

NANO IDEA

Open Access



The Antibacterial Activity of Ta-doped ZnO Nanoparticles

Bing-Lei Guo¹, Ping Han¹, Li-Chuan Guo², Yan-Qiang Cao¹, Ai-Dong Li^{1*}, Ji-Zhou Kong¹, Hai-Fa Zhai¹ and Di Wu¹

Abstract

A novel photocatalyst of Ta-doped ZnO nanoparticles was prepared by a modified Pechini-type method. The antimicrobial study of Ta-doped ZnO nanoparticles on several bacteria of Gram-positive *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) were performed using a standard microbial method. The Ta-doping concentration effect on the minimum inhibitory concentration (MIC) of various bacteria under dark ambient has been evaluated. The photocatalytical inactivation of Ta-doped ZnO nanoparticles under visible light irradiation was examined. The MIC results indicate that the incorporation of Ta⁵⁺ ions into ZnO significantly improve the bacteriostasis effect of ZnO nanoparticles on *E. coli*, *S. aureus*, and *B. subtilis* in the absence of light. Compared to MIC results without light irradiation, Ta-doped ZnO and pure ZnO nanoparticles show much stronger bactericidal efficacy on *P. aeruginosa*, *E. coli*, and *S. aureus* under visible light illumination. The possible antimicrobial mechanisms in Ta-doped ZnO systems under visible light and dark conditions were also proposed. Ta-doped ZnO nanoparticles exhibit more effective bactericidal efficacy than pure ZnO in dark ambient, which can be attributed to the synergistic effect of enhanced surface bioactivity and increased electrostatic force due to the incorporation of Ta⁵⁺ ions into ZnO. Based on the antibacterial tests, 5 % Ta-doped ZnO is a more effective antimicrobial agent than pure ZnO.

Keywords: Ta-doped ZnO; Photocatalytical inactivation; Minimum inhibitory concentration; Antibacterial activity

Background

In recent years, ZnO has received increasing attention, owing to its unique optical, electrical, and chemical properties [1]. Among these properties, degradation of pollutants catalyzed by ZnO has been studied widely so far [2–4]. Furthermore, ZnO appears to strongly resist microorganisms [5, 6]. Some work on the considerable antibacterial activity of bulk and nanosized ZnO materials has been reported [7–10].

Antibacterial agents are of relevance to a number of industrial sectors including environmental, water disposal, food, synthetic textiles, packaging, healthcare, medical care, as well as construction and decoration. They can be classified into two types, organic and inorganic ones. Most organic antibacterial agents are sensitive to temperature or pressure [11], while inorganic

materials such as metal and metal oxide [12] have received more recognition over the past decade due to superior durability, less toxicity, greater selectivity, and heat resistance [13, 14]. TiO₂ and ZnO semiconductors have been extensively studied as antimicrobial agents due to their photocatalytic activity under UV light [15, 16].

ZnO, as a semiconductor with a wide band gap (3.3 eV), is abundant in nature and environmentally friendly with low price. ZnO nanoparticles have been reported to exhibit strong antibacterial activities on a broad spectrum of bacteria [7–11]. A comparative study showed that for *Bacillus subtilis* and *Escherichia coli*, biocidal activity generally increased from SiO₂ to TiO₂ to ZnO [8]. ZnO has higher bactericidal efficacy on *B. subtilis* than on *E. coli* [5]. The minimum inhibitory concentration (MIC) was dependent on the ZnO particle size, ranging from 2000 to 12,500 ppm for *B. subtilis* and 50,000 to 100,000 ppm for *E. coli* [6]. However, contradictory results have been reported on the impact of particle size on the antibacterial activity of ZnO [17, 18]. Up to now, the antibacterial mechanism of ZnO is still

* Correspondence: adli@nju.edu.cn

¹National Laboratory of Solid State Microstructures, Materials Science and Engineering Department, College of Engineering and Applied Sciences, Collaborative Innovation Center of Advanced Microstructures, Nanjing University, Nanjing 210093, People's Republic of China

Full list of author information is available at the end of the article

under investigation and not well understood [19]. Several possible mechanisms, including the penetration of the cell envelope and disorganization of bacterial membrane [10], the photocatalytically generated reactive oxygen species (ROS) on oxide surface [11], and Zn^{2+} ion binding to the membranes of microorganisms [20], have been suggested.

