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Core–shell–shell nanorods for controlled release of silver that can serve as a nanoheater for photothermal treatment on bacteria

Bo Hu [a], Ning Wang [a], Lu Han [a], Ming-Li Chen [a,*], Jian-Hua Wang [a,b,*]

[a] Research Center for Analytical Sciences, College of Sciences, Northeastern University, Box 332, Shenyang 110819, People's Republic of China
[b] Collaborative Innovation Center of Chemical Science and Engineering, Tianjin 300071, People's Republic of China

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ABSTRACT

A novel bactericidal material comprising rod-shaped core–shell–shell Au–Ag–Au nanorods is constructed as a nanoheater in the near-infrared (NIR) region. The outer Au shell melts under laser irradiation and results in exposure of the inner Ag shell, facilitating the controlled release of the antibacterial Ag shell/layer or Ag^+. This results in the Au–Ag–Au nanorods having a favorable bactericidal ability as it combines the features of physical photothermal ablation sterilization of the outer Au shell and the antibacterial effect of the inner Ag shell or Ag^+ to the surrounding bacteria. The sterilizing ability of Au–Ag–Au nanorods is investigated with *Escherichia coli* O157:H7 as a model bacterial strain. Under low-power NIR laser irradiation (785 nm, 50 mW cm^{-2}), the Au–Ag–Au nanoheater exhibits a higher photothermal conversion efficiency (with a solution temperature of 44 °C) with respect to that for the Au–Ag nanorods (39 °C). Meanwhile, a much improved stability with respect to Au–Ag nanorods is observed, i.e., 16 successive days of monitoring reveal virtually no change in the ultraviolet–visible spectrum of Au–Ag–Au nanorods, while a significant drop in absorption along with a 92 nm red shift of Localized Surface Plasmon Resonance is recorded for the Au–Ag nanorods. This brings an increasing bactericidal efficiency and long-term stability for the Au–Ag–Au nanorods. At a dosage of 10 μg ml^{-1}, a killing rate of 100% is reached for the *E. coli* O157:H7 cells under 20 min of irradiation. The use of Au–Ag–Au nanorods avoids the abuse of broad-spectrum antibiotics and reduces the damage of tissues by alleviating the toxicity of silver under controlled release and by the use of low-power laser irradiation. These features could make the bimetallic core–shell–shell nanorods a favorable nanoheater for in vivo biomedical applications.

1. Introduction

Recently, much effort has been directed to the development of antimicrobial materials in order to avoid the overuse of broad spectrum antibiotics in the treatment of drug-resistant bacteria. Photothermal therapy is an emerging approach in the clinical treatment of hemostasis and disinfection. Its non-invasive nature, fast recovery capability and less severe adverse reactions/complications make the photothermal converting material ideal for in vivo antimicrobial treatment and diagnostics [1–3]. Its study is focused on the localized photo-induced hyperthermia and thermotherapy. Briefly, the photothermal converting materials are first modified with biomolecules or polymers, and then attached to the nidus or focus on the infection by target recognition, e.g., electrostatic interaction, immune recognition and magnetic aggregation. When irradiated by a laser beam, it absorbs incident photon energy and converts into heat in ~1 ps, and dissipates the heat into surrounding media in 10 ps [4–6]. An elevated temperature may bring excessive local heating, which triggers intracellular protein denaturation and a change in the membrane permeability, causes mucosal tissue coagulation and capillary occlusion, destroys the membrane and finally kills bacteria [7]. The efficacy of photothermal therapy relies on energy absorption from laser irradiation and thermal conversion efficiency of the nanomaterials [8]. Near infrared (NIR) laser irradiation in the range 650–950 nm and 1000–1350 nm offers two biological transparency windows in which water, blood and soft tissues are penetrable to the maximum extent possible [9,10]. NIR has been recognized as the most suitable laser irradiation in photothermal therapy, and can bring maximum radiation penetration in deep tissues [11–13].

