USING VANCOMYCIN BOUND Fe3O4@Au NANOPARTICLES AS THE PHOTOTHERMAL AGENTS FOR SELECTIVE-KILLING OF PATHOGENIC BACTERIA

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Introduction

The rapid emergence of antibiotic-resistant bacterial strains has become serious problems for bacterial infections. Thus, exploring new therapeutics to solve the problems is urgent. An effective photothermal approach for selectively killing of pathogenic bacteria is presented in this study. Vancomycin, which is capable of interacting with D-Ala-D-Ala moieties of the peptide units on the cell wall of bacteria, was bound to the surfaces of gold nanoshell silica NPs embedded with magnetic iron oxide nanoparticles (Fe_3O_4@Au NPs). The vancomycin-bound Fe_3O_4@Au (van-Fe_3O_4@Au) NPs with multi-functions including absorption capacity at NIR region, magnetic characteristics, and targeting capability for bacteria are demonstrated as effective photothermal agents for pathogenic bacteria under illumination of NIR light in this study.

Methods

Magnetic iron oxide nanoparticles were prepared via co-precipitation. The nanoparticles were then modified with a thin layer of silane. Gold seeds were prepared based on Baiker's method [1]. After adjusting the pH of a gold seed solution obtained above to pH 7, the nanoparticles modified with silane with amino terminal functional group were then slowly added into the gold seed solution under stirring. The mixture was continually stirred for 12 h. The nanoparticle suspension was then added to a gold growth solution, which contained potassium carbonate, aqueous tetrachloroaurate solution, and deionized water. Formaldehyde was then slowly dropped into the mixture. When the color of the suspension changed from pale-pink to dark-green, the reaction was completed within 3 min. The details of the preparation steps for the nanoparticles are shown in Figure 1. The suspension was centrifuged at 1200 rpm for 15 min and excess gold NPs with pale-purple color were then removed. The remaining magnetic gold nanoshell NPs (Fe_3O_4@Au) were re-suspended in deionized water. Bis(vancomycin)-cystamide was then bound to the surface of Fe_3O_4@Au NPs via S-Au bonding.

All the pathogenic bacteria were collected from the patients at the General Tzu-Chi Hospital, Hualien, Taiwan. Bacteria (10^5~10^6 cfu/mL, 60 μL) prepare in PBS buffer (0.1 M) were mixed with van-Fe_3O_4@Au NPs. The mixture was then irradiated by a diode laser (808 nm; ca. 250 mW/cm^2). After irradiation, the bacteria remaining in the mixture were cultured on a Petri dish that contained TSBY medium at 37 ^{o}C for 12-16 h. TSBY was prepared by mixing TSB (12 g), yeast extract (2 g), and granulated agar (7.4 g) in deionized water (400 mL).

Results

Previous studies [2-5] have demonstrated vancomycin-bound nanoparticles are capable of recognizing pathogenic bacteria. Figures 2a-h display the TEM images of the samples obtained by incubation of bacteria including *Acinetobacter baumannii*, *Escherichia coli* O157: H7, *Staphylococcus pyogenes*, *S. saprophyticus*, pan-drug resistant *A. baumannii* (PDRAB), methicillin-resistant *S. aureus* (MRSA), vancomycin-resistant *Enterococcus Faecalis* (VRE1), and vancomycin-resistant *E. Faecium* (VRE 4), respectively, with vancomycin-bound Fe_3O_4@Au NPs. Apparently, these bacteria are fully covered with Van-Fe_3O_4@Au NPs. After these bacteria targeted by Van-Fe_3O_4@Au NPs were irradiated by NIR light for 3 min, less

than 1% of the bacterial cells could survived. The results indicate that this photothermal approach for inhibiting the cell growth of pathogenic bacteria is very effective.

