Progress in Natural Science: Materials International xxx (xxxx) xxx–xxx

HOSTED BY

ELSEVIER

Contents lists available at ScienceDirect

Progress in Natural Science: Materials International

journal homepage: www.elsevier.com/locate/pnsmi

Original Research

Efficient inactivation of *Staphylococcus aureus* by silver and copper loaded photocatalytic titanate nanotubes☆

Bhupendra Joshi[a], Chhabilal Regmi[b], Dipesh Dhakal[c], Gobinda Gyawali[a], Soo Wohn Lee[b,*]

[a] Division of Basic Science Basic Engineering, Sun Moon University, Chungnam 31460, Republic of Korea
[b] Department of Environment and Bio-Chemical Engineering, Sun Moon University, Chungnam 31460, Republic of Korea
[c] Department of Life Science and Bio-Chemical Engineering, Sun Moon University, Chungnam 31460, Republic of Korea

ARTICLE INFO

Keywords:
Titante nanotubes
Staphylococcus aureus
Antibacterial
Photocatalytic inactivation
Reactive oxygen species

ABSTRACT

One dimensional titanate nanotubes (TNTs) were synthesized by microwave assisted alkaline hydrothermal process. The process was followed by UV-photodeposition of Ag and Cu on the surface of TNTs to enhance the photocatalytic activity in visible light spectrum. The loading of Ag and Cu (single and combination mode) offered a new insight to inactivate multi-drug resistant micro-organisms. The antibacterial properties of these samples were studied on Gram positive bacteria, *Staphylococcus aureus* (*S. aureus*) using well diffusion method. The TNTs with Ag and Cu loading showed a clear zone of inhibition after overnight incubation of *S. aureus*. The bacterial inactivation efficiency of nanoparticles in the visible light was further analyzed by kill kinetics. TNTs with Ag and/or Cu loading showed a significant reduction in bacterial growth. Cu co-loaded with Ag sample showed the highest inactivation efficiency within 90 min of visible light irradiation. To elucidate the mechanism of bactericidal properties of samples under visible light irradiation, the formation of reactive oxygen species (ROS), particularly, superoxide radical anion was determined by nitro blue tetrazolium (NBT) assay and the protein degradation by each samples were measured. Based on overall results, it was observed that the Cu co-loaded with Ag on TNTs samples were found to be more effective as compared to either Ag or Cu loaded TNTs. It provides new avenues for utilizing the combination of Cu and Ag for enhancing the antimicrobial efficacies for different nanoparticles.

1. Introduction

The infection caused by antibiotic resistant Gram positive bacteria such as methicillin-resistant superbug *Staphylococcus aureus* (MRSA) is a worldwide problem in clinical medicine, particularly case of transplanted, severe burns and immunosuppressed patients in hospitals [1]. These Gram positive bacteria can survive on various surfaces, such as fabrics, plastics, glass, aluminum foil, bed rails and stethoscopes [2]. Thus, the most of nosocomial infections are caused by multi-drug resistant *S. aureus*. Even these bacteria show resistance to vancomycin, the last resort antibiotic that is only used when other antibiotics are unresponsive [3]. Moreover, mutants of *S. aureus* have less susceptibility to different kind of chemical biocides such as triclosan, centrimide, chlorhexidine, benzalkonium chloride, parahydroxybenoates, hypochlorite, and betadine [4].

To overcome the above mentioned problems, the nanoparticles and inorganic photocatalysts are being developed as novel antibacterial material or alternative to biological or synthetic chemicals. One of the most used metal nanoparticle is silver that shows effective bactericidal properties on different kinds of bacteria [5] but the use of silver as a biocide is not economically suitable. Even the cheaper copper nanoparticles are used as an antibacterial material but are not effective at low concentration as compare to silver nanoparticles [6]. The bactericidal properties of Ag and Cu nanoparticles are reported by many researchers [5–7]. However, these nanoparticles residue in the treated water may have adverse effect on human health [8].

Semiconductor photocatalysts are not only considered as green technology for degrading organic pollutants, but also emerging as antimicrobial material [9–12]. The reported efficient antimicrobial photocatalyst are synthesized by loading metal nanoparticles on the photocatalyst's surface [13,14]. The loading of the metal nanoparticles on photocatalytic semiconductor can reduce the chance of liberating metal nanoparticles.

