ACS APPLIED MATERIALS & INTERFACES

Research Article

www.acsami.org

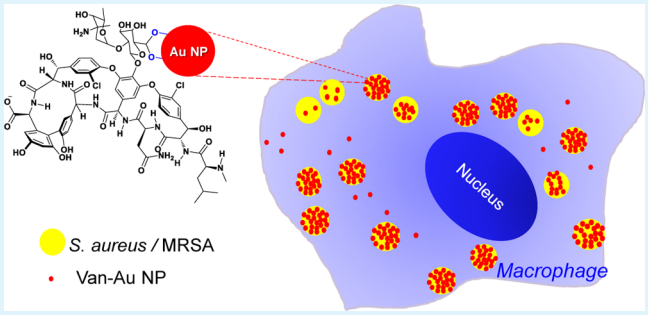
Potent Antibacterial Nanoparticles for Pathogenic Bacteria

Hong-Zheng Lai, Wei-Yu Chen, Ching-Yi Wu, and Yu-Chie Chen*

Department of Applied Chemistry, National Chiao Tung University, Hsinchu 300, Taiwan

Supporting Information

ABSTRACT: Antibiotic-resistant bacteria have emerged because of the prevalent use of antibacterial agents. Thus, new antibacterial agents and therapeutics that can treat bacterial infections are necessary. Vancomycin is a potent antibiotic. Unfortunately, some bacterial strains have developed their resistance toward vancomycin. Nevertheless, it has been demonstrated that vancomycin-immobilized nanoparticles (NPs) are capable to be used in inhibition of the cell growth of vancomycin-resistant bacterial strains through multivalent interactions. However, multistep syntheses are usually necessary to generate vancomycin-immobilized NPs. Thus, maintaining the antibiotic activity of vancomycin when the drug is immobilized on the surface of NPs is challenging. In this study, a facile approach to generate vancomycin immobilized gold (Van-Au) NPs through one-pot stirring of vancomycin with aqueous tetrachloroauric acid at pH 12 and 25 °C for 24 h was demonstrated. Van-Au NPs (8.4 ± 1.3 nm in size) were readily generated. The generated Van-Au NPs maintained their antibiotic activities and inhibited the cell growth of pathogens, which included Gram-positive and Gram-negative bacteria as well as antibiotic-resistant bacterial strains. Furthermore, the minimum inhibitory concentration of the Van-Au NPs against bacteria was lower than that of free-form vancomycin. *Staphylococcus aureus*-infected macrophages were used as the model samples to examine the antibacterial activity of the Van-Au NPs. Macrophages have the tendency to engulf Van-Au NPs through endocytosis. The results showed that the cell growth of *S. aureus* in the macrophages was effectively inhibited, suggesting the potential of using the generated Van-Au NPs as antibacterial agents for bacterial infectious diseases.



KEYWORDS: *pathogenic bacteria, antibiotics, gold nanoparticles, vancomycin, Staphylococcus aureus, macrophage*

1. INTRODUCTION

Infectious diseases resulting from pathogenic bacteria may cause irreversible damage when diagnostics and medical treatment are not promptly administered. The majority of these diseases are caused by bacterial infections that may lead to sepsis, bacteremia, pneumonia, endocarditis, or even death.[1−3] The proliferation and high environmental endurance of bacteria can lead to serious diseases.[4] Antibiotics have been administered to treat bacterial infections. However, many pathogenic bacteria have developed resistance against antibiotics because of the extensive use of these antibacterial agents.[5,6] Therefore, new types of antibiotics and effective therapeutic strategies should be developed to solve this emerging problem.

Vancomycin is one of the potent antibiotics against Gram-positive bacteria.[7] Antibacterial activity is caused by its specific recognition of the peptidoglycan terminus D-Ala-D-Ala on the cell wall of Gram-positive bacteria, leading to the inhibition of the synthesis of the bacterial cell wall. However, the extensive use of vancomycin has resulted in the emergence of vancomycin-resistant bacterial strains. Recent studies have shown that vancomycin-immobilized nanoparticles (Van-NPs)[8−15] can be used to effectively target pathogenic bacteria, such as Gram-positive, Gram-negative, and vancomycin-resistant bacterial strains. NPs with different shapes and compositions, such as spherical gold (Au) NPs,[8,9] magnetic NPs,[10,11] polygonal Au NPs,[12] Fe_3O_4@Au nanoeggs,[13] silica beads,[14] and spherical Ag NPs,[15] immobilized with vancomycin have been investigated to probe pathogenic bacteria. The trapping capacity was varied with the orientation/architecture of vancomycin on the surface of the NPs.[16]

Several types of NP-based antimicrobial therapeutics[16−18] and agents[16,19−22] have been explored lately. The most challenging part in the generation of effective Van-NPs against bacteria is to maintain the antibiotic activity of vancomycin on the surface of Van-NPs. The active binding site of vancomycin specific for bacteria should not be blocked when immobilized on the surface of NPs, that is, vancomycin activity mainly relies on the orientation of the vancomycin structure anchored on the surface of the Van-NPs.[23] The complex synthesis steps when generating Van-NPs may lead to the loss of antibiotic activity. Thus, implementing simple immobilization procedures could reduce the probability of losing the antibacterial activity during the preparation of Van-NPs. One-pot reactions using β-lactam antibiotic as reducing agent and protective group to generate Au NPs have been proposed.[22] The results suggested that

Received: November 13, 2014
Accepted: December 23, 2014
Published: January 13, 2015

DOI: 10.1021/am507919m
ACS Appl. Mater. Interfaces 2015, 7, 2046−2054

antibiotic-immobilized NPs can be generated through a facile step if the antibiotic contains suitable functional groups for directing the NP syntheses. Vancomycin is a glycopeptide composed of glycosides and a peptide sequence. The peptide is responsible for the binding with the target bacteria. If the glycosides could react with Au salts to generate Au NPs, the active binding site, particularly the peptide unit, may maintain its antibiotic activity after being immobilized on Au NPs. Thus, a one-pot reaction can be achieved by simply stirring vancomycin with aqueous tetrachloroaurate at suitable reaction conditions to generate vancomycin-immobilized Au NPs (Van-Au NPs). Although this reaction is quite straightforward, the activity of vancomycin on the Au NPs (Van-Au NPs) still remains. The feasibility of using these generated Van-Au NPs through one-pot reactions as antibiotic agents to inhibit the cell growth of pathogenic bacteria, such as Gram-positive, Gram-negative, and antibiotic-resistant bacterial strains, was investigated.

Macrophages are the first line of defense against pathogen attacks. However, several threatening microorganisms, such as *Staphylococcus aureus* and *Mycobacterium tuberculosis*, can survive inside the macrophages even after being engulfed.[24] Medical treatment becomes even more difficult when macrophages are infected by antibiotic-resistant bacterial strains. Thus, potent medical treatments should be explored to treat this type of infection. Macrophages tend to engulf NPs,[25−27] making NPs suitable carriers to deliver antibacterial agents into macrophages. Thus, the possibility of using the generated Van-Au NPs as antibiotic agents to inhibit the cell growth of bacteria-infected macrophages was also examined.

2. EXPERIMENTAL SECTION

2.1. Reagents and Materials. Vancomycin hydrochloride hydrate, sodium hydroxide, dimethyl sulfoxide (DMSO), disodium phosphate, α-cyno-4-hydroxycinanamic, trifluoroacetic acid, Roswell Park Memorial Institute (RPMI)-1640 medium (with glutamine), Dulbecco's Modified Eagle Medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-3,5-diphenylformazan (MTT), and monopotassium phosphate were purchased from Sigma (St. Louis, MO, USA). Monosodium phosphate was purchased from Mallinckrodt (Kentucky, USA), while hydrogen tetrachloroaurate(III) was purchased from Showa (Tokyo, Japan). Hydrochloric acid and sodium chloride were purchased from J. T. Baker (Phillipsbur, NJ, USA). Potassium chloride and citric acid monohydrate were obtained from Riedel-de Haën (Seelze, Germany). Luria-Bertani (LB) broth and tryptic soya broth (TSB) were purchased from Becton Dickinson (Franklin Lakes, NJ, USA). Acetonitrile was purchased from Merck (Darmstadt, Germany). Yeast extract was purchased from Alpha Bioscience (Baltimore, MD, USA), and agarose was purchased from Amresco (Solon, OH, USA). *S. aureus*, methicillin-resistant *S. aureus* (MRSA), *Escherichia coli* O157:H7, *Acinetobacter baumannii*, pandrug-resistant *A. baumannii* (PDRAB), *Enterococcus faecalis*, *E. faecium*, *Pseudomonas aeruginosa*, vancomycin-resistant *E. faecalis* (VRE 1), vancomycin-resistant *E. faecium* (VRE 4), and *Klebsiella pneumoniae* were collected from the patients in the Tzu-Chi General Hospital (Hualien, Taiwan) and kindly provided by Prof. P. J. Tsai (NCKU, Taiwan). *E. coli* J96 was kindly provided by Prof. H. L. Peng (NCTU, Taiwan). Phosphate-buffered saline (PBS) solution (10× without calcium and magnesium) was purchased from Biowest (Nuaillé, France). Macrophage cells (RAW 264.7) were purchased from the Food Industrial Research and Development Institute (Hsinchu, Taiwan). Filter cartridges (MP003C46) with a cutoff mass of ∼3 kDa were purchased from Pall Corp. (PN, USA).

