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Protocols

Multifunctional nanocomplex for surface-enhanced Raman scattering imaging and near-infrared photodynamic antimicrobial therapy of vancomycin-resistant bacteria

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ABSTRACT

Since vancomycin (Van)-resistant *enterococci* (VRE) strains first emerged as a serious threat to public health, extensive studies focused on optical imaging and antimicrobial therapy have been performed for monitoring and microbiological control. In this study, we developed silicon 2,3-naphthalocyanine dihydroxide (Nc) and Van functionalized silica-encapsulated, silver-coated gold nanoparticles (Au@AgNP@SiO_2@Nc-Van) as a novel theranostic system for surface-enhanced Raman scattering (SERS) detection and antimicrobial photodynamic therapy (aPDT) of VRE strains. The silver-coated gold nanoparticle, as the SERS-active core, exhibited excellent Raman enhancement efficacy. Results of *in vitro* bacterial SERS imaging revealed Van-enhanced specific binding affinity toward VRE. Meanwhile, Si(IV) naphthalocyanine, serving as a near-infrared (NIR) photosensitizer, was axially linked to the nanoparticle surface, yielding nanostructured hybrid materials that could photoinactivate VRE. Almost 4–5 logs of bacterial reduction were obtained upon *in vitro* photodynamic therapy of VRE treated with a nanomolar concentration of the nanocomplex. Mouse infection assays were applied for an *in vivo* evaluation of VRE lethality. Upon near-infrared light irradiation, this hybrid nanomaterial caused obvious infection regression and even complete eradication compared to the findings in the non-treated groups. Therefore, this novel nanosystem integrating SERS imaging and noninvasive aPDT has huge potential for applications in theranostics with regard to VRE management.

1. Introduction

Vancomycin (Van), a symbolic glycopeptide antibiotic, is recognized as the last resort against bacterial infection caused by methicillin-resistant gram-positive pathogens. The skeleton of Van specifically binds with the D-alanyl-D-alanine moieties of peptidoglycan to inhibit bacterial cell wall synthesis, leading to cell death [1,2]. However, because of an overuse of antibiotics in clinics and animal feeds, Van-resistant *enterococci* (VRE) have emerged as a serious threat to public health [3–5]. Thus, the development of novel alternative approaches has become one of the most challenging tasks for treating drug-resistant pathogens. One strategy based on antimicrobial photodynamic therapy (aPDT) has received considerable attention in anti-infection treatment. In this approach, photosensitizers (PS) generate cytotoxic reactive oxygen species (ROS, e.g., singlet oxygen ($_1O^2$)) upon light irradiation, leading to cell damage and death [6–12]. To date, aPDT has been extensively applied to treat a variety of bacterial infections. In our previous work, we reported a specific multifunctional divalent Van derivative in which Van was employed as the affinity ligand and porphyrin (Por) was chosen as the bridging moiety to generate a Por-Van dimer conjugate [1]. This divalent Por-Van, as a theranostic compound, exhibited photodynamic antimicrobial activity and served as a fluorescent labeling approach for VRE pathogens. However, *in vivo* theranostic treatments have been hampered greatly by some intrinsic shortcomings of this divalent compound, such as low solubility and stability in biological buffers, a weak fluorescent quantum yield, and strong photo-bleaching.

In the present study, silica-encapsulated silver-coated gold nanoparticles (Au@AgNPs@SiO_2) were synthesized and conjugated with 2,3-naphthalocyanine dihydroxide (Nc) and Van molecules to form multivalent nanoparticles for SERS imaging and aPDT of VRE

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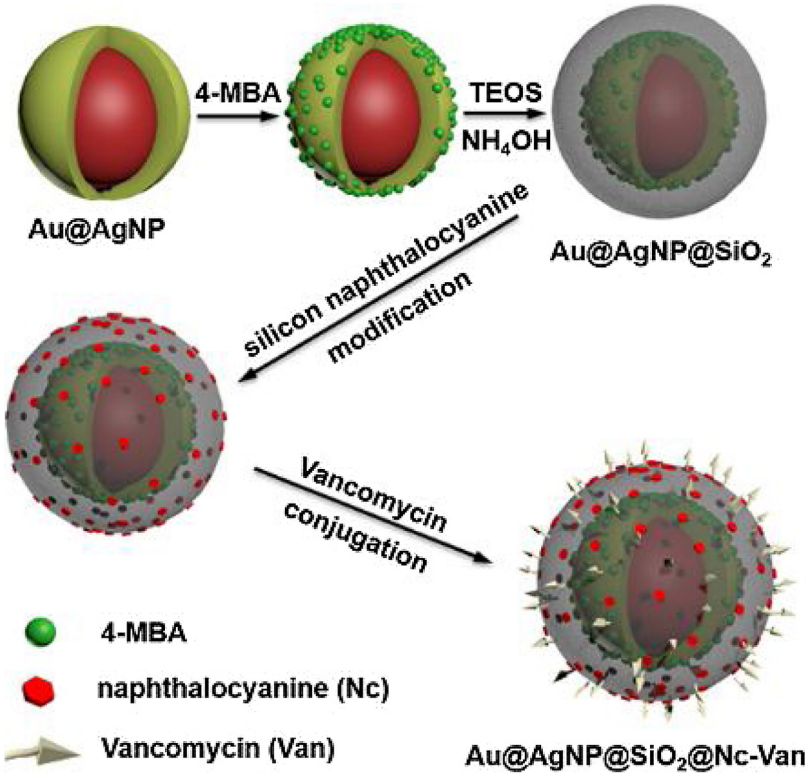