The most studied low cost and stable photocatalyst for antimicrobial purpose is titanium dioxide (TiO_2) and its polymorphs [15,16]. The mechanism of microbes inactivation by photocatalysts in presence of

☆ Peer review under responsibility of Chinese Materials Research Society.

* Corresponding author.
E-mail addresses: joshibhupen@gmail.com (B. Joshi), swlee@sunmoon.ac.kr (S.W. Lee).

https://doi.org/10.1016/j.pnsc.2018.01.004
Received 23 August 2017; Received in revised form 4 January 2018; Accepted 4 January 2018

light is due to the generation of reactive oxygen species (ROS). The ROS such as $OH^{\bullet}$, $HO_2^{\bullet}$, H_2O_2, and $O_2^{\bullet-}$ interact with sulfhydryl (–SH) groups of proteins and nucleotide base pair of DNA causing DNA unwinding [17].

Titanate nanotube is one of the polymorph of TiO_2, mostly synthesized by anodization method and studied for antibacterial properties [16,18]. However, there are few reports on photoinactivation of bacteria by microwave assisted hydrothermally synthesized TNTs powder and this method is the efficient method to synthesize nanopowders in short time [13,14]. TNTs show poor photocatalytic activity in visible light unless some nobel metal particles such as Ag, Au, Pt and Cu are loaded on their surface. A little amount of these metal nanoparticles on the surface of photocatalysts show a broad visible absorption based on localized surface plasmon resonance (LSPR) [18,19].

Herein, the study is aimed to investigate the antibacterial property as well as photoinactivation of *S. aureus* by Ag and Cu loaded TNTs synthesized by microwave assisted hydrothermal process. The TNTs are known to have very high surface area and loading of Cu along with Ag nanoparticles can be cost-effective by minimizing the amount of Ag, an expensive metal. The highlight of this study is the comparison of Cu-TNTs and Ag-TNTs and Ag/Cu-TNTs for inactivation of *S. aureus*. MRSA is the least studied bacteria as compared to *E. coli*. Moreover, the antimicrobial properties of metal loaded TNTs on Gram positive bacteria like *S. aureus* are not yet studied.

2. Materials and methods

2.1. Sample fabrication

A commercial titanium dioxide, the P25 Degussa of 2 g was mixed with 50 mL of 10 M NaOH and the mixture was sonicated for 5 min. Then the mixture was stirred (700 rpm) for 10 min and transferred into the Teflon-line vessel for microwave hydrothermal treatment at 150 °C for 4 h with 400 rpm maintaining power of 195 W. The obtained powder was neutralized with 5 N HCl and washed the solution with distilled water until the pH of the solution was ~7. The product (TNT) was then filtered and dried in oven for 12 h at 80 ^{0}C.

The microwave assisted hydrothermally synthesized TNTs (1 g) was dispersed in 50 mL of ethanol by sonication for 5 min. The calculated amount of $AgNO_3$ was dissolved in minimum amount of water separately to obtain 2 wt% of metallic Ag. The prepared $AgNO_3$ solution was added drop wise to the sonicated mixture of TNTs that was vigorously stirred. Then in stirred condition, the mixture was exposed to UV light for 2 h. The Ag photodeposited TNTs (2AgTNT) was dried in oven for 12 h at 80 °C. Similar process was followed to prepare 2 wt% copper loaded TNTs (2CuTNT) using $CuSO_4$ precursor. In addition, $CuSO_4$ and $AgNO_3$ solutions were prepared separately as to obtain total of 2 wt% loading varying Ag and Cu ratio on TNTs i.e. 0.5 wt% Ag and 1.5 wt% Cu (0.5Ag/1.5CuTNT), 1 wt% Ag and 1 wt% Cu (1Ag/1CuTNT), and 1.5 wt% Ag and 0.5 wt% Cu (1.5Ag/0.5CuTNT).

