



Surface modification with zwitterionic cysteine betaine for nanoshell-assisted near-infrared plasmonic hyperthermia



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ABSTRACT

Nanoparticles decorated with biocompatible coatings have received considerable attention in recent years for their potential biomedical applications. However, the desirable properties of nanoparticles for in vivo uses, such as colloidal stability, biodistribution, and pharmacokinetics, require further research. In this work, we report a bio-derived zwitterionic surface ligand, cysteine betaine (Cys-b) for the modification of hollow gold-silver nanoshells, giving rise to hyperthermia applications. Cys-b coatings on planar substrates and nanoshells were compared to conventional (11-mercaptoundecyl)tri(ethylene glycol) (OEG-thiol) to investigate their effects on the fouling resistance, colloidal stability, environmental tolerance, and photothermal properties. The results found that Cys-b and OEG-thiol coatings exhibited comparable antifouling properties against bacteria of gram-negative *Pseudomonas aeruginosa* (*P. aeruginosa*) and gram-positive *Staphylococcus epidermidis* (*S. epidermidis*), NIH-3T3 fibroblasts, and bovine serum albumin. However, when the modified nanoshells were suspended at a temperature of 50 °C in aqueous 3 M NaCl solutions, shifts in the extinction maximum of the OEG-capped nanoshells with time were observed, while the corresponding spectra of nanoshells capped with Cys-b generally remained unchanged. In addition, when the nanoshells were continuously exposed to NIR irradiation, the temperature of the solution containing nanoshells capped with Cys-b increased to a plateau of 54 °C, while that of the OEG-capped nanoshells gradually decreased after reaching a peak temperature. Accordingly, the Cys-b nanoshells were conjugated with anti-HER2 antibodies for targeted delivery to HER2-positive MDA-MB-453 breast cancer cells for hyperthermia treatment. The results showed the selective delivery and effective photothermal cell ablation with the antibody-conjugated Cys-b nanoshells. Therefore, this work demonstrated the promise of bio-derived zwitterionic Cys-b as a stable and biocompatible surface coating for materials in nanomedicine.

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1. Introduction

Hyperthermia, which involves the introduction of moderate heat to a specific target, has become an important method for tumor therapy because of the limited tolerance of tumor cells to a temperature range of 41–47 °C [1,2]. These elevated temperatures cause irreversible cell damage by loosening cell membranes and denaturing proteins. The heating sources applied include radio frequency, microwaves, and ultrasound waves. However, these sources suffer from drawbacks because of their associated damage to the surrounding healthy tissues. An alternative strategy is photothermal

therapy (PTT) in which photothermal agents are employed for heat generation in a local environment [3–6]. The agents can be dye molecules such as naphthalocyanines, indocyanine, and porphyrins coordinated to transition metals. However, these chromophores suffer from low absorption coefficients and poor photostability [7,8]. In recent years, tremendous advances have been witnessed in the development of nanomaterials with unique optical properties [9–11]. More specifically, novel metal nanoparticles have been employed as powerful agents for PTT because of their robust photostability and strong optical response via the surface plasmon resonance (SPR). A variety of plasmonic nanostructures including nanospheres [12,13], nanoshells [14,15], nanorods [16,17], and nanocages [18], have been developed that respond to wavelengths in the visible and near infrared (NIR) regions. In PTT applications, in addition to a strong extinction, the nanoparticle agents should possess additional properties, such as nontoxicity, long-term colloidal

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stability, high biocompatibility, and facile functionalization [19]. These requirements shed light on the critical role of surface chemistry for decorating plasmonic nanomaterials for their effective and safe implementations.

Commonly, thiolated oligo(ethylene glycol) (OEG) adsorbates are employed as capping ligands, in which thiol groups are adsorbed onto the surface of gold nanostructures via thiol-gold bonds [20]. OEG-modified nanoparticles can significantly enhance colloidal stability, biocompatibility, and biodistribution [21]. Because of its steric repulsion with an elastic and osmotic component [22–24], OEG coatings serve as antifouling materials to repel nonspecific adsorptions. Nevertheless, several factors must be considered when using OEG coatings under complex conditions [24]. For example, OEG adsorbates form hydrogen bonds with water molecules, and thus the conformational change and packing density of the oligomeric ethylene glycol can significantly affect the interfacial water layers [25]. In addition, enhanced temperature and ionic strength in the environment induce changes in the OEG conformation from a helical to an all-trans form, which leads to weakening of the bonding to interfacial water molecules [26]. Furthermore, the poor hydration of OEG eventually gives rise to energetically favorable nonspecific adsorptions [27]. Additionally, OEG can undergo degradation under the stresses of heat and light irradiation, and the possible formation of hydroperoxides [21,28,29]. Taken together, an alternative coating material to OEG for use in particular cases, such as plasmonic nanoparticles for the photothermal therapy, remains highly desirable.