Conclusion

For the first time, Van-Fe_3O_4@Au NPs are demonstrated to be effective photothermal agents for selective-killing of pathogenic bacteria under illumination of NIR light. The results show the current approach can effectively inhibit the cell growth of nosocomial bacteria. Furthermore, this photothermal approach is also effective in inhibiting the cell growth of antibiotic-resistant bacterial strains. The results suggest a quite promising hope for the future in treating patients suffering from tedious bacterial infections.

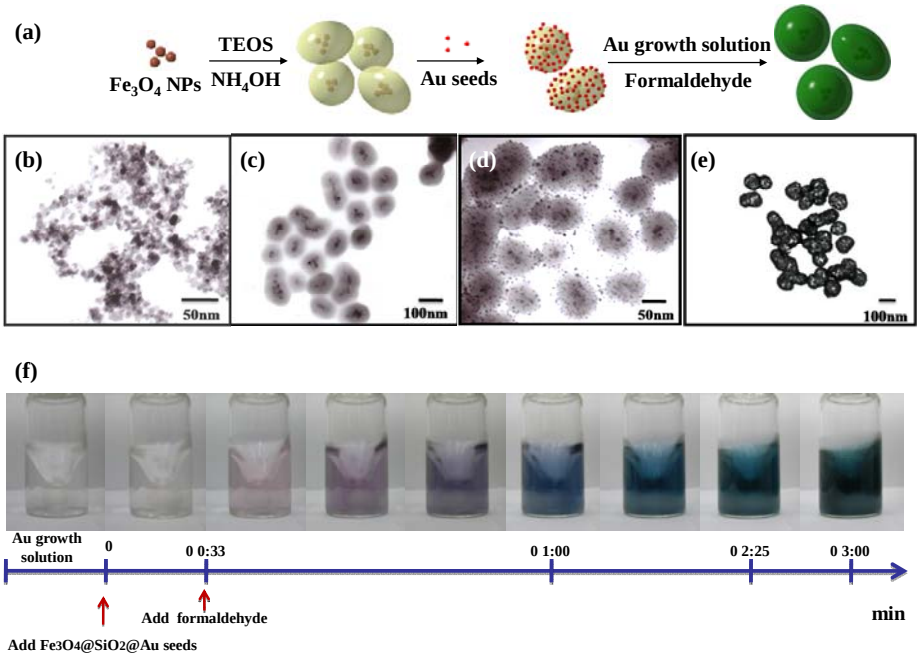

Figure 1. **(a) Schematic of the steps for preparation of Fe_3O_4@Au NPs. TEM images of (b) magnetic iron oxide nanoparticles, (c) magnetic iron oxide nanoparticles embedded silica NPs (Fe_3O_4@SiO_2), (d) Fe_3O_4@SiO_2 attached with gold nanoseeds, (e) Fe_3O_4@Au NPs. (f) The reaction process of generating Fe_3O_4@Au NPs by adding Fe_3O_4@SiO_2@Au seeds into an Au growth solution followed by reducing with formaldehyde.**

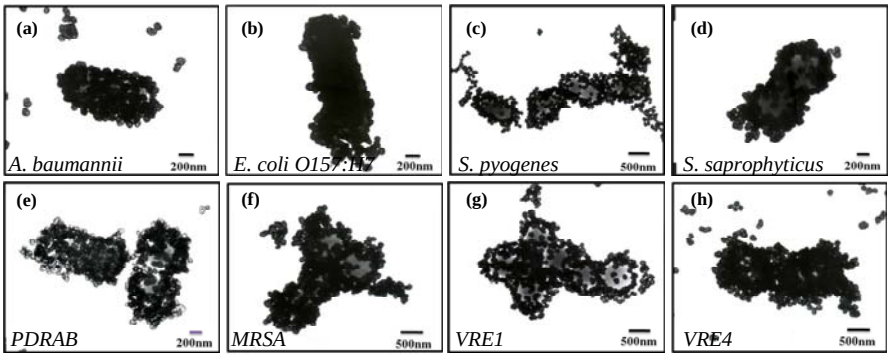

Figure 2. **TEM images of the bacterial cells targeted by van- Fe_3O_4@Au NPs.**

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