Fig. 1. Scheme for the synthesis of Nc- and Van-modified silica-encapsulated silver-coated gold nanoparticle (Au@AgNP@SiO_2@Nc-Van/NP@Nc-Van).

pathogens (Fig. 1). This novel hybrid theranostic system has several unique advantages over the previously reported Por-Van dimer: (i) The silica shell is a good substitute for Por to bridge Van derivatives, because it has a similar rigid structure to afford entropically enhanced binding and the steric hindrance necessary for multivalent interactions; (ii) it likely lacks many of the limitations that have hampered the application of the Por-Van dimer, such as biocompatibility, solubility, stability, and complicated synthesis procedures; (iii) the reliable near-infrared (NIR) photodynamic efficiency of Nc makes it possible to substitute Por in noninvasive antimicrobial therapy; (iv) satisfactory SERS signals generated by the Au@AgNP core could serve as a promising alternative approach for fluorescence of the Por-Van dimer to label and monitor bacterial strains in a highly effective manner.

2. Experimental methods

2.1. Synthesis and characterization

Chemical reagents and solvents were used as received from commercial sources unless otherwise stated. The chemical structure was determined by ^{1}H nuclear magnetic resonance (NMR) spectra obtained on a 300 MHz Bruker Avance system (Bruker, Fallanden, Switzerland) in $CDCL_3$. Liquid chromatography-mass spectrometry (LC–MS) analyses were obtained on a TSQ Quantum Ultra quadrupole mass spectrometer (Thermo Fisher Scientific, San Jose, CA, USA) Ultraviolet-visible (UV–vis) spectra were recorded in a 5-mm path quartz cell on a TU-1810PC spectrometer (Purkinje General Instrument, Beijing, China). Raman spectra were collected from the sample solution on an R-3000HR spectrometer (Raman system Inc, Austin, USA) with a red light-emitting diode (LED) laser (λ = 785 nm) at 290 mW. An elemental analysis was performed using a SU 8010 scanning electronic microscope (SEM) (Hitachi, Tokyo, Japan) under vacuum at an acceleration voltage of 15 kV coupled to an BRUKER XFlash6010 energy dispersive spectrometry (EDS) detector.

Compound **1** was synthesized according to a previously reported method with some modifications [13]. The synthetic route is shown in Fig. S1. To a cooled (0 °C) solution of *N*-hydroxysuccinimide (NHS, 3.42 g; 27.7 mmol, 1.1 eq) in dry tetrahydrofuran (THF) was added triethyl amine (TEA, 3.83 mL, 27.7 mmol, 1.1 eq) followed by dropwise addition of glutaryl dichloride (1.61 mL, 12.6 mmol, 1 eq). The resulting white suspension was stirred for 2 h at room temperature (23–25 °C). The solvent was evaporated and the residue was dissolved in CH_2Cl_2, washed with water, and dried over $MgSO_4$. Filtration and evaporation of the solvent yielded a white solid (3.95 g, 96%), which was recrystallized from isopropyl alcohol (85%). Fig. S2 shows the result of ^{1}H NMR (300 MHz, $CDCL_3$, 25 °C): δ = 2.147-2.243 (2H, m), 2.775–2.842 (12H, m).

Synthesis of compound **2**: Fig. S1 shows the synthetic route of compound **2**. 20 mg of Van (0.027 mmol, 1 eq) and 46 mg of compound **1** (0.141 mmol, 5 eq) were dissolved in 4 mL of *N*,*N*-dimethylformamide (DMF). The mixture was cooled in an ice bath, and 10 μL of *N*,*N*-diisopropylethylamine (DIPEA) was then added. After overnight stirring, the reaction was quenched by acetone and a white solid precipitate was obtained (40.6 mg). Fig. S3 shows the LC–MS data, in which the peak at m/z 1661 corresponds to M^+.

Au@AgNPs (approximately 60 nm) were synthesized using a previously reported method [14]. In a typical reaction, $HAuCl_4$ (164.4 μL, 0.01%) was added to 100 mL of pure water and heated for 30 min at 100 °C under vigorous magnetic stirring. Sodium citrate (1 mL, 1% w/w) was then added and heated for 15 min. $AgNO_3$ (470 μL, 0.1 M) and sodium citrate (1.6 mL, 1% w/w) were then added dropwise. The mixture was kept boiling for 1 h, resulting in orange-colored solution.

Au@AgNPs (120 mL, 60 nm) were concentrated into 20 mL by centrifugation at 5900*g* for 10 min. The obtained solution was slowly transferred into 100 mL of 2-propanol under vigorous stirring. 4-mercaptobenzoic acid (4-MBA, 400 μL, 5 mM in ethanol)