ZnO has high UV absorption efficiency and good transparency to visible light. In order to improve its photocatalytic properties, some metal ions or doping nitrogen have been added into ZnO to narrow or split the band gap and enhance interfacial electron-transfer rate [21–23]. Some transition metal ions, e.g., Co^{2+} [24], Mn^{2+} [22], Ti^{4+} [25], La^{3+} [26], and Fe^{3+} [27], have been doped into ZnO. At present, most research focused on the influence of transition metal ions on the photocatalytic efficiency rather than the antimicrobial activity [22, 23].

Our group has first prepared Ta-doped ZnO nanopowders by a modified Pechini-type method and deeply investigated the photoinduced degradation of organic dye of methylene blue using Ta-doped ZnO with various Ta contents under visible light irradiation [28, 29]. In this work, we performed the antibacterial study of Ta-doped ZnO nanoparticles on several bacteria of Gram-positive *Bacillus subtilis* (*B. subtilis*) and *Staphylococcus aureus* (*S. aureus*) and Gram-negative *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) using a standard microbial method. The Ta concentration effect on the MIC of various bacteria has been evaluated. The antimicrobial activity of Ta-doped ZnO under visible light and dark conditions was examined and compared. The possible antimicrobial mechanism in Ta-doped ZnO systems was also proposed.

Methods

Preparation of Ta-doped ZnO Nanoparticles

A photocatalyst of Ta-doped ZnO with various Ta contents of 1, 3, and 5 % (mol. percentage) and pure ZnO was prepared by a modified Pechini-type method [27]. All chemicals were analytical grade and used without further purification. Zinc nitrate $Zn(NO_3)_2 \cdot 6H_2O$ and the water-soluble peroxy-citrato-tantalum were chosen as precursors of Zn and Ta, respectively. The synthesis of home-made water-soluble peroxy-citrato-tantalum has been described elsewhere in details [30]. Deionized water was used as a dispersing agent in all the experiments.

Citric acid (CA) as chelating agent was dissolved into 50 ml water with 2.98 g $Zn(NO_3)_2 \cdot 6H_2O$ (in 2:1 molar ratio with respect to the zinc nitrate). Ethylene glycol (EG) as cross-linking additive was added into the aqueous Ta precursor solution at the molar ratio of EG to CA of 5:1. As surfactant and catalyst, 1 g polyvinyl pyrrolidone (PVP, average mol. wt. 100,000) and 0.5 ml HNO_3 were added to the mixture of solutions

containing Zn and Ta, respectively. Hydroxypropyl cellulose (HPC, average mol. wt. 100 000, Aldrich) with a concentration 3.5×10^{-3} g/ml and 1 g acetylacetone were put into the above solution as steric dispersant and stabilizer, respectively. A colorless transparent sol was obtained and subsequently baked at 140 °C for 12 h for polyesterification. The resultant dark gray glassy resin finally underwent a two-step heat treatment to yield the final products: firstly, pyrolysis at 400 °C for 2 h and then annealing at 700 °C for 1 h in air.

In addition, control sample of Ta_2O_5 (99.5 wt %) powders was purchased from Shanghai Chemicals Corporation for control experiment.

Characterization of Ta-doped ZnO Nanoparticles

The structure of the particles was characterized by a powder X-ray diffraction (XRD) equipment (D/max2000, Rigaku) using Cu K α radiation. The Ta-doping content was determined by inductively coupled plasma resonance (ICP, JY 38S, JY). The morphology and microstructure of nanoparticles were examined by scanning electron microscope (SEM, Philips XL-30, The Netherlands) and transmission electron microscope (TEM, Tecnai G² F20, Philips). The surface chemical composition of the nanoparticles were investigated by X-ray photoelectron spectroscopy (XPS, Thermo Fisher K-Alpha) with standard Al K α (1486.7 eV) X-ray source. The binding energy scale was calibrated using the energy position of the adventitious C 1s peak at 284.6 eV.