2.2. Generation of Van-AuNPs. Van-Au NPs were generated through one-pot reactions. Tetrachloroauric acid (10 mM, 100 μL) and vancomycin (10 mM, 25 μL) were stirred in a reaction vial for 15 min followed by addition of aqueous sodium hydroxide (2 M, 20 μL). The mixture was stirred at 25 °C for another 24 h under room light. The resulting Van-Au NPs solution was mixed with deionized water (2 mL) and filtrated through a membrane filter (cutoff mass ≈ 3 kDa) under centrifugation at 6000 rpm for 35 min to rinse the generated Au NPs. The rinse step was repeated 3 times. The resultant suspension was neutralized by adding HCl (2 M, 2 μL) to have pH ≈ 7.

2.3. Estimation of the Binding Amount of Vancomycin on Van-Au NPs. The binding amount of vancomycin on the Van-Au NPs was estimated by examining the change in the intensity of the wavelength at 282 nm ($\lambda_{282\ nm}$) in the absorption spectra of the supernatant before and after the one-pot reactions. The product solution was initially centrifuged at 100 000 rpm at 4 °C for 10 h. The intensity at $\lambda_{282\ nm}$ in the absorption spectrum of the supernatant was then obtained. The Van-Au NPs were rinsed with deionized water (2 mL) by loading the NPs in a tube with a membrane filter (cutoff mass of ∼3 kDa) under centrifugation at 6000 rpm for 20 min. The absorption intensity at $\lambda_{282\ nm}$ of the rinse solution was then measured. The binding amount of vancomycin on the Van-Au NPs was estimated based on the absorption intensity of the supernatant and rinse solution at $\lambda_{282\ nm}$.

2.4. Characterization of the Generated Van-Au NPs. The absorption spectrum of the Van-Au NPs was obtained using a Varian Cary 50 ultraviolet−visible (UV−Vis) spectrophotometer. The size and morphology of Van-Au NPs were examined using a JEOL 2000 FX transmission electron microscope (TEM) (Tokyo, Japan) (accelerating voltage of the electron beam, 120 kV). TEM samples were prepared by depositing a droplet (1 μL) of the generated Van-Au NPs onto a carbon-coated copper disk and dried overnight. Then, the samples were placed in a vacuum chamber (∼4×10^{-3} Torr) for 12 h. The size distribution of the Van-Au NPs was estimated from the TEM images using ImageJ software. The zeta potential of the Van-Au NPs prepared in PBS solution (pH 6, 7, and 8) was estimated using a Beckman Coulter DelsaTM Nano C (Fullerton, CA, USA). Three replicates were performed. To prepare the Van-Au NP sample for X-ray photoelectron spectroscopy (XPS) (UL VAC-PHI, Kanagawa, Japan), aqueous Van-Au NPs (1.5 mg/mL) were deposited on a silicon wafer that has been washed with piranha solution consisting of sulfuric acid (H_2SO_4, 95−98%) and hydrogen peroxide (H_2O_2, 35%) (3:1, v/v). To prepare the piranha solution, hydrogen peroxide was carefully added to sulfuric acid, and the sample was allowed to dry on the wafer under ambient conditions. The procedure was repeated several times until the amount of sample on the silicon wafer was sufficient.

To prepare the sample for infrared (IR) absorption spectroscopic analysis, Van-Au NPs (0.5 mg) were mixed with dried KBr powder (2 mg) and finely ground. The resultant fine powder was placed in an oven at 60 °C for 12 h to remove the remaining moisture. The fine powder was then pressed in a mechanical press to form a translucent pellet under a high pressure of 4000 psi. The resulting pellet was then analyzed using a PerkinElmer IR spectrophotometer (Waltham, MA, USA).

2.5. Preparation of Bacterial Samples. All bacteria were freshly harvested from the bacterial culture after incubation at 37 °C for 12 h. *E. coli* J96, *K. pneumonia*, *P. aeruginosa*, PDRAB, and *A. baumannii* were cultured in LB broth (10 μg/mL, 10 mL). *S. aureus*, MRSA, *E. faecalis*, *E. faecium*, *E. coli* O157:H7, vancomycin-resistant *E. faecalis*, and vancomycin-resistant *E. faecium* were cultured in the mixture of TSB (30 μg/mL, 10 mL) and yeast extract (5 μg/mL, 10 mL) (TSBY) broth. The resulting bacterial samples were centrifuged using a Thermo centrifuge (model D-37520; Waltham, MA, USA) equipped with a rotor (radius = 8.0 cm) at 6000 rpm for 10 min. The supernatant was removed. The bacterial cells were rinsed with PBS solution (0.15 M, 1 mL × 2) through centrifugation at 6000 rpm for 10 min. The obtained bacterial cells were resuspended in PBS solution (1 mL, 0.15 M, pH 7.4).

2.6. Examination of Cell Compatibility of the Van-Au NPs. Macrophage cells (RAW 264.7) were selected as the model cells to investigate the cell compatibility of the generated Van-Au NPs. The cells (∼8000 cells) were incubated in DMEM (0.1 mL). After 24 h

DOI: 10.1021/am507919m
ACS Appl. Mater. Interfaces 2015, 7, 2046−2054

incubation, the medium was replaced with the medium containing different concentrations of Van-Au NP (or free-form vancomycin) and incubated for another 2 h. Cell viability was estimated through MTT assay.[28] Briefly, the supernatant was removed, and the cells were added with MTT (1 mg/mL, 0.1 mL) and incubated for 4 h in an incubator (5% CO_2, 37 °C). DMSO (99.9%, 0.15 mL) was then added to the cell samples. The resulting solution was analyzed using UV−Vis absorption spectroscopy. The difference in the intensities of the solution between the absorbance at 560 and 630 nm was recorded to estimate cell viability.

2.7. Examination of Binding Affinity and Antibiotic Activity. Van-Au NPs (2.24 mg/mL, 0.2 mL) were mixed and shaken (~200 rpm) with bacterial samples (~10^9 cells/mL) prepared in PBS solution (0.8 mL, 0.15 M, pH 6) for 2 h. The samples were centrifuged at 3000 rpm for 10 min, and the binding affinity was examined by the naked eye. Examination of the antibiotic activity of the Van-Au NPs toward pathogenic bacteria was conducted based on the procedures shown as follows. Bacteria samples (~10^9 cell/mL, 10 μL) were mixed well with broth (1.49 mL) followed by addition of different concentrations of Van-Au NPs (0.5 mL). The mixtures were shaken at 37 °C for 9 h. The optical density at a wavelength of 600 nm (OD_{600}) of the sample solution was monitored by a WPA biowave C08000 cell density meter every half hour. The growth curves resulted from bacteria only, and Van-Au NPs incubated with bacteria were plotted accordingly. The bacterial samples were also incubated with/without antibiotic agents on Petri dishes in parallel, that is, bacteria (~10^4 cells/mL, 0.2 mL) were mixed with the Van-Au NPs (~480 μg/mL, 0.2 mL) and gently shaken at room temperature (~25 °C) for 2 h. Bacteria prepared in PBS solution (~10^4 cells/mL, 0.2 mL), bacteria incubated with citrate salt-directed synthesis of Au NPs (0.2 mL), and bacteria incubated with vancomycin (0.2 mL) were conducted in parallel at 37 °C. After incubation for 14 h, the bacterial colonies on the Petri dishes were counted.