In recent years, attention has been paid to zwitterionic materials, which contain both positively and negatively charged groups. These materials interact strongly with water molecules through ionic solvation, leading to stable configurations at high temperature and ionic strength [27,30–33]. Analogously, in nature, cell membranes are comprised largely of amphiphilic lipids containing polar zwitterionic groups that resist nonspecific adsorptions and allow highly selective biorecognition at interfaces. Therefore, an increasing number of applications utilizing zwitterionic materials as biocompatible and antifouling coatings for complex environments have been explored [30,31,34–36]. Our group recently reported the study and development of a novel zwitterionic surface ligand, cysteine betaine (Cys-b), which is derived from the natural organosulfur compound, cysteine, by converting its primary amine to a quaternary ammonium [37]. We showed that this branched zwitterionic group has a high tolerance to pH changes and resistance to photooxidation in the presence of oxygen and light irradiation [37]. Moreover, self-assembled monolayers (SAMs) of Cys-b on gold exhibit better repellence than cysteine against proteins, bacteria, and mammalian cells. Therefore, the unique features of Cys-b make it promising as a new nanoscale coating material for potential implementation in the modification of plasmonic nanomaterials for PTT.

In the research reported here, we conducted a comparative study between Cys-b and thiolated OEG coatings on both planar “flat” gold surfaces and on plasmonic gold-based nanoparticles. The formation of SAMs on the flat gold surfaces was examined by contact angle measurements and X-ray photoelectron spectroscopy (XPS). Fouling tests on modified substrates were carried out with protein, bacteria, and NIH 3T3 cells. Importantly, the colloidal stability of plasmonic hollow gold-silver nanoshells coated with Cys-b and OEG-thiol was investigated by UV–vis spectroscopy and dynamic light scattering (DLS) to follow the changes in light absorbance/scattering and hydrodynamic sizes, respectively. The photothermal properties of modified nanoparticles as PTT agents were confirmed by NIR irradiation and the measurement of temperature changes in solutions. Finally, we demonstrated the effectiveness of Cys-b modified hollow gold-silver nanoshells conjugated with anti-HER2 antibodies against MDA-MB-453 breast

cancer cells in hyperthermia treatments. These studies provided not only insight into the potential benefits of Cys-b as a zwitterionic surface ligands for plasmonic nanoparticles, but also evidence of a robust surface strategy for maintaining bioinertness in complex environments.

2. Experimental section

2.1. Materials

L-Cysteine, potassium hydroxide (KOH), dimethyl sulfate, trifluoroacetic acid (TFA), glacial acetic acid, acetone, silver nitrate, potassium carbonate, N-hydroxysuccinimide (NHS), and (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) (EDC) were purchased from Sigma-Aldrich. (11-Mercaptoundecyl)tri(ethylene glycol) (OEG-thiol) and thiol-PEG6-acid (COOH-thiol) were purchased from Broadpharm. Anti-HER2 mouse antibodies and anti-mouse IgG (H + L) conjugated with Alexa Fluor 488 were obtained from Cell Signaling Technology. Trisodium citrate dihydrate and nitric acid were obtained from EM Science. Hydrogen tetrachloroaurate(III) hydrate ($\text{HAuCl}_4 \cdot \text{H}_2\text{O}$) was purchased from Strem. Bovine serum albumin (BSA) was obtained from MDBio Inc. Dulbecco's Modified Eagle's Medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco. LIVE/DEAD Viability/Cytotoxicity Kit containing calcein AM and EthD-1 was purchased from Thermo Fisher Scientific. Luria-Bertani broth (LB broth) was obtained from BD. Water was purified to a resistivity of 18 $\text{M}\Omega \cdot \text{cm}$ using the Academic Milli-Q Water System (Millipore Corporation) and filtered using a 0.22 μm filter.

2.2. Cys-b synthesis

The detailed experimental procedure for Cys-b synthesis has been described [37]. Briefly, a flask containing 1 g of cysteine in 3 mL of deionized water was immersed in an ice bath and stirred under nitrogen. An 8.5 mL aliquot of 6.5 M KOH was introduced dropwise until the cysteine dissolved. The residual KOH and 5.2 mL of dimethyl sulfate were dropped in simultaneously over 1 h with stirring. Afterward, the flask was kept for another 20 min at rt, and then 1.2 mL of glacial acetic acid was added. The solution was evaporated *in vacuo* to a volume of around 2 mL. The byproduct potassium methyl sulfate was precipitated by adding 40 mL of ethanol and then filtered. The filtrate was concentrated using a rotovap to a volume of around 2 mL, and then precipitated by adding 50 mL of acetone. The white product was washed with acetone 5 times to afford pure cysteine betaine, which was reduced in 0.1 M dithiothreitol (DTT) in deionized water and stirred at 65 °C for 2 h. After cooling, 50 mL of acetone was added to precipitate the white product of pure Cys-b (70% yield).

2.3. Formation of SAMs on planar substrates

Au thin films with a thickness of 50 nm on glass slides were prepared by thermal evaporation (I Shien SPS-302). The substrates were cleaned in a sonication bath of 0.1% SDS, acetone, and ethanol for 10 min of each, followed by drying under a stream of nitrogen. The substrates were transferred to a plasma cleaner (PDC-001, Harrick Plasma, NY) to expose O_2 plasma twice with a power of 10.5 W for 10 min. The clean substrates were immediately immersed into a 1 mM Cys-b solution in DI water containing 2% TFA or 1 mM OEG-thiol solution in ethanol, and shaken at 50 rpm at room temperature for 12 h. The modified substrates were removed and cleaned with deionized water, ethanol, and water, followed by drying under a stream of nitrogen [38].

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