Bacterial Strains and Culture Conditions

For antibacterial experiments, Gram-positive *B. subtilis* and *S. aureus* and Gram-negative *E. coli* and *P. aeruginosa* were four etiological agents of several infective diseases in humans, chosen as the target organism. *E. coli* (ATCC25922), *S. aureus* (ATCC6538), *B. subtilis* (CMCC63501), and *P. aeruginosa* (ATCC 27853) were purchased from Guangzhou Microbiology Research Center. All tubes and materials were sterilized in an autoclave before the experiments. Nutrient broth was used to culture *E. coli*, *S. aureus*, *B. subtilis*, and *P. aeruginosa* at 37 °C for 2 days on a rotary platform in an incubator. The liquid cultures were finally diluted to obtain bacterial cell concentration of approximately 10^7 colony-forming units (CFU)/ml for following antibacterial test.

Evaluation of Antibacterial Activity

The MIC of Ta-doped ZnO nanoparticles was measured by broth dilution test in dark condition. Phosphate buffered saline (PBS, 2.5 ml) and broth solution (2.5 ml) were added to the tubes containing the 10^7 CFU inoculation. Then, Ta-doped ZnO nanoparticles with various amounts of 100–1000 μ g/ml were added into the tubes.

The negative control tube did not contain any inoculum, and positive control tube was free of antibacterial agent. The tubes were incubated at 37 °C for 48 h on a rotary platform. The visual turbidity of the tubes was noted before and after incubation. The MIC was defined as the endpoint where no visible turbidity could be detected.

The antibacterial activity of Ta-doped ZnO nanoparticles was also evaluated under visible light illumination. The tube samples containing various bacteria were prepared as above. Based on the MIC results, the concentration of antibacterial agent suspensions in tubes was set to 160 µg/ml for *B. subtilis* and 100–200 µg/ml for *E. coli*, *S. aureus*, and *P. aeruginosa*, respectively. A 300-W Xe arc lamp (300W, Ushio) was located 15 cm away from the tubes, providing the visible light above 425 nm through a cut-off filter. After visible light irradiation for various durations of 0–240 min, the tubes were cultured for at 37 °C on a rotary platform. The cultivation time in an incubator was equal to the irradiation time. The growth curve was determined by measuring the time evolution of the optical density (OD) of the sample contained in a 10-mm optical path length quartz cuvette. The density of bacterial cells in the liquid cultures was estimated by OD measurements at 600 nm wavelength using a UV-vis-NIR spectrophotometer (UV-3600, Shimadzu) at a frequency of once every 20 min.

In order to make a comparative study, pure ZnO and Ta₂O₅ powders were also used as control samples for antibacterial test under visible light and dark ambient. The control tubes were prepared and incubated under the similar conditions.

To investigate the antibacterial behavior of Ta-doped ZnO nanoparticles, SEM were also used to examine the morphology of some bacteria samples with and without antimicrobial treatment under visible light and dark ambient.

Results and Discussion

Characterization of Ta-doped ZnO Nanoparticles

Figure 1 shows the XRD patterns of pure ZnO and 5 % Ta-doped ZnO nanoparticles annealed at 700 °C. One and 3 % Ta-doped ZnO samples show pure hexagonal wurtzite structure of polycrystalline ZnO (JCPDS card No.16-1451) with slightly increasing *a* and *c* lattice constant (not shown here), indicating that the larger Ta⁵⁺ ions occupy the Zn²⁺ sites to form Ta-doped ZnO solid solution [27]. When Ta-doping content is 5 %, an evident peak at 23.1° and 32.6° from Ta₂O₅ can be observed, and meanwhile, some weak peaks at 30.2°, 52.8°, and 58.3° can be indexed to secondary phase of orthorhombic ZnTa₂O₆ (JCPDS card 39-1484). For comparison, the XRD pattern of commercial Ta₂O₅ powders is also given in Fig. 1 with orthorhombic phase (JCPDS card No.71-0639). The crystallite sizes are estimated