2.8. Estimation of Minimum Inhibitory Concentration (MIC). Bacterial samples (~10^4 cells/mL, 0.2 mL) prepared in PBS solution (0.15 M, pH 7.4) were suspended in either LB broth or TSBY (1.4 mL) and then added with different concentrations of Van-Au NP (0.4 mL). The final bacterial concentration was ~10^3 cells/mL. The OD_{600} of the sample solution was recorded before and after incubation at 37 °C for 8 h. To count the colony, the broth solution (0.2 mL) was cultured on a Petri dish with the proper medium. The colony number was counted after incubation at 37 °C for 14 h.

2.9. Examination of Antibiotic Activity of the Van-Au NPs toward *S. aureus* in Macrophages. *S. aureus*-infected macrophages were prepared as model samples to examine the antibiotic activity of the Van-Au NPs inside of macrophage cells. For ease of vitalization of *S. aureus*, *S. aureus* were labeled with dextran-AuNCs, which has been demonstrated to be suitable fluorescence labeling agents for bacteria.[29] The generation of dextran-AuNCs was based on the procedures stated elsewhere.[29] Dextran-Au NCs (1 mg/mL, 0.1 mL) were incubated with *S. aureus* (~10^9 cells/mL, 0.1 mL) for 1 h followed by centrifugation at 8000 rpm for 20 min. The supernatant was removed, and the precipitates were resuspended in PBS solution (0.1 mL, 0.15 M, pH 6). After being rinsed by PBS solution three times, *S. aureus* cells labeled with the dextran-Au NCs were readily used for cellular uptake by macrophage cells. Macrophages (RAW 264.7) (~10^6 cells/mL) were incubated in DMEM (1 mL) at 37 °C. After incubation for 12 h, the medium was replaced by new medium (1 mL) containing dextran-AuNCs-labeled *S. aureus* (~10^7 cell/mL) and incubated for another 2 h. The medium was then removed by a pipet, and the remaining species were rinsed with PBS solution (0.15 M, pH 7.4, 1 mL × 4). The macrophage cells were investigated under a microscope.

Subsequently, the medium was replaced by new DMEM medium containing Van-Au NPs (0.217 mg/mL, 1 mL) and incubated for another 2 h. The medium was then removed by a pipet, and the remaining species were rinsed with PBS solution (0.15M, pH 7.4, 1 mL × 4). Furthermore, the dextran-AuNCs-labeled *S. aureus*-infected macrophage treated with/without Van-Au NPs, macrophage only, and dextran-AuNCs labeled *S aureus* were stained with Hoechst stain (0.1%). Methanol (1 mL) was incubated with the sample at 37 °C for 1 h. The supernatant was removed, and the cell samples were rinsed with PBS solution (0.15 M, pH 7.4, 1 mL × 2). The cell samples were incubated with block solution (0.1 mL) at 37 °C for 1 h. The block solution was composed of 10% fetal bovine serum and 0.25% Triton X-100 in PBS solution. The supernatant was removed, and the cell samples were rinsed with wash buffer (0.1 mL × 3) composed of 0.25% Triton X-100. Subsequently, 0.1% Hoechst dyes (0.1 mL) were added to the cell sample and incubated at 37 °C for 1 h. The resultant cell samples were observed under a fluorescent microscope.

To investigate the antibiotic activity of the Van-Au NPs, the antibiotic test of *S. aureus*-infected macrophages treated with/without the Van-Au NPs was also conducted. Macrophages (RAW 264.7) (~10^6 cells/mL) were incubated in DMEM (1 mL) at 37 °C. After incubation for 12 h, the medium was replaced by medium (1 mL) containing *S. aureus* (~10^7 cell/mL) and incubated for another 2 h. The medium was then removed by a pipet, and the remaining species were rinsed with PBS solution (0.15 M, pH 7.4, 1 mL × 4). Subsequently, the medium was replaced by new DMEM medium containing Van-Au NPs (0.23 mg/mL, 1 mL) and incubated for another 2 h. The medium was then removed by a pipet, and the remaining species were rinsed with PBS solution (1 mL × 2). Furthermore, the fresh TSBY (1 mL) broth was added and incubated with cell samples at 37 °C for 16 h. The macrophage sample was diluted 10^6 times serially by PBS solution. The resultant solution (0.1 mL) was directly cultured on a Petri dish containing TSBY agar at 37 °C for 16 h to investigate the growth condition of the bacterial cells. The control sample of *S. aureus*-infected macrophages without being treated by the Van-Au NPs was also conducted in parallel.

3. RESULTS AND DISCUSSION

It has been demonstrated that glucose can be used to reduce Au ions for generation of Au NPs at high pH with addition of aqueous NaOH.[30−32] Vancomycin contains glycosides. Thus, we believed that vancomycin can be used to reduce Au ions and be directly capped on the generated Au NPs. Therefore, facile one-pot reactions involving simple stirring of tetrachloroaurate with vancomycin in aqueous solution at alkaline condition (pH 12) was conducted. The generated product solution was red in color (inset in Figure 1A). The absorption spectrum of the resulting Van-Au NPs had a maximum absorption band at ~518 nm (Figure 1A). Figure 1B shows the TEM image of the Van-Au NPs. The average size of the Van-Au NPs was estimated to be 8.4 ± 1.3 nm (Figure 1C). Furthermore, the binding amount of vancomycin on the Van-Au NPs was estimated to be ~190 nmol/mg, while the reaction yield was ~64%.

Figures S1A and S1B, Supporting Information, show the IR spectra of the free-form vancomycin and Van-Au NPs, respectively. The two spectra primarily differ in the fingerprint region from 900 to 1200 cm^{-1}, which corresponds to C−O stretching. Two peaks at 1155 and 1176 cm^{-1} in the IR spectrum of vancomycin (Figure S1A, Supporting Information) were not found in the IR spectrum of the Van-Au NPs (Figure S1B, Supporting Information). Furthermore, the small peak at 1016 cm^{-1} in Figure S1A, Supporting Information, was not found in Figure S1B, Supporting Information. These results indicated that functional groups with C−O stretching were involved in the binding, that is, the glycoside on the vancomycin (Scheme S1, Supporting Information) might be involved in the generation of the Van-Au NPs. XPS results were also examined to confirm further this possibility. Figures S2A, S2B, and S2C, Supporting Information, show the XPS spectra of O 1s, N 1s, and C 1s in vancomycin (black) and Van-Au NPs (red), respectively. Apparently, the XPS peaks in the spectra of O 1s and C 1s shifted, whereas the peaks in the N 1s spectra

