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In situ fabrication of mesoporous silica-coated silver-gold hollow nanoshell for remotely controllable chemo-photothermal therapy via phase-change molecule as gatekeepers

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ABSTRACT

This study reports a new strategy for *in situ* fabrication of plasmonic hollow silver-gold nanoshell (with resonance tuned to NIR region) encased in the hollow mesoporous silica as an efficient platform to efficiently and precisely regulate the release of 5-fluorouracil (anticancer drug) for prostate cancer therapy and photothermal therapy. The mesopores were capped with thermosensitive phase-change material lauric acid, which allowed for remote, precise, and spatiotemporal control of drug release via external heating or photothermal heating of plasmonic silver-gold nanoshell via NIR laser irradiation. The system was nanometric, monodispersed, and showed negative surface charge. The nanocarrier showed better pH stability and thermodynamic stability compared to dense silica-coated gold nanoshells. The drug release could be triggered remotely by applying low powered continuous wave NIR laser (λ = 808 nm). The nanocarrier showed improved internalization by cancer cells, which was further enhanced by laser irradiation. High powered laser directly killed the cancer cells via photothermal effect in the region irradiated. Thus, this system fabricated by novel synthetic strategy provided efficient chemo- and phototherapy.

1. Introduction

In the last decade, an unprecedented attention has been given to the development of gold nanostructures with various anisotropic morphologies (e.g. rod, shell, cage, hollow shell, stars) for various multimodal bio-applications including therapy, diagnostics, delivery of small biological molecules, bio-sensing, and bio-imaging (Choi and Han, 2018; Gupta et al., 2017; Kim and Lee, 2017; Tran et al., 2016). They exhibit a unique photo-physical property called localized surface plasmon resonance (LSPR) that is caused by the confinement of collective resonant oscillations of free electron cloud to their metallic lattice upon irradiation with photons of resonant frequency. Depending on its size, plasmon resonance relaxes either by radiative process (light absorption and/or scattering) or by non-radiative process in the form of photothermal heating. This distinct optical versatility and other desirable characteristics, such as convenient surface modifiability, biocompatibility, flexibility of fabricating different morphological forms, and ease of facile functionalization with active ligands via Au-S chemistry, have made gold nanoparticles attractive in cancer therapy. They can be used as exogenous plasmonic photothermal therapy (PTT)

Abbreviations: 5-FU, 5-fluorouracil; AgNO3, silver nitrate; AgNPs, silver nanoparticles; AgNPs-MS, silver nanoparticles coated with mesoporous silica; APTES, (3-aminopropyl)triethoxysilane; DLS, dynamic light scattering; EDX, energy dispersive X-ray; EG, ethylene glycol; FITC, fluorescein isothiocyanate; FTIR, Fourier Transform Infrared; HAuCl4, gold tetrachloroauric acid; LA, lauric acid; LSPR, localized surface plasmon resonance; MSNs, mesoporous silica nanoparticles; MTT, 3-(4, 5-dimethyl-2-thiazoyl)-2,5-diphenyl tetrazolium bromide; MUA, 11-mercaptoundecanoic; NIR, near-infrared; NPs, nanoparticles; PCM, phase change material; PTT, photothermal therapy; PVP 55000, polyvinylpyrrolidone 55000; SGNS, silver-gold nanoshells; SGNS-MS, mesoporous silica coated silver-gold nanoshells; SGNS-MS-LA, lauric acid immobilized SGNS-MS; TEOS, tetraethyl orthosilicate; TGA, thermogravimetric analysis; UV–Vis, ultraviolet visible; XRD, X-ray diffraction

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agents with LSPR tuned to near-infrared (NIR) range ($\lambda > 700$ nm) (Guo and You, 2017; Hong and Choi, 2018). In the NIR range, biological tissue transmissivity is the highest and the laser suffers minimal absorption and scattering by tissue components, which enables penetration up to 10 cm (for deep seated tumors), and minimizes non-specific and collateral heating of adjacent tissues (Weissleder, 2001; Guo and You, 2017). Among gold nanostructures, hollow gold nanoshells (GNS) are prominent as their interior voids can be used to deliver molecules of interest to cancer sites (Poudel et al., 2017). They are easy to synthesize, and provide LSPR tunability, enhanced colloidal and thermodynamic stability, and do not require toxic surfactants for fabrication.

Despite their tremendous potential in biomedical applications, unmodified GNS are susceptible to aggregation and precipitation in solution; even the PEGylated GNS has been found to decompose substantially in low pH environment and in physiological pH of the serum (Goodman et al., 2014). Furthermore, plasmonic nanoparticles (NPs) can melt and deform under laser irradiation that significantly alters the optical properties like absorption and scattering of light from lasers (Zijlstra et al., 2009). In addition, standard "soft" coatings, like PEG, surfactants, or polymers, can also melt at the elevated photothermal temperature of plasmonic NPs when irradiated by laser. To minimize these problems, the NPs can be coated with silica, which not only provides excellent colloidal stability via electrostatic and steric protection, but also resists shape change and provides thermodynamic stability. Silica coating has been frequently applied to many inorganic nanoparticles (e.g., metallic NPs, semiconductor NPs, ceramic NPs) owing to its biocompatibility, easy thickness tunability, chemical inertness, controllable porosity, convenient surface derivatization via silane chemistry, and optical transparency (Guerrero-Martínez et al., 2010). The mesoporous silica nanoparticles (MSNs) provide augmented surface area ($> 900\ m^2/g$), tunable pore sizes, and large pore volume ($> 0.9\ cm^2/g$) for higher adsorption and loading of drugs irrespective of their hydrophilicities or ionic charge. Although both PEGylated and non-PEGylated GNS were previously reported to successfully load and deliver very high percentage of model drug doxorubicin, such high loading was possible mainly because of electrostatic interactions of the cationic drug (doxorubicin) to negatively charged GNS surfaces (You et al., 2010).

