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Bactericidal mechanisms of Au@TNBs under visible light irradiation

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ABSTRACT

Au@TNBs nanocomposites were synthesized by depositing Au nanoparticles onto the surfaces of TiO_2 nanobelts (TNBs). The disinfection activities of Au@TNBs on model cell type, Gram-negative *Escherichia coli* (*E. coli*), were examined under visible light irradiation conditions. Au@TNBs exhibited stronger bactericidal properties toward *E. coli* than those of TNBs and Au NPs under visible light irradiation. The bactericidal mechanisms of Au@TNBs under light conditions were explored, specifically, the specific active species controlling the inactivation of bacteria were determined. Active species (H_2O_2, diffusing $^{\bullet}OH$, $^{\bullet}O_2^-$, $^1O_2$, and e^-) generated by Au@TNBs were found to play important roles on the inactivation of bacteria. Moreover, the concentrations of H_2O_2, $^{\bullet}OH$, $^{\bullet}O_2^-$, and $^1O_2$ generated in the antimicrobial system were estimated. Without the presence of active species, the direct contact of Au@TNBs with bacterial cells was found to have no bactericidal effect. The reusability of Au@TNBs were also determined. Au@TNBs exhibited strong antibacterial activity toward *E. coli* even in five consecutively reused cycles. This study indicated that the fabricated Au@TNBs could be potentially utilized to inactivate bacteria in water.

1. Introduction

Pathogenic microbe contamination of water has been the source of numerous disease outbreaks worldwide especially in developing countries [1]. Drinking water safety thus has attracted great concern over past decades. Chemicals such as chlorine, chloramines, and ozone are commonly utilized to disinfect microbe in water. However, harmful disinfection byproducts (DBPs), many of which are carcinogens, could be generated in these traditional disinfection processes [2,3]. Therefore, great efforts have been recently devoted to developing new techniques to inactivation of pathogenic microbes in water. Since they can produce reactive oxygen species (ROS) to inactivate microbe, TiO_2 nanomaterials have recently attracted increasing attention in bacteria decontamination in water [4,5]. Due to their wide band gap, low electron transfer rate to oxygen, and the high electron–hole recombination rate, TiO_2 nanomaterials could not generate ROS efficiently under visible light irradiation conditions [6,7]. To enhance the generation of ROS under visible light conditions, noble metals such as Pd [8], Pt [9], Ag [10], and Au [11–13] have been utilized to dope TiO_2 particles. Among these noble metals, Previous studies [14,15] showed that Au nanoparticles (NPs) could also generate ROS under light conditions. Thereby, Au NPs have recently attracted great attention for doping TiO_2 to inactivate bacteria. The bactericidal effects of Au NPs doped TiO_2 are expected to be greatly improved under light irradiation conditions [12,13].

It is well known that the photocatalytic activity of TiO_2 is affected by the size, morphology, crystalline structure, and surface structure [16,17]. One-dimensional TiO_2 nanobelts (TNBs) has been found to have higher photocatalytic activity relative to TiO_2 nanoparticles due to the enhanced visible-light harvesting capabilities, less grain boundaries, and lower $e^- - h^+$ recombination rate [18]. The amount of ROS generated by TNBs would be greater than TiO_2 nanospheres under visible light conditions. Thus, the antibacterial effects of TNBs are expected to be more significant. By acting as "transition metal impurities", Au NPs depositing on the surfaces of TNBs could stimulate the generation of active species [19]. Thereby, Au NPs doped TNBs (Au@TNBs) are expected to have improved bactericidal property under visible light irradiation. However, to date, the disinfection activities of Au@TNBs have not been investigated and thus require examination. Although the antibacterial activities of Au@TiO_2 have been investigated [12,13],

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the mechanisms involved in the photocatalytic disinfection processes of Au NPs loaded TiO_2 nanospheres yet have not been addressed. The possible bactericidal mechanisms driving to the bactericidal activities of Au@TNBs thus require systematical investigation.

Hence, the objective of this manuscript is to synthesize the Au NPs deposited TNBs nanocomposites (Au@TNBs) and systematically investigate their bactericidal mechanisms. The disinfection effects of Au@TNBs for *Escherichia coli* (*E. coli*) under visible light conditions were determined. The bactericidal mechanisms were systematically discussed, and the major active species contributing to antimicrobial activity were proposed. In addition, the reusability of Au@TNBs was also explored.

2. Materials and methods

2.1. Materials

TiO_2 nanoparticles (diameter of 25–35 nm) were purchased from Degussa (Hanau, Germany). Potassium iodide (KI), 4-hydroxy-2,2,6,6-tetramethylpiperidine 1-oxyl (TEMPOL), isopropanol, potassium dichromate ($K_2Cr_2O_7$), furfuryl alcohol (FFA), 2, 3-bis (2-methoxy-4-nitro-5-sulfophehyl)-2H-tetrazolium-5-carboxanilide (XTT), and para-chlorobenzoic acid (pBCA) were all purchased from Sigma–Aldrich (USA). All other chemicals were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China) and Xilong Chemical Group (Shantou, Guangdong, China). All the chemicals were analytical grade and used without further purification.

