxorubicin (DOX). Then, a leak-tight gold layer with good NIR light-to-heat conversion was grown on the vesicular surface *via* the *in situ* Au(III) reduction approach. It is expected that the DOX-loaded gold nanoshell may avoid drug loss in bloodstream, and meanwhile the pH and reduction dual sensitivity may allow the vesicle to quickly release DOX by responding to the intratumoral/intracellular microenvironments after the gold layer was broken by a NIR light irradiation. Consequently, a NIR light-triggered photothermo-chemotherapy may be achieved precisely at the tumor site to improve the therapeutic efficacy and meanwhile to lower side effects. To demonstrate this potential, the drug delivery efficiency and photothermo-chemotherapeutic effect of gold nanoshell administered through intravenous injection was evaluated in nude mice bearing human Bel-7402 hepatoma xenografts.

2. Experimental section

2.1. Materials

The diblock copolymer, polyethylenimine-b-poly(2-diisopropylamino/2-mercaptoethylamine) ethyl aspartate denoted as PEI-PAsp(DIP/MEA), was synthesized as previously reported [12]. $HAuCl_4$ (99.9%, Sigma–Aldrich, St. Louis, MO, USA) and hydroxylamine solution (50 wt.% in H_2O) (Sigma–Aldrich, St. Louis, MO, USA) of analytical grade were used as received. Dimethylsulfoxide (DMSO) from Sigma–Aldrich (St. Louis, MO, USA) was dried over CaH_2 and distilled. Doxorubicin hydrochloride (DOX·HCl) was purchased from Zhejiang Hisun Pharmaceutical Co., Ltd., China and used as received. Human hepatoma Bel-7402 cells were purchased from the Experimental Animal Center of Sun Yat-sen University (Guangzhou, China). Cell culture media, penicillin-streptomycin, fetal bovine serum (FBS) and 0.25% trypsin were purchased from Gibco BRL (Carlsbad, CA, USA). (3-(4,5-Dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) was obtained from Sigma–Aldrich. Annexin V-APC/7-AAD double labeling kit was obtained from BD Bioscience (5 μL per test, San Jose, CA, USA).

2.2. Preparation of DOX-loaded polymeric vesicles (DOX-loaded PNV)

In brief, 10 mg of PEI-PAsp(DIP/MEA) and DOX·HCl (0.5 mg, 1.0 mg, 2.0 mg or 3.0 mg according to experimental design) were co-dissolved in 1 mL of DMSO. The solution was then emulsified by sonication in 10 mL of Phosphate Buffered Saline (PBS, pH 8 ~ 9) in an ice bath. The mixture was stirred under bubbling of an oxygen flow for 1 h to form the shell-crosslinked vesicles. Subsequently, the solution was dialyzed (MWCO: 14 kDa) against PBS (pH 7.4) for 3 days to remove free DOX. Finally, the solution was filtered through a 0.45 μm filter to remove large aggregates.

2.3. Preparation of DOX-loaded gold nanoshell (DOX-loaded GNS@PNV)

As outlined in Fig. 1, the DOX-loaded gold nanoshell was prepared using the DOX-loaded PNV as template. The reactions were carried out under nitrogen and away from light [13]. First, the gold seeds were grown on the surface of the polymeric vesicles using PEI as a reducing agent. The polymeric vesicle solution (1 mg/mL, 1 mL) was diluted to 0.1 mg/mL with deionization water, followed by addition of 100 μL aqueous solution of $HAuCl_4$ (10 mg/mL) whose pH had been adjusted to ~9 with NaOH solution. The reaction proceeded for 6 h to obtain gold seeds-decorated vesicles. Then, 10 mL of the above solution was added with 100–2500 μL of $NH_2OH·HCl$ (40 mM) and 20–500 μL of 10 mg/mL $HAuCl_4$ solution at different $M_{copolymer}/M_{Au}$ values of 1/0.1, 1/0.25, 1/0.5, 1/1, and 1/2.5 according to experimental design. The reaction was conducted for 15 min at 4 °C, after which the particles were purified by centrifugation and then washed four times with pure water. PEG_{5k}-SH was added to enhance the colloidal solution stability [14]. Finally, five DOX-loaded gold nanoshells denoted as GNS@PNV-1, GNS@PNV-2, GNS@PNV-3, GNS@PNV-4 and GNS@PNV-5 respectively were obtained. DOX-free GNS@PNV was prepared by

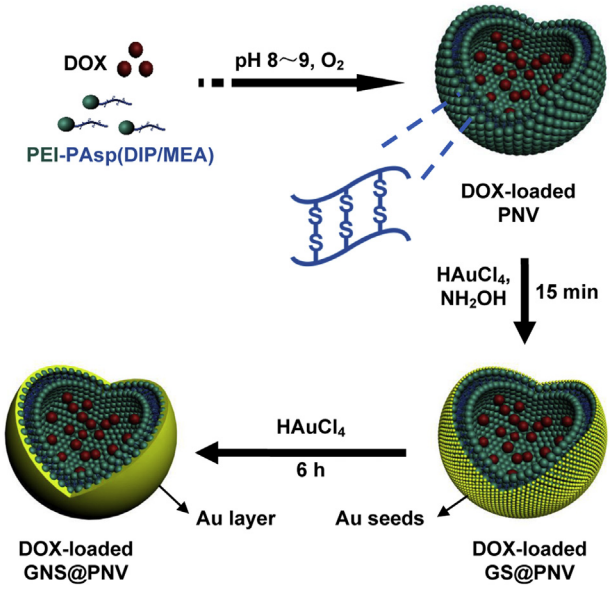

Fig. 1. The schematic diagram of the preparation of DOX-loaded polymeric vesicle (PNV) and gold nanoshell (GNS@PNV).