As a kind of NIR-activated nanomaterial, nanorod-structural precious metals (Au or Ag) have attracted extensive attention. They usually exhibit two characteristic absorptions due to the excitation

* Corresponding authors at: Research Center for Analytical Sciences, College of Sciences, Northeastern University, Box 332, Shenyang 110819, People's Republic of China. Tel.: +86 24 83688944; fax: +86 24 83676698.
E-mail addresses: chenml@mail.neu.edu.cn (M.-L. Chen), jianhuajrz@mail.neu.edu.cn (J.-H. Wang).

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of surface plasmon, transverse surface plasmon resonances (TSPR) and longitudinal surface plasmon resonances (LSPR). The longitudinal absorbance can be regulated with the aspect ratio within a wide spectrum, e.g., from visible to the NIR region [14–16]. Silver nanoparticles have favorable antibacterial properties and serve as an efficient antibacterial nanomaterial by binding to the bacteria surface or migrating into the cell [17]. At the beginning, nanorod-structural silver is prepared to study in vivo toxicological effects. The rod-shaped structure is easily distinguished in cells [18] and this endows a superior optical response with tunable LSPR within 600–750 nm. But the non-uniform anisotropic silver nanorods are rather unstable in aqueous medium and may even turn into nanospheres [19,20]. On the contrary, gold nanorods are stable, nontoxic and have a stronger NIR-responsive LSPR absorption than silver nanorods in the biological NIR-transparent window [21]. Au nanorods have been applied as nanoheaters for photothermal therapy and controllable release under NIR lasers for pathogenic bacteria treatment [22–26]. However, for most bacteria, e.g., *Escherichia coli*, an effective antibacterial effect can only be obtained at a certain temperature, while the temperature generated by photothermal ablation of native Au nanorods under low energy NIR laser irradiation is not sufficient for this purpose. A new type of silver nanorod, i.e., nanorods with a gold core and silver shell structure (Au–Ag nanorods), was recently proposed [27–30]. This structure endows silver nanorods with a more uniformed shape and size; however, the outer silver shell is still unstable in aqueous medium and tends to disappear in a few days. In addition, the nonspecific biological toxicity of silver not only kills the bacteria cells but also causes apoptosis of the normal cells. This feature thus limits the use of silver nanorods as therapeutic agents [31–37].

In the present work we present a novel core–shell–shell rod-shaped bimetallic bactericidal material, i.e., Au–Ag–Au nanorods, for the purpose of eliminating the above drawbacks of the Au, Ag and Au–Ag nanorods for photothermal therapy to bacteria. With Au nanorods as a core and template, a thin silver shell grows on the template Au nanorod surface followed by the growth of another gold shell on the middle silver layer. Under NIR irradiation, the photothermal effect arising from the outer Au shell causes the ablation of the bacteria cells. Meanwhile NIR laser irradiation results in the melting of the outer Au shell and the exposure of the inner Ag shell, which facilitates controlled release of the silver shell or Ag^+. In this mode, the Au–Ag–Au nanorods combine physical photothermal sterilization of the outer Au shell and the antibacterial effect of the inner Ag shell in addition to the released Ag^+. The bimetallic core–shell–shell Au–Ag–Au nanorods provide a better bactericidal efficiency with respect to the other two kinds of nanorods, Au and Au–Ag nanorods, and offer a significantly improved stability in aqueous medium over Au–Ag nanorods. *E. coli* EHEC O157:H7 (*E. coli* O157:H7) is chosen as a target bacterial strain to demonstrate the sterilizing effect of the nanoheater. The use of Au–Ag–Au nanorods avoids the risk of abuse of broad-spectrum antibiotics and biological system damage by physical sterilization with a low power NIR laser.