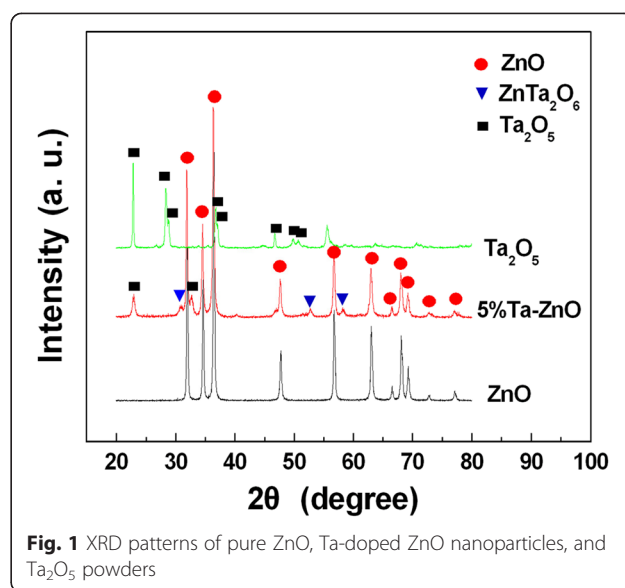


Fig. 1 XRD patterns of pure ZnO, Ta-doped ZnO nanoparticles, and Ta₂O₅ powders

from the Scherer formula to be around 38, 30, and 53 nm, corresponding to pure ZnO, 5 % Ta-doped ZnO, and Ta₂O₅ powders, respectively.

Typical SEM images of pure ZnO, 5 % Ta-doped ZnO nanoparticles, and Ta₂O₅ powders are shown in Fig. 2a–c. Figure 2d displays a TEM image of 5 % Ta-doped ZnO nanoparticles. Pure ZnO and Ta-doped ZnO nanoparticles are approximately spherical shaped with the dispersive diameters of 10–60 nm. These smaller nanoparticles easily assemble together to form some clusters of several hundred nanometers. Commercial Ta₂O₅ powders exhibit smaller grain of ~40–60 nm. A HRTEM image (not shown here) of 5 % Ta-doped ZnO nanoparticle records the disorder regions and some dislocations in the surface layer and grain boundary. Therefore, it can be deduced that there exist more defects on the surface of the 5 % Ta-doped ZnO nanocrystals by a modified PC method.

Antibacterial Test Results

MIC was determined after incubation at 37 °C for 48 h in dark ambient by the standard microbial method, as summarized in Table 1. It is easily seen that all samples including ZnO, Ta-doped ZnO, and Ta₂O₅ exhibit better biocidal effect on *B. subtilis* and worse antibacterial activity on *P. aeruginosa* bacteria. Meanwhile, with increasing the Ta content to 3–5 %, the growth of *E. coli* and *S. aureus* is effectively inhibited. For *E. coli* and *S. aureus*, no significant antibacterial activity is observed for pure ZnO, 1 % Ta-doped ZnO, and Ta₂O₅ samples at maximum concentration of 1000 µg/ml. The lowest MIC for *E. coli*, *S. aureus*, and *B. subtilis* is 180, 200, and 160 µg/ml after adding the 5 % Ta-doped nanoparticles into the tubes, respectively. When continuing to increase the Ta-doping amount from 5 to 10 % in ZnO, the