DOI: 10.1021/am507919m
ACS Appl. Mater. Interfaces 2015, 7, 2046−2054

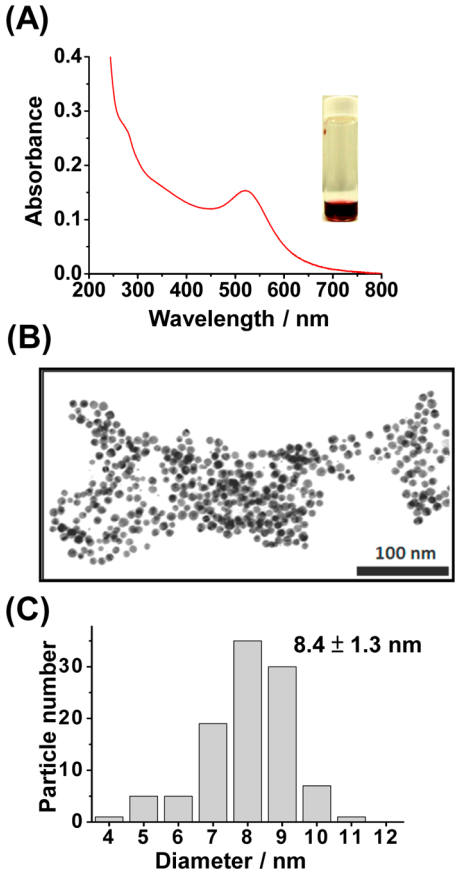
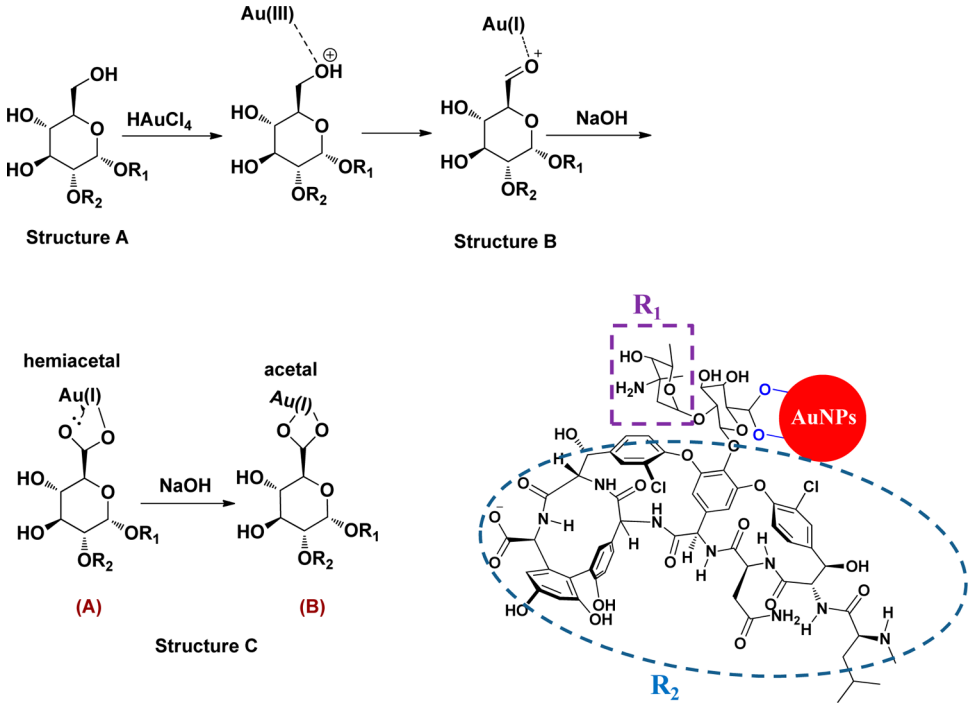


Figure 1. (A) Absorption spectrum of Van-Au NPs. (Inset) Photograph of Van-Au NPs. (B) TEM image of Van-Au NPs and (C) corresponding particle size distribution estimated using ImageJ.

were kept the same in the spectra of vancomycin and Van-Au NPs. The results indicated that C−O-related functional groups were involved in the generation of the Van-Au NPs. By contrast, nitrogen-containing functional groups were not involved in the generation of the Van-Au NPs. Hence, we proposed the putative reaction mechanism of the generation of Van-Au NPs, as shown in Scheme 1. The reaction of vancomycin (Structure A) and tetrachloroauric acid occurred in the presence of sodium hydroxide (pH 12). Thus, glycoside was oxidized at high pH (Structure B) and reacted with tetrachloroauric acid to generate Van-Au NPs (Structure C). On the basis of the proposed reaction mechanism, the binding site (dashed lines shown in Structure A) was not blocked during the Van-Au NP generation. Thus, the binding affinity toward bacteria and antibiotic activity of vancomycin on the Van-Au NPs was unaltered.

The binding affinity of the generated Van-Au NPs toward bacteria was further examined. Figure 2A, 2B, 2C, 2D, 2E, 2F, 2G, 2H, 2I, 2J, 2K, 2L, and 2M displays the photographs obtained after vortex mixing the Van-Au NPs alone, Van-AuNPs with PDRAB, vancomycin-resistant *E. faecium* (VRE 4), vancomycin-resistant *E. faecalis* (VRE1), *E. faecalis*, MRSA, *S. aureus*, *P. aeruginosa* (PA), *A. baumannii* (AB), *E. faecium*, *E. coli* O157:H7 (O157:H7), *K. pneumoniae* (KP), and *E. coli* J96 (J96), respectively, for 2 h, which was followed by centrifugation at 3000 rpm for 10 min. All samples were prepared in PBS solution (pH 7.4). Precipitates were observed in all samples except the vial containing the Van-Au NPs alone (Figure 2A). The results shows that red precipitates only appeared in the samples containing Van-Au NPs and bacteria, which indicated the binding affinity arising between the Van-Au NPs and the bacteria. The heavy conjugates resulted from the binding of Van-Au NPs with bacteria settled at a low centrifugation speed. Figure 3 shows the TEM images obtained after incubating different bacteria with Van-Au NPs. Van-Au

Scheme 1. Proposed Reaction of Vancomycin-Directed Synthesis of Au NPs



DOI: 10.1021/am507919m
ACS Appl. Mater. Interfaces 2015, 7, 2046−2054

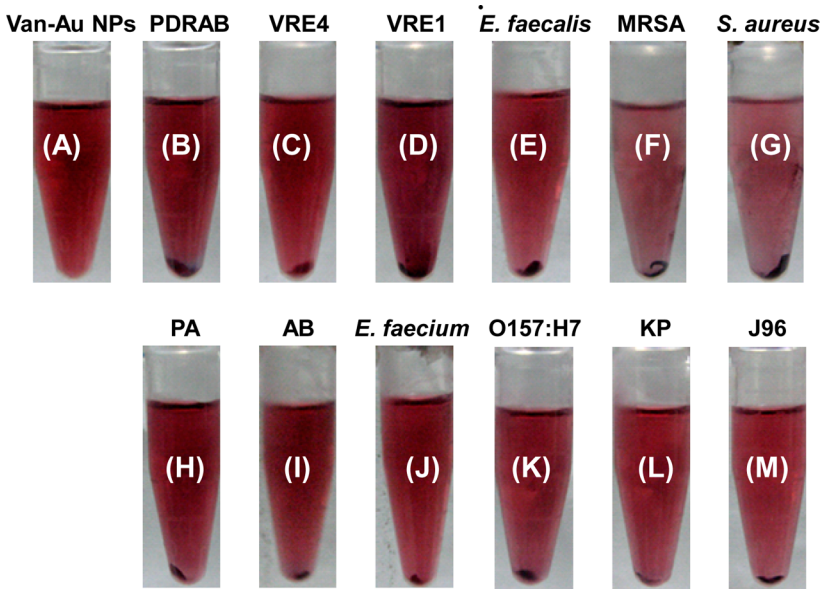


Figure 2. Photographs obtained after vortex mixing (A) Van-Au NPs alone, Van-Au NPs with (B) PDRAB, (C) VRE4, (D) VRE1, (E) *E. faecalis*, (F) MRSA, (G) *S. aureus*, (H) *P. aeruginosa* (PA), (I) *A. baumannii* (AB), (J) *E. faecium*, (K) *E. coli* O157:H7 (O157:H7), (L) *K. pneumonia* (KP), and (M) *E. coli* J96 (J96) for 2 h followed by centrifugation at 3000 rpm for 10 min.

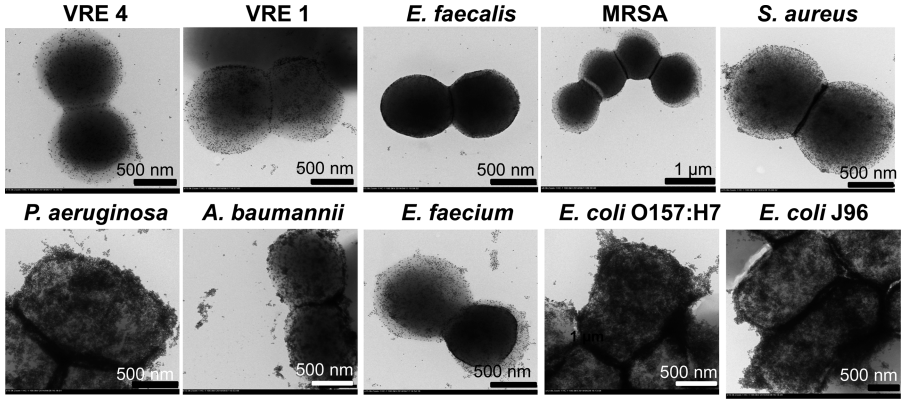


Figure 3. TEM images obtained by incubating different bacteria with the Van-Au NPs. All scale bars on the images are equal to 500 nm except the scale bar in the image of MRSA is 1 μm.