2.2. Characterization

The phase and crystallinity were determined by powder X-ray diffraction (XRD) by using X-ray Diffractometer (Rigaku, D/Max 2000HR diffractometer, Japan). Diffuse reflectance spectra (DRS) were recorded by the UV–Vis DRS spectrophotometer (V 570, Jasco International Co. LTD., Japan). XPS measurement was taken from the Multilab system with Al Kα source at 15 kV and 200 W. The morphologies of different samples were observed by the Transmission Electron Microscopy (TEM, JEM-2010, Jeol). The antibacterial performance of samples were evaluated in *S. aureus* in a photo reactor by using solar simulator (portable solar simulator PEC-L01, Pecell) (Am 1.5 G) as a light source. An ultraviolet cutoff ($\lambda < 420$ nm) filter in solar simulator was applied for visible light source for antibacterial activity.

2.3. Antibacterial activity

The antibacterial activities were evaluated against a Gram-positive bacterium *Staphylococcus aureus*. *S. aureus* was procured in frozen form from American Type Culture Collection (ATCC-BAA-1687). The pathogen was regenerated in plate by scraping on LB agar plate and incubating overnight. A single colony of bacteria was selected using a 10-μL loop and inoculated into a falcon tube containing 5 mL of LB broth. Bacteria in the falcon tube were then incubated at 37 °C under agitation at 200 rpm for another 16 h. Then the antibacterial activity of the nanoparticles was investigated using the well diffusion method. Triplicate plates were swabbed with the overnight culture (10^7 cells/mL) of pathogenic *S. aureus* bacteria. The solid medium was gently punctured with the help of cork borer to make a well. Finally, the TNTs samples (10 mg/mL and 20 mg/mL) were added from the stock into each well and incubated for 24 h at 37 ± 2 °C. After 24 h of incubation in dark, the zone of inhibition was measured and expressed as a diameter in mm.

Furthermore, antibacterial activity was performed by kill kinetics studies under the visible light for various TNTs samples. *S. aureus* were incubated in LB broth at 37 °C for 12 h with shaking and centrifuged at 3000 rpm. The cell pellet was washed by phosphate buffer saline (PBS) (0.2×10^{-3} M, pH = 7.2) by centrifuging at 3000 rpm. The treated cells were re-suspended and diluted with phosphate buffer saline. After diluting the treated cells with PBS, the reaction volume was maintained 5 mL adjusting the photocatalyst concentration 10 mg/mL to maintain the bacteria concentration around 1×10^7 CFU/mL. An ultraviolet cutoff ($\lambda < 420$ nm) filter was fitted in solar simulator for visible light irradiation. During the visible light irradiation period, 10 μL aliquot was immediately diluted to make 1 mL solution and 100 μL from the solution was pipetted and plated on the LB agar. The aliquot were taken in intervals of 30 min and finally the CFU/mL was calculated after overnight incubation.

2.4. ROS (super oxide radical anion) determination

The superoxide radical anion was determined by NBT assay using previously reported protocol with slight modifications [20]. Photocatalytically treated cells were washed thrice with PBS and the cells were incubated with an equal volume of NBT working reagent (1: 10 diluted by PBS from 0.01% NBT stock; Sigma-Aldrich, St Louis, MO, USA) at 37 °C for 45 min. Then the samples were washed and centrifuged at 3000 rpm for 10 min in PBS to remove the residual NBT solution. The cell pellet containing only formazan was solubilized in 100 μL of 2 M KOH and dimethyl sulfoxide (DMSO). Then ROS was quantified based on the absorbance at 620 nm wavelength by Optizen Pop NanoBio UV–Vis spectrophotometer, Mecasys Co. Ltd, South Korea.

2.5. Protein degradation

The slight modification in Bradford assay was used as standard protocol to determine the protein degradation. Bovine serum albumin (BSA) standard (Biorad) was taken in the amount of 50 μL from 1 mg/mL stock that was incubated with 100 μL of 10 mg/mL concentration of TNTs samples and treated for 90 min in visible light. After 90 min, 1.4 mL of Bradford reagent was added. Then, the samples were gently mixed and incubated at room temperature for 45 min and transferred into cuvettes. The absorbance of different samples was recorded in Optizen Pop NanoBio UV–Vis spectrophotometer (Mecasys Co. Ltd, South Korea) at 595 nm.

3. Results and discussion

3.1. Structure and morphology

The broader peaks of XRD reflections as shown in Fig. 1a confirmed