2.2. Synthesis and characterization of Au@TNBs

TNBs were synthesized by a hydrothermal process [20] with slight modification, and then Au NPs were deposited on the surface of TNBs via a self-assembly procedure similar to that described in a previous study [21]. The detailed fabrication protocols for TNBs, Au NPs, as well as Au@TNBs fabrication are provided in the Supplementary Information. Powder X-ray diffraction (XRD), Energy dispersive X-ray spectroscopy (EDS) and X-ray photoelectron spectroscopy (XPS), high resolution transmission electron microscope (HRTEM), and inductively coupled plasma optical emission spectroscopy (ICP-OES), were employed to characterize the fabricated materials. Detailed information can be found in Supplementary Information.

2.3. Bacteria preparation

Gram-negative *Escherichia coli* ATCC15597 (*E. coli*), has been employed as model cell type in previous study [20], was used to determine the bactericidal activities of the Au@TNBs. The detailed bacterial growth and stock suspension preparation protocols can be found in previous study [20] as well as in Supplementary Information. The prepared bacterial stock concentration was typically 10^9–10^{10} colony forming unit (CFU) per milliliter, which was diluted to a concentration of 6.5×10^7 CFU mL^{-1} prior to addition to the Au@TNBs suspension.

2.4. Bactericidal experiments

The photocatalytic disinfection was carried out by using the setup described in Fig. S1. Specifically, a xenon lamp (300 W, Osram Instruments, USA) with a UV cutoff filter ($\lambda \geq 400$ nm) was used as a light source, which was put 15 cm above the reaction system. A double-walled beaker with volume of 100 mL, which filled with constant-temperature water between the double walls, was employed as reaction container for disinfection experiments. The reaction suspensions were well-mixed by stirring and maintained at 25 °C for all experiments. Specifically, a constant-temperature water circulator was employed to keep the temperature of the suspension maintaining at 25 °C. The light intensity at the surface of the reaction solution was measured to be 30 mW/cm^2 by a solar power meter (TM-207, TENMARS, Taiwan). The irradiance spectrum (Fig. S2) of the light at the position of the surface of the reaction solution in the wavelength range from 290 to 700 nm was measured with a spectrometer (HR4000CG-UV-NIR, Ocean Optics, FL, USA) calibrated with a radiometric reference light source (HL2000-CAL, Ocean Optics, FL, USA). The filter completely cut off the UV portion of the irradiation, and the visible light portion absorbed by the reaction solution was calculated to be 4.0 mW/cm^2 according to the method described in previous studies [22,23]. Thus, the power in the visible light region absorbed by the whole reaction solution was 60.8 mW.

Prior to the photocatalytic disinfection experiments, all glass apparatuses used in the experiments were autoclaved at 121 °C for 20 min to ensure sterility. Following that, 5 mg of Au@TNBs was placed into 45 mL sterilized water and sonicated for 5 min to fully disperse the nanomaterials in solution. After that, 5 mL of bacterial stock solution (6.5×10^7 CFU mL^{-1}) was added into the sonicated mixture to obtain the target initial cell concentration of 6.5×10^6 CFU mL^{-1} (pH = 7.0 ± 0.1, ionic strength = 3.0 mM). At different time intervals, 0.5 mL of the reaction solution was sampled and serially diluted with sterilized water. Then, 0.1 mL of the diluted samples were immediately streaked on nutrient agar plates and incubated at 37 °C for 24 h. The number of colonies formed was counted to determine the number of viable cells. Each set of experiments that utilized the newly cultured bacteria was performed in triplicate at pH 7. FEI Quanta 200 FEG environmental scanning electron microscopic (ESEM) was employed to observe the morphological change of the bacterial during the disinfection process. The bacterial pretreatment protocol prior to ESEM characterization can be found in Supplementary Information.

Blank control experiment, bacterial solution without Au@TNBs, under visible light irradiation was also conducted. Moreover, the bactericidal activity of Au NPs (3–7 nm, 5 mg L^{-1}, similar size and concentration of Au particles on 0.1 g L^{-1} Au@TNBs), TNBs (95 mg L^{-1}, similar concentration of TNBs on 0.1 g L^{-1} Au@TNBs), and Au NPs (5 mg L^{-1}) + TNBs (95 mg L^{-1}) mixture on *E. coli* were also investigated with visible light irradiation.

To clarify the antibacterial effects of different active species that generated by Au@TNBs on the inactivation of bacterial cells, experiments with addition of various scavengers to remove the corresponding active species were performed under visible light irradiation. Specifically, KI was used to remove h^+ and surface bounded •OH [24]. Isopropanol, Cr (VI), and 4-hydroxy-2,2,6,6-tetramethylpiperidin 1-oxyl (TEMPOL) were used to remove diffusing •OH, e^-, and $•O_2^-$ in the solution bulk, respectively [25]. Fe (II)-EDTA was used as an enhancer of the bulk phase •OH (Fe (II) could react with H_2O_2 (Fenton reaction) to generate bulk phase •OH) to indirectly verify the existence of H_2O_2 [26]. To further elucidate the bactericidal activities of active species, experiments conducted in a partition system were implemented. The detailed information for partition system experiments can be found in previous study [20], as well as in Supplementary Information.

To further explore the roles of e^- in the disinfection system, and investigate whether $^1O_2$ had disinfection effect on bacterial cells, disinfection experiments were conducted under anaerobic conditions to avoid the generation of oxidative radicals (such as H_2O_2, $^1O_2$, and $•O_2^-$). The water utilized in anaerobic experiments was boiled for 30 min before use. High purity nitrogen (>99.999%) was continuously purged during the disinfection process to ensure the absence of O_2 in the inactivation system.