NPs were apparently well anchored on the bacterial cell surface, indicating that Van-Au NPs have binding affinity with these model bacteria, including Gram-positive, Gram-negative, and antibiotic-resistant bacteria.

One might suspect that the binding affinity might have resulted from electrostatic interactions. Thus, the zeta potentials of the Van-Au NPs at pH 6, 7, and 8 were investigated. The corresponding zeta potentials were ca. −17.7, −25.6, and −34.6 mV, which showed that the Van-Au NPs have negative charges at pH > 6. Bacteria, including Gram-positive and Gram-negative bacteria, have negatively charged surfaces because of the presence of teichoic acid[33] and lipopolysaccharides,[34] respectively. Thus, electrostatic interactions have a negligible influence on the binding interactions between the Van-Au NPs and bacteria. The binding interactions may have resulted from the molecular recognition similar to that between free-form vancomycin and bacteria. In other words, the binding site of vancomycin for bacteria was not blocked after vancomycin was immobilized on the Van-Au NP surface. On the basis of the results obtained from IR absorption spectroscopic analysis and XPS analysis shown in Figures S1 and S2, Supporting Information, respectively, the C−O group of glycoside on the vancomycin structure was involved in the binding between vancomycin and Au NPs, as proposed (Scheme 1).

The aforementioned binding tests showed that Van-Au NPs could recognize different bacteria, implying that the antibiotic activity of the Van-Au NP might be unaltered. Thus, *S. aureus* was primarily selected as the model sample to demonstrate the feasibility of using the Van-Au NPs as antibacterial agents. Figure 4A shows the growth curves of *S. aureus* (~5×10^6 cells mL^{-1}, 2 mL) obtained by culturing bacteria in broth containing different concentrations of Van-Au NPs. The results show that the cell growth of *S. aureus* was effectively inhibited when the concentration of vancomycin loaded on the Van-Au NPs was 53 μg/mL (red curve) compared with the curve (black) obtained from culturing *S. aureus* in Van-Au NP free broth. *S. aureus* cell growth was completely inhibited when the concentration of the vancomycin loaded on the Van-Au NPs reached ~106 μg/mL. By contrast, less effective inhibition of the bacterial cell growth was observed when the bacterial sample was treated with free-form vancomycin (Figure 4B).

DOI: 10.1021/am507919m
ACS Appl. Mater. Interfaces 2015, 7, 2046−2054

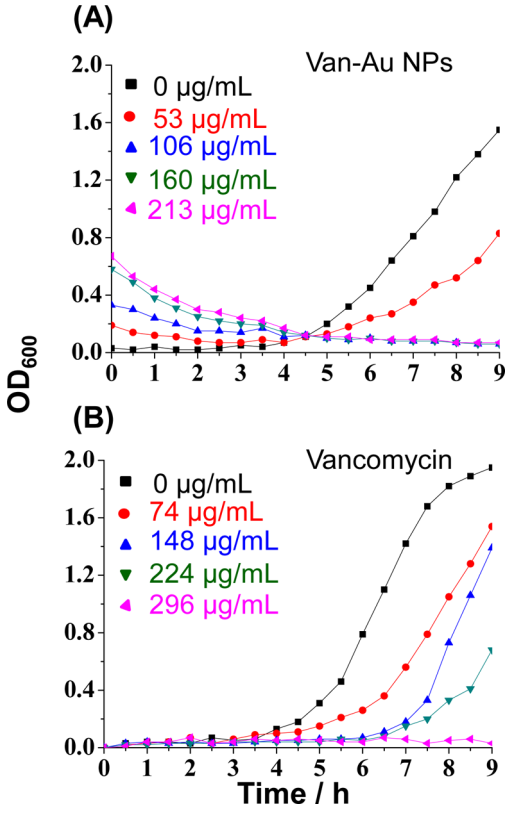


Figure 4. Growth curves of *S. aureus* (~5 × 10^6 cells/mL, 2 mL) obtained by culturing bacteria in TSBY broth containing different concentrations of (A) Van-Au NPs and (B) vancomycin.

The inhibition of the cell growth of *S. aureus* was observed when the concentration of free-form vancomycin concentration was 74 μg/mL. When the concentrations of vancomycin were increased to 148 (blue curve) and 224 μg/mL (green curve), bacterial growth continued. The cell growth of *S. aureus* was completely inhibited when the concentration of vancomycin reached 296 μg/mL, which was higher than that of Van-Au NPs. When the OD_{600} was over 1.0, the samples were diluted to prepare the curves shown in Figure 4, that is, the dilution factor was taken into account when plotting Figure 4. It was noticed that the presence of the Van-Au NPs in the sample solution also contributed to the OD_{600}, so the OD_{600} value was not close to 0 in the first few hours of the incubation in those samples used for plotting Figure 4A. Within the first 4 h, the OD_{600} value gradually decreased. Presumably, the Van-AuNPs can bind to the bacterial cells, leading to formation of heavy conjugates. Even when we vortex mixed the sample before OD measurement, the concentration of the Van-AuNPs in the solution still decreased, leading to the decrease of the OD_{600} in the first few hours. After 4.5 h incubation, the antibiotic activity of the Van-Au NPs was quite apparent and the Van-Au NPs seemed degraded. Therefore, the OD_{600} was close to 0 after incubation for certain hours. The results show that the Van-Au NPs are more potent than that of free-form vancomycin used to inhibit the cell growth of *S. aureus*. One may suspect that the growth curve cannot present the real growth condition because of the formation of heavy conjugates of the Van-Au NP-bacterium and may lead to wrong conclusions. Thus, we further checked the antibiotic activity of the Van-Au NPs by culturing the samples on Petri dishes.

To check the antibiotic activity of the Van-Au NPs closely, bacteria with or without antibiotic agents were cultured on Petri dishes. These cultures included *S. aureus* mixed with PBS solution, *S. aureus* incubated with the Van-Au NPs, *S. aureus* with bare Au NPs, and *S. aureus* with vancomycin. These cultures were gently shaken at room temperature (~25 °C) for 2 h followed by growing the culture (0.2 mL) on individual Petri dishes (~37 °C) overnight. Figure 5A and 5B shows the

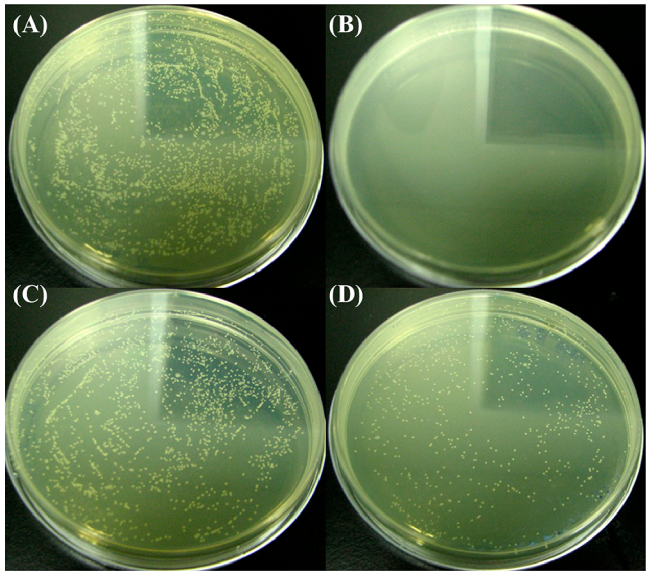


Figure 5. Photographs obtained by culturing the sample solutions (0.2 mL) from (A) *S. aureus* (~10^4 cells/mL, 0.2 mL) mixed with PBS solution (0.2 mL), (B) *S. aureus* (~10^4 cells/mL, 0.2 mL) incubated with Van-Au NPs (0.48 mg/mL, 0.2 mL), (C) *S. aureus* (~10^4 cells/mL, 0.2 mL) with bare Au NPs, and (D) *S. aureus* (~10^4 cells/mL, 0.2 mL) with free-form vancomycin (0.15 mg/mL, 0.2 mL) on individual Petri dishes.