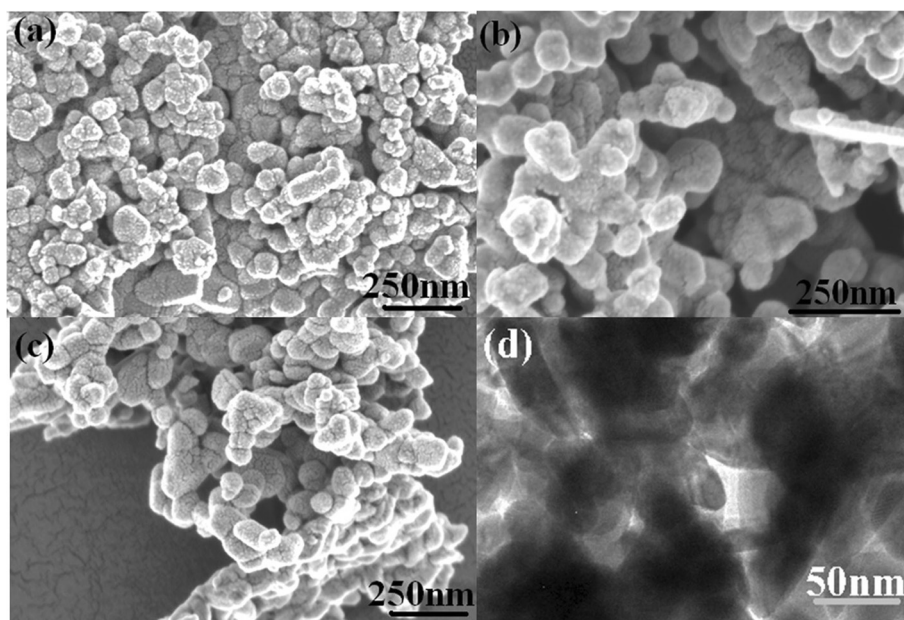


Fig. 2 SEM and TEM images of various powders. **a** pure ZnO. **b** 5 % Ta-doped ZnO nanoparticles. **c** Ta₂O₅ powders. **d** 5 % Ta-doped ZnO nanoparticles (TEM image)

antibacterial effect is similar. The MIC results indicate that the Ta-doping ion can improve the antibacterial effect of ZnO nanoparticles on *E. coli*, *S. aureus*, and *B. subtilis* under dark conditions.

Many bacteriological tests have shown that ZnO suspensions in the lower concentration range (0.01–1 mM, i.e., 0.8–80 µg/ml) exhibit less antimicrobial activity [31, 32]. Our MIC results also confirm this point. Fewer Zn²⁺ ions might act as nutrient-a supplement promoting the metabolic action of bacteria at trace concentrations. So, usually, ZnO are believed to be nontoxic, biosafe, and biocompatible and have been used in many applications in daily life, such as drug carriers, in cosmetics, and fillings in medical materials.

The antibacterial activity of Ta-doped ZnO nanoparticles was also evaluated under visible light illumination. Spectrophotometry were explored to assess bacterial growth because spectrophotometric readings

are generally more precise and reproducible [33–35]. Figure 3 shows the growth curves of four bacteria with time evolution in the presence of Ta-doped ZnO nanoparticles with different Ta contents under visible light. All samples of Ta-doped ZnO and pure ZnO nanoparticles with 100–200 µg/ml concentration of antibacterial agent exhibit evident antibacterial effect on *P. aeruginosa*, *E. coli*, and *S. aureus* under visible light irradiation in Fig. 3a–c; however, quite a few of these samples such as pure ZnO and 1 % Ta-doped ZnO nanoparticles do not show antibacterial activity in dark conditions, even if at 1000 µg/ml concentration. Moreover, the biocidal effect on *P. aeruginosa* is quite different under visible light and dark ambient. The visible light irradiation greatly improves antibacterial activity of *P. aeruginosa*.