2. Materials and methods

2.1. Preparation of Au nanorods

The Au nanorod templates are prepared by following a seed-mediated and silver-assisted growth method [28]. Firstly, an Au seed solution is obtained by rapid addition of a freshly prepared ice-cold $NaBH_4$ aqueous solution (Sinopharm Chemical Reagent Co., China, 0.01 M, 0.6 ml) into a mixture solution containing 0.25 ml $HAuCl_4$ aqueous solution (0.01 M) and 9.75 ml cetyltrimethyl ammonium bromide (CTAB) aqueous solution (0.1 M). The mixture is allowed to react at 30 °C for 1.5–2 h before use. Afterwards, a growth solution (100 ml aqueous solution, pH 1–2) containing CTAB (Sinopharm Chemical Reagent Co., China, 0.1 M), $HAuCl_4$ (AR, Sinopharm Chemical Reagent Co., China, 5×10^{-4} M), $AgNO_3$ (AR, Sinopharm Chemical Reagent Co., China, 1×10^{-4} M) and ascorbic acid (AA) (AR, Sinopharm Chemical Reagent Co., China, 8×10^{-4} M) is prepared and mixed with 24 μl of the Au seed solution, and the mixture is allowed to react at 30 °C for overnight. Au nanorods with different aspect ratios are obtained by controlling the pH value of the growth solution with HCl (Tianjin Damao Chemicals, China, 0.1 M). The Au nanorod solution is centrifuged (9,000 rpm, 10 min) twice before use.

2.2. Preparation of Au–Ag nanorods

A certain amount of $AgNO_3$ (0.01 M) (0, 0.01, 0.05, 0.10, 0.20, 0.25, 0.30, 0.35 and 0.40 ml) aqueous solution is injected into the previously treated Au nanorod solution and then 0.22 ml AA (0.01 M) is added. Afterwards, 0.4 ml NaOH (Tianjin Damao Chemicals, China, 0.05 M) is introduced into the mixture to improve the reduction performance of AA. The 20 ml of mixture is magnetically stirred for 6 h and centrifuged at 9,000 rpm for 10 min twice to remove the excessive amount of reducing agent, NaOH and surfactant before further use.

2.3. Preparation of Au–Ag–Au nanorods

After centrifugation, the prepared Au–Ag nanorods are re-dispersed in 0.1 M CTAB and then mixed with a certain amount of $HAuCl_4$ solution (0.01 M, 0.1–0.8 ml) and AA (0.01 M, 0.22 ml) to make a 20 ml growth solution. The mixture is afterwards allowed to further react for 10 h under magnetic stirring and stored at room temperature for future use.

2.4. The modification of Au–Ag–Au nanorods with polymers

20 ml of the prepared Au–Ag–Au nanorod solution is centrifuged at 10,000 rpm for10 min and re-dispersed in 18 ml pure water. The solution is mixed with 2 ml of poly(sodium-p-styrenesulfonate) (PSS, average Mw ~70,000) (Sigma–Aldrich, Milwaukee, USA) solution (20 mg ml^{-1}) and stirring for overnight. The obtained Au–Ag–Au–PSS solution is then washed twice with pure water by centrifugation at 10,000 rpm for 10 min and dispersed in 20 ml water. 0.04 g poly(allylamine hydrochloride) (PAH, average Mw ~15,000) (Sigma–Aldrich, Milwaukee, USA) is added into the re-dispersed solution and a further reaction is conducted overnight under magnetic stirring. The obtained Au–Ag–Au–PSS–PAH solution is centrifuged at 10,000 rpm for 10 min twice to remove the excessive PAH and then stored at room temperature for further use. The modification of Au and Au–Ag nanorods is performed similarly by following the same procedure to obtain Au–PSS–PAH and Au–Ag–PSS–PAH nanorods.

2.5. Characterization of polymer-modified Au–Ag–Au nanorods

Ultraviolet–visible (UV–vis) spectra are recorded with a U-3900 UV–vis spectrophotometer (Hitachi Ltd, Japan). X-ray photoelectron spectroscopy (XPS) analysis is performed on an ESCALAB 250 X-ray photoelectron spectrometer (Thermo Ltd, USA). Surface charge property of the materials is investigated by measuring the zeta potential with a Zetasizer Nano ZS/ZEN3690 (Malvern, UK). Transmission electron microscopy (TEM) images are acquired with an H-7650 microscope (Hitachi, Japan) operated at 200 kV. Fourier transform infrared (FT-IR) spectra are obtained by using a Nicolet-6700 FT-IR spectrometer (Thermo Ltd, USA) within a range of 4000–500 cm^{-1}. The photothermal transfer property of the materi-

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