overnight culture results of *S. aureus* cells and *S. aureus* cells mixed with Van-Au NPs, respectively. Apparently, many bacterial colonies were observed on the Petri dish, as shown in Figure 5A. However, no colonies were observed in Figure 5B. The loading amount of vancomycin on the Van-Au NPs was ~0.277 μg/μg. By considering the volume including bacterial solution, the final concentration of vancomycin in the sample was ~66 μg/mL. These results indicated that Van-Au NPs could suppress the cell growth of *S. aureus*. However, one might wonder if the inhibition capability may be caused by the toxicity of Au NPs. Thus, *S. aureus* cells were cultured in a Petri dish with bare Au NPs generated from the overnight reduction of sodium citrate (Figure 5C). The bacterial cells grew well, similar to those shown in Figure 5A, indicating that the presence of these Au NPs did not affect the cell growth of *S. aureus*. Figure 5D shows the photograph obtained after gently shaking *S. aureus* with free-form vancomycin for 2 h, which was followed by culturing on a Petri dish. The final concentration of vancomycin in the bacterial sample was ~75 μg/mL, which was slightly higher than the vancomycin used for Figure 5B. Nevertheless, bacterial colonies were observed in Figure 5D. The colony number was slightly smaller than that observed in the control sample in Figure 5A. From these results, we concluded that the Van-Au NPs are more effective in inhibiting the cell growth of *S. aureus* than free-form vancomycin. Furthermore, bare Au NPs do not cause apparent toxicity

DOI: 10.1021/am507919m
ACS Appl. Mater. Interfaces 2015, 7, 2046−2054

toward bacteria. Additionally, we also used a Gram-negative bacterial strain, PDRAB, as the model sample to investigate the antibiotic activity of the Van-Au NPs on Gram-negative bacteria. Figure S3A, Supporting Information, shows the result obtained by incubating PRDAB alone overnight, while Figure S3B, Supporting Information, was obtained when incubating the same bacterial sample with Van-Au NPs for the same time. It is clear that the growth of PDRAB is completely inhibited. Figure S3C, Supporting Information, shows the result obtained from another control sample by overnight incubating unmodified Au NPs with the same bacterial sample. The result showed that the presence of the unmodified Au NPs did not affect the growth of PDRAB. Figure S3D, Supporting Information, shows the result obtained after incubating free vancomycin with the sample for obtaining Figure S3B, Supporting Information. The result shows that the bacterial growth was not affected too much in this same concentration of vancomycin as loaded on the Van-NPs, that is, the Van-Au NPs were effective for inhibiting the growth of PDRAB, while bare Au NPs has no effect on the bacterial cells. The results show that the Van-Au NPs not only inhibit the cell growth of Gram-positive bacteria but also are also capable to inhibit the growth of Gram-negative bacteria as well as antibiotic-resistant bacterial strains such as PDRAB. The results are similar to what was discovered in previous studies.[8,11−13]

After demonstrating the antibacterial activity of the Van-Au NPs, the MIC_{50} of the Van-Au NPs was further examined using different bacteria as the samples, which included Gram-positive, Gram-negative, and antibiotic-resistant bacterial strains. To fully examine the effect, the cell concentration of bacteria ($\sim 10^3$ cells/mL, 2 mL) was significantly lower than that used for Figure 5. Table 1 shows a summary of the MIC_{50} of free-form vancomycin and Van-Au NPs. Apparently, the MIC_{50} values of the Van-Au NPs against all the tested bacteria were significantly lower than those obtained using free-form vancomycin as antibiotic. The results suggested the inhibition capacity of the Van-Au NP against the cell growth of the bacteria is more potent than that of free-form vancomycin. Furthermore, Van-Au NPs could be used to effectively inhibit the cell growth of Gram-negative bacteria, such as *E. coli*, *P. aeruginosa*, and *A. baumannii*. In addition, the results showed that Van-Au NPs not only could be used to effectively inhibit the cell growth of vancomycin-resistant bacterial strains, such as VRE1 (*E. faecalis*) and VRE4 (*E. faecium*), but are also effective antibiotic agents against other antibiotic-resistant bacterial strains, which include MRSA and PDRAB.

Table 1. MIC_{50} of the Vancomycin and Van-Au NPs against Different Pathogenic Bacteria

bacteria strains	MIC_{50} (μg/mL)	
	vancomycin	Van-AuNP[a]
S. aureus	32	8
MRSA	64	8
E. coli J96	64	8
E. coli O157:H7	128	8
P. aeruginosa	64	8
E. faecalis	64	16
E. faecium	>128	16
A. baumannii	128	8
VRE1(*E. faecalis*)	64	16
VRE4 (*E. faecium*)	>128	32
PDRAB	>128	8

[a]Amount of vancomycin loaded on the Van-Au NPs suspended in solution.

The cell biocompatibility of the Van-Au NPs was also investigated. Macrophage cells (RAW 264.7) were used as the model samples. Figure S4, Supporting Information, shows the plot of cell viability obtained by incubating the macrophage cells with different concentrations of Van-Au NPs at 37 °C for 2 h, followed by MTT assay. The results show the toxicity of the Van-Au NPs was not apparent. The cell viability was ~80%; even the concentration of Van-Au NPs was increased to >400 μg/mL. The results indicated that the Van-Au NPs have acceptable cell biocompatibility.

Pathogenic bacteria that can survive inside macrophages are more difficult to treat. Considering that macrophages tend to engulf NPs,[24−27] Van-Au NPs could effectively treat macrophages infected by pathogenic bacteria. First, we examined whether the macrophage cells (RAW 264.7) can readily engulf Van-Au NPs. Figure 6A and 6B shows the fluorescence image and the optical image in bright field of the macrophage cells

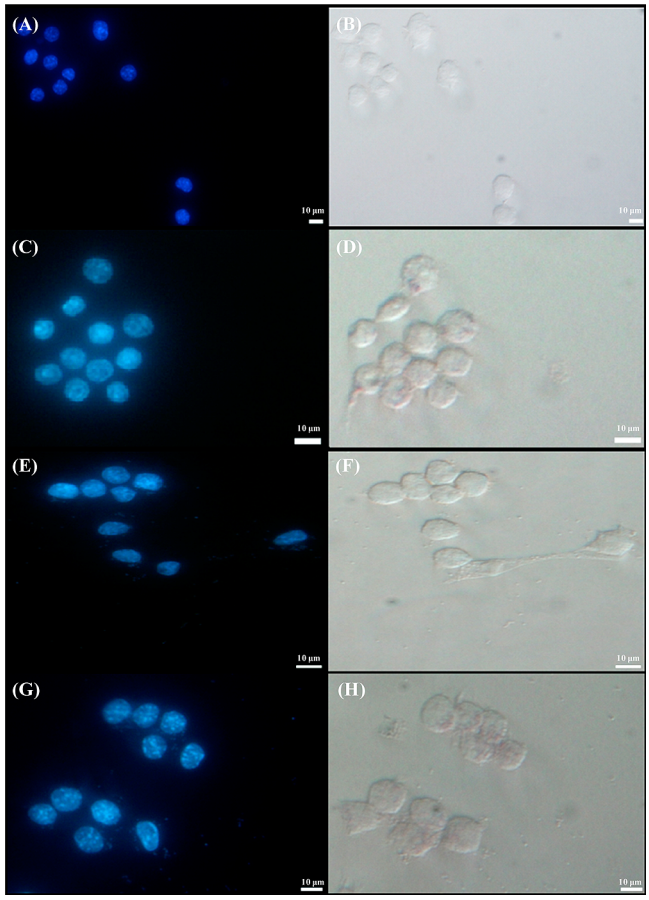


Figure 6. (A) Fluorescence image and (B) optical image in bright field of the macrophage cells. (C) Fluorescence image and (D) optical image in bright field of the macrophage cells obtained after being incubated with Van-Au NPs for 2 h followed by rinsing with new medium. Images obtained using (E) a fluorescence microscope and (F) an optical microscope of the dextran-AuNC-labeled *S. aureus*-infected macrophage cells. (G) Fluorescence image and (H) optical image in bright field of AuNCs-dextran-labeled *S. aureus*-infected macrophage after being incubated with Van-Au NPs for 2 h. Excitation wavelength was 330−380, and a 420 nm filter was used for fluorescence imaging. Exposure time was set at 60 ms.