The drug release from MSN upon administration follows diffusion controlled kinetics due to its open porosity (Jadhav et al., 2016). The release rate is drug solubility-dependent, which means that highly water-soluble drugs may show burst release in the off-target sites. To prevent this kind of premature release, the mesopores can be capped using stimuli sensitive "gatekeepers" (Jadhav et al., 2017a; Jadhav et al., 2017b; Ramasamy et al., 2018). By virtue of these gatekeepers, MSN can deliver the loaded cargo intracellularly to a specific target. This reduces acute and chronic toxicities and facilitates safe dose-escalation of even highly cytotoxic drugs. In this study, lauric acid (dodecanoic acid) was used as gatekeeper of mesopores for temperature-sensitive drug release from the system. Lauric acid (LA) is a medium chain fatty acid and biocompatible phase change material (PCM) with a melting point of ~44 °C. Solid-liquid PCMs, such as LA, undergo reversible and rapid solid-liquid transition with temperature changes and are being extensively studied for their high latent heat storage properties with a great potential for applications ranging from solar cells, and food processing to thermo-regulating textiles (Sariera and Onderb, 2012; Oróa et al., 2012). The temperature-regulated, on-demand drug release was first reported by Choi et al. (2010) who used 1-tetradecanol (melting point: 39 °C) and lauric acid as PCMs for coating gelatin microbeads (Choi et al., 2010). Moon et al. (2011) used PCM as stopper for pores of gold nanocages for ultrasound and/or direct heating assisted release of encapsulated dye. They also used this system for remotely controllable, NIR-laser assisted photothermal release of drug from gold nanocages (Sun et al., 2014). Lee et al. (2014) and Liu et al. (2015) showed that PCM could serve as an efficient gatekeeper for mesoporous silica shell with gold nanorod core for NIR-irradiation controllable release of drugs with nearly zero premature release at normal body temperature.

In this study, we report the *in situ* fabrication of silver-gold nanoshells (SGNS) within hollow mesoporous silica shell via galvanic chemistry. We used this system to efficiently and precisely regulate the release of drug (5-fluorouracil) by plugging the mesopores with thermo-responsive PCM lauric acid for prostate cancer therapy. Our new synthetic strategy involved the galvanic reaction of precursor silver nanoparticle (AgNP) encased inside the hollow mesoporous silica shell with gold chloride salt solution to fabricate SGNS coated with mesoporous shell i.e., SGNS-MS, which showed better pH stability and thermodynamic stability compared to bare SSGNS. This system was investigated for remotely controllable drug delivery and photothermal therapy. Physicochemical characterization, photo-and thermo-responsive drug release, and biological studies, including intracellular uptake, cytotoxicity and apoptosis assays, and live dead assay in prostate cancer cells (DU-145 and PC-3), were performed.

2. Materials and methods

2.1. Materials

5-Fluorouracil (5-FU) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Gold tetrachloroauric acid ($HAuCl_4$), silver nitrate ($AgNO_3$), polyvinylpyrrolidone 55000 (PVP 55000), lauric acid (LA), ethylene glycol (EG), tetraethyl orthosilicate (TEOS), (3-aminopropyl)triethoxysilane (APTES), fluorescein isothiocyanate (FITC), and 11-mercaptoundecanoic acid (MUA) were purchased from Sigma-Aldrich (St Louis, MO, USA). Prostate cancer DU-145 and PC-3 cell lines were obtained from the Korean Cell Line Bank (Seoul, South Korea). All other chemicals were of reagent grade and used without further purification.

2.2. Synthesis of PVP-stabilized silver nanoparticles (AgNP)

The PVP stabilized monodispersed AgNP were synthesized by polyol method (Li et al., 2012). In a typical reaction, 7.5 g of PVP (Mw = 55000) in EG (75 mL) was slowly heated to ~180 °C at the rate of 1 °C/min under vigorous stirring for 30 min until the color of the solution turned golden yellow. Then, the temperature was adjusted to and kept at 140 °C. $AgNO_3$ (0.50 g) in EG (25 mL) was added dropwise into the above solution over 5 min. The reaction was continued for another 10 min at 140 °C. The solution was cooled down to 25 °C and the viscous brown colloidal dispersion suggesting the formation of AgNP was precipitated by adding excess acetone (400 mL). The products were purified through repeated washing with deionized water and centrifugation. Finally, AgNPs were dispersed in 100 mL water for further modification.

2.3. Coating of AgNP with dense silica shell

First, before dense silica coating, AgNP were coated with MUA in ethanol. The process was adapted from Zhang et al. (2013). One mL of PVP-coated AgNP (3.175 mg/mL) was centrifuged and reconstituted in 10 mL of deionized water in an amber colored vial. Thirty microliters of 100 nM MUA ethanolic solution was added into AgNP dispersion and stirred for 12 h at room temperature. Since the NPs were already coated with PVP, no physical adsorption with Tween was deemed necessary. Then, the dispersion was washed with deionized water by multiple cycles of centrifugation-redistribution. Next, solid silica shell was grown on MUA-coated AgNP by Stöber method. Briefly, 10 mL of NP dispersion in water was dispersed in 40 mL isopropanol, and 1 mL of 30 wt% ammonia was added under vigorous stirring. Next, 200–400 μL of 20% TEOS-isopropanol solution was added in small increments over 1 h. The mixture was stirred at 600 RPM overnight, after which it was