DOI: 10.1021/am507919m
ACS Appl. Mater. Interfaces 2015, 7, 2046−2054

only, respectively. The nuclei were stained with Hoechst stain. Comparing the images on the left- and right-hand side, it is clear where the nuclei are. Figure 6C and 6D shows the fluorescence image with blue emission and the optical image in bright field of the macrophage cells obtained after being incubated with Van-Au NPs for 2 h followed by rinsing with new medium. The image under bright field shows that the Van-Au NPs with red color (Figure 6D) were readily engulfed to the cytoplasm of the cells after 2 h of incubation, indicating the capability of the macrophage cells in engulfment of the Van-Au NPs. Next, macrophages infected by *S. aureus*, which is known to survive inside of macrophages,[35] were used as the model system to examine the antibiotic activity of the Van-Au NPs. We first prepared the sample of macrophages infected by *S. aureus*. To make the results more observable, *S. aureus* cells were first labeled with dextran-Au NCs, which have bright fluorescence and negligible toxicity toward bacteria and cells as demonstrated previously.[29] Images were obtained using a fluorescence microscope (Figure 6E) and an optical microscope in bright field (Figure 6F). The bacteria labeled with dextran-Au NCs were apparently engulfed into the macrophages and evidently observed in the cytoplasm by comparing the images obtained from fluorescence microscope and bright field. Figure 6G and 6H shows the fluorescence image and optical images obtained under bright field of dextran-AuNCs-labeled *S. aureus*-infected macrophage after being incubated with Van-Au NPs for 2 h. Apparently, the Van-Au NPs were engulfed by the macrophage cells and engulfed to the cytoplasm.

After demonstrating that macrophage cells can engulf Van-Au NPs, we further examined whether the cell growth of *S. aureus* inside of the macrophage cells can be inhibited by the engulfed Van-Au NPs. The *S. aureus*-infected macrophages ($\sim 10^6$ cells) were treated with Van-Au NPs (0.23 mg/mL, l mL in DMEM) at 37 °C for 2 h. After removing the supernatant and rinsing with PBS solution, the remaining macrophage cells were cultured in TSBY broth (1 mL) at 37 °C with CO_2 (5%) for 16 h. The resultant sample was 10^6-fold diluted by PBS solution followed by culturing the sample (0.1 mL) on a Petri dish containing TSBY at 37 °C for 16 h. The control sample contained *S. aureus*-infected macrophage cells only. Figure 7A and 7B shows the photographs of the control and experimental samples obtained after overnight incubation, respectively. The colony number of *S. aureus* was effectively decreased in the Van-Au NP-treated macrophages (Figure 7B), indicating the cell growth of the bacteria inside the macrophages was inhibited. Additionally, we also used MRSA-infected macrophage cells as the model sample to examine the effectiveness of the Van-Au NPs. Sample preparation was the same as that used to obtain Figure 7. Figures S5A and S5B, Supporting Information, show the photographs obtained after overnight night cell culture of the control (MRSA-infected macrophages) and experimental samples (MRSA-infected macrophages treated with the Van-Au NPs), respectively. Apparently, few colonies were observed in Figure S5B, Supporting Information, although the bacteria grew well in the control sample (Figure S5A, Supporting Information). These results indicated that Van-Au NPs could be used to effectively kill bacteria such as *S. aureus* and MRSA inside macrophages.

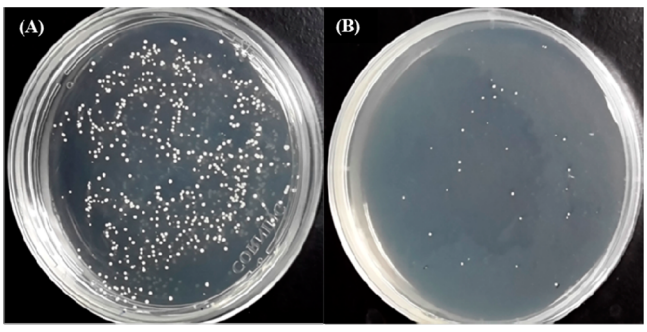


Figure 7. The *S. aureus* infected macrophage cells were incubated (A) without and (B) with Van-Au NPs at 37 °C for 2 h followed by removing the supernatant and rinsed with the PBS buffer. The remaining macrophage cells were cultured in TSBY broth (1 mL) at 37 °C with CO_2 (5%) for 16 h. The resultant sample was 10^6-fold diluted by PBS followed by culturing the sample (0.1 mL) on Petri dishes containing TSBY at 37 °C for 16 h.

4. CONCLUSIONS

In this study, a facile approach for generating Van-Au NPs from one-pot reaction has been successfully developed. These Van-Au NPs could effectively inhibit the cell growth of pathogenic bacteria, such as Gram-positive, Gram-negative, and antibiotic-resistant bacterial strains, indicating the potential of the generated Van-Au NPs as universal antibacterial agents. Generation of the Van-Au NP was straightforward, and the antibiotic activity of the generated Van-Au NPs was unaltered. The Van-Au NP MIC_{50} against bacteria, which included vancomycin-resistant bacterial strains, was significantly lower than that of free-form vancomycin. In addition, the feasibility of using Van-Au NPs as antibacterial agents to treat *S. aureus*-infected macrophages was demonstrated. The Van-Au NPs could be readily internalized by macrophages to inhibit the cell growth of *S. aureus* inside the cells, that is, Van-Au NPs not only serve as drug carriers but also act as effective antibiotics. Furthermore, Van-Au NPs are effective against antibiotic-resistant bacterial strains, such as MRSA, that have been engulfed by macrophages. Further studies should be conducted to investigate the antibiotic activity of Van-Au NPs in vivo. The success of this approach may potentially lead to new therapeutics for treating bacterial infections. Thus, the potential use of Van-Au NPs in bacterial infectious diseases can be anticipated.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Binding site between vancomycin and Gram-positive bacteria, IR absorption and XPS spectra of vancomycin and Van-AuNPs, cell comparability examination, PDRAB inhibition experiments, and bacterial growth results from MRSA-infected macrophage cells (10^6 cell mL^{-1}) incubated without and with Van-Au NPs. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: +886-3-5131527. Fax: +886-3-5723764. E-mail: yuchie@mail.nctu.edu.tw.

Notes

The authors declare no competing financial interest.

DOI: 10.1021/am507919m
ACS Appl. Mater. Interfaces 2015, 7, 2046−2054

■ ACKNOWLEDGMENTS

We thank the Ministry of Science and Technology of Taiwan (NSC 102-2113-M-009-019-MY3 and NSC 102-2627-M-009-002) for financial support of this research.

■ REFERENCES

(1) Hoffman, L. R.; D'Argenio, D. A.; MacCoss, M. J.; Zhang, Z.; Jones, R. A.; Miller, S. I. Aminoglycoside Antibiotics Induce Bacterial Biofilm Formation. *Nature* **2005**, *436*, 1171−1175.

(2) Furno, F.; Morley, K. S.; Wong, B.; Sharp, B. L.; Arnold, P. L.; Howdle, S. M.; Bayston, R.; Brown, P. D.; Winship, P. D.; Reid, H. J. Silver Nanoparticles and Polymeric Medical Devices: A New Approach to Prevention of Infection. *J. Antimicrob. Chemother.* **2004**, *54*, 1019−1024.

(3) Klevens, R. M.; Edwards, J. R.; Richards, C. L.; Horan, T. C.; Gaynes, R. P.; Pollock, D. A.; Cardo, D. M. Estimating Health Care-Associated Infections and Deaths in US Hospitals, 2002. *Public Health Rep.* **2007**, *122*, 160−166.

(4) Tamar, L.; Jay, G.; Frank, R. E.; Liliana, G. Pediatric Vancomycin Use in 421 Hospitals in the United States, 2008. *PLoS One* **2012**, *7*, e43258.

(5) von Nussbaum, F.; Brands, M.; Hinzen, B.; Weigand, S.; Häbich, D. Antibacterial Natural Products in Medicinal Chemistry—Exodus or Revival? *Angew. Chem., Int. Ed.* **2006**, *45*, 5072−5129.

(6) Coque, T. M.; Tomayko, J. F.; Ricke, S. C.; Okhyusen, P. C.; Murray, B. E. Vancomycin-Resistant *Enterococci* from Nosocomial, Community, and Animal Sources in the United States. *Antimicrob. Agents Chemother.* **1996**, *40*, 2605−2609.

(7) Levine, D. P. Vancomycin: A History. *Clin. Infect. Dis.* **2006**, *42* (Suppl 1), S5−S12.

(8) Gu, H.; Ho, P.; Tong, E.; Wang, L.; Xu, B. Presenting Vancomycin on Nanoparticles to Enhance Antimicrobial Activities. *Nano Lett.* **2003**, *3*, 1261−1263.

(9) Kell, A. J.; Stewart, G.; Ryan, S.; Peytavi, R.; Boissinot, M.; Huletsky, A.; Bergeron, M. G.; Simard, B. Vancomycin-Modified Nanoparticles for Efficient Targeting and Preconcentration of Gram-Positive and Gram-Negative Bacteria. *ACS Nano* **2008**, *2*, 1777−1788.

(10) Gu, H.; Ho, P.-L.; Tsang, K. W.; Wang, L.; Xu, B. Using Biofunctional Magnetic Nanoparticles to Capture Vancomycin-Resistant *Enterococci* and Other Gram-Positive Bacteria at Ultralow Concentration. *J. Am. Chem. Soc.* **2003**, *125*, 15702−15703.

(11) Lin, Y.-S.; Tsai, P.-J.; Weng, M.-F.; Chen, Y.-C. Affinity Capture Using Vancomycin-Bound Magnetic Nanoparticles for the MALDI-MS Analysis of Bacteria. *Anal. Chem.* **2005**, *77*, 1753−1760.

(12) Huang, W.-C.; Tsai, P.-J.; Chen, Y.-C. Functional Gold Nanoparticles as Photothermal Agents for Selective-Killing of Pathogenic Bacteria. *Nanomedicine* **2007**, *2*, 777−787.

(13) Huang, W.-C.; Tsai, P.-J.; Chen, Y.-C. Multifunctional Fe_3O_4@ Au Nanoeggs as Photothermal Agents for Selective Killing of Nosocomial and Antibiotic-Resistant Bacteria. *Small* **2009**, *5*, 51−56.

(14) Qi, G.; Li, L.; Yu, F.; Wang, H. Vancomycin-Modified Mesoporous Silica Nanoparticles for Selective Recognition and Killing of Pathogenic Gram-Positive Bacteria Over Macrophage-Like Cells. *ACS Appl. Mater. Int.* **2013**, *5*, 10874−10881.

(15) Yu, T.-J.; Li, P.-H.; Tseng, T.-W.; Chen, Y.-C. Multifunctional Fe_3O_4/Alumina Core/Shell MNPs as Photothermal Agents for Targeted Hyperthermia of Nosocomial and Antibiotic-Resistant Bacteria. *Nanomedicine* **2011**, *6*, 1353−1363.

(16) Arnold, J. K.; Gale, S.; Shannon, R.; Regis, P.; Maurice, B.; Ann, H.; Michel, G. B.; Benoit, S. Vancomycin-Modified Nanoparticles for Efficient Targeting and Preconcentration of Gram-Positive and Gram-Negative Bacteria. *ACS Nano* **2008**, *2*, 1777−1788.

(17) Chen, W.-C.; Tsai, P.-C.; Chen, Y.-C. Functional Fe_3O_4/TiO_2 Core/Shell Magnetic Nanoparticles as the Photo-Killing Agents for Pathogenic Bacteria. *Small* **2008**, *4*, 485−491.

(18) Sun, J.; Li, J.; Fan, H.; Ai, S. Ag Nanoparticles and Vancomycin Comodified Layered Double Hydroxides for Simultaneous Capture and Disinfection of Bacteria. *J. Mater. Chem. B* **2013**, *1*, 5436−5442.

(19) Lai, B.-H.; Chen, D.-H. Vancomycin-Modified LaB_6@ SiO_2/Fe_3O_4 Composite Nanoparticles for Near-Infrared Photothermal Ablation of Bacteria. *Acta Biomater.* **2013**, *9*, 7573−7579.

(20) Choi, S. K.; Myc, A.; Silpe, J. E.; Sumit, M.; Tinmoi, P.; McCarthy, W. K.; Desai, A. M.; Thomas, T. P.; Kotlyar, A.; Holl, M. M. B.; Orr, B. G.; Baker, J. R. Dendrimer-Based Multivalent Vancomycin Nanoplatform for Targeting the Drug-Resistant Bacterial Surface. *ACS Nano* **2013**, *7*, 214−228.

(21) Demurtas, M.; Perry, C. C. Facile One-Pot Synthesis of Amoxicillin-Coated Gold Nanoparticles and Their Antimicrobial Activity. *Gold Bull.* **2014**, *47*, 103−107.

(22) Rai, A.; Prabhune, A.; Perry, C. C. Antibiotic Mediated Synthesis of Gold Nanoparticles with Potent Antimicrobial Activity and Their Application in Antimicrobial Coatings. *J. Mater. Chem.* **2010**, *20*, 6789−6798.

(23) Kell, A. J.; Simard, B. Vancomycin Architecture Dependence on the Capture Efficiency of Antibody-Modified Microbeads by Magnetic Nanoparticles. *Chem. Commun.* **2007**, 1227−1229.

(24) Granger, D. Macrophage Production of Nitrogen Oxides in Host Defence Against Microorganisms. *Res. Immunol.* **1991**, *142*, 570−572.

(25) Xie, J.; Xu, C.; Kohler, N.; Hou, Y.; Sun, S. Controlled PEGylation of Monodisperse Fe_3O_4 Nanoparticles for Reduced Non-Specific Uptake by Macrophage Cells. *Adv. Mater.* **2007**, *19*, 3163−3166.

(26) Zahr, A. S.; Davis, C. A.; Pishko, M. V. Macrophage Uptake of Core-Shell Nanoparticles Surface Modified with Poly(ethylene glycol). *Langmuir* **2006**, *22*, 8178−8185.

(27) Cui, Z.; Hsu, C.-H.; Mumper, R. J. Physical Characterization and Macrophage Cell Uptake of Mannan-Coated Nanoparticles. *Drug Dev. Ind. Pharm.* **2003**, *29*, 689−700.

(28) Mosmann, Tim. Rapid Colorimetric Assay for Cellular Growth and Survival: Application to Proliferation and Cytotoxicity Assays. *J. Immunol. Methods* **1983**, *65*, 55−63.

(29) Chiu, W.-J.; Chen, W.-Y.; Lai, H.-Z.; Wu, C.-Y.; Chiang, H.-L.; Chen, Y.-C. Dextran-Encapsulated Photoluminescent Gold Nanoclusters: Synthesis and Application. *J. Nanopart. Res.* **2014**, *16*, 2478.

(30) Liu, J.; Qin, G.; Raveendran, P.; Ikushima, Y. Facile "Green" Synthesis, Characterization, and Catalytic Function of*β*-d-Glucose-Stabilized Au Nanocrystals. *Chem.—Eur. J.* **2006**, *12*, 2131−2138.

(31) Shervan, Z.; Yamamoto, Y. Carbohydrate-Directed Synthesis of Silver and Gold Nanoparticles: Effect of the Structure of Carbohydrates and Reducing Agents on the Size and Morphology of the Composites. *Carbohydr. Res.* **2011**, *346*, 651−658.

(32) Genga, D.; Chen, L.; Lu, G. pH Induced Size-Selected Synthesis of PtRu Nanoparticles, Their Characterization and Electrocatalytic Properties. *J. Mol. Catal. A: Chem.* **2007**, *265*, 42−49.

(33) Berry, V.; Gole, A.; Kundu, S.; Murphy, C. J.; Saraf, R. F. Deposition of CTAB-Terminated Nanorods on Bacteria to Form Highly Conducting Hybrid Systems. *J. Am. Chem. Soc.* **2005**, *127*, 17600−17601.

(34) Berry, V.; Saraf, R. F. Self-Assembly of Nanoparticles on Live Bacterium: An Avenue to Fabricate Electronic Devices. *Angew. Chem., Int. Ed.* **2005**, *44*, 6668−6673.

(35) Watanabe, I.; Ichiki, M.; Shiratsuchi, A.; Nakanishi, Y. TLR2-Mediated Survival of *Staphylococcus aureus* in Macrophages:A Novel Bacterial Strategy Against Host Innate Immunity. *J. Immunol.* **2007**, *178*, 4917−4925.

DOI: 10.1021/am507919m
ACS Appl. Mater. Interfaces 2015, 7, 2046−2054