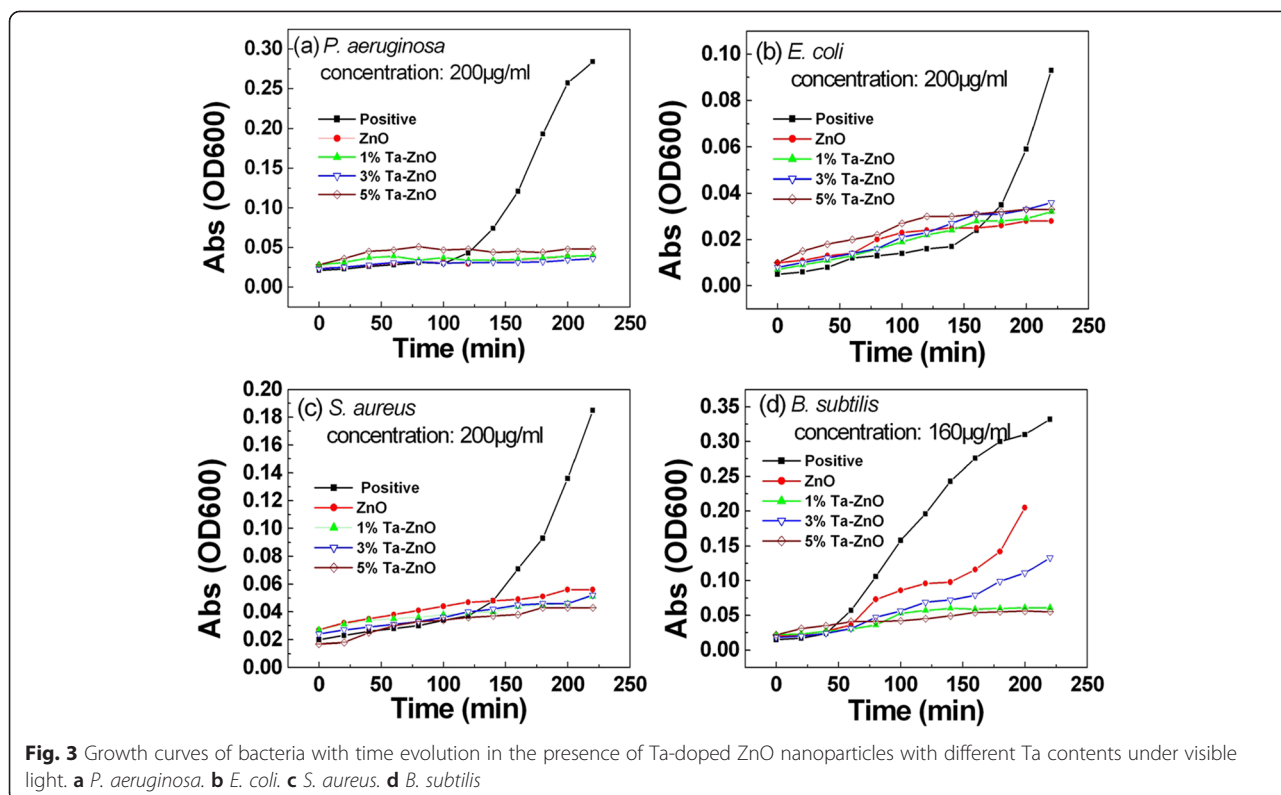
At present, for *P. aeruginosa*, *E. coli*, and *S. aureus*, Ta-doped ZnO nanoparticles show almost the same antibacterial activity as ZnO under visible light illumination. Our previous work on visible photodegradation of methylene blue with Ta-doped ZnO nanocrystals indicates that pure ZnO nanoparticles with defects also exhibits photocatalytic activity under visible light [27, 28], as indicated in Fig. 4. Maybe, the present exposure dose of antimicrobial agent is not low enough to distinguish the antibacterial activity between Ta-doped ZnO and ZnO nanoparticles.

In addition, compared to MIC result under dark conditions, the visible light irradiation has a hardly significant influence on antibacterial activity of *B. subtilis* at

Table 1 MIC results of ZnO and Ta-doped ZnO with various Ta contents (unit: µg/ml)

Bacteria	ZnO	1 % Ta-doped	3 % Ta-doped	5 % Ta-doped	Ta ₂ O ₅
<i>P. aeruginosa</i>	N.D.	N.D.	N.D.	N.D.	N.D.
<i>E. coli</i>	N.D.	N.D.	200–250	180	N.D.
<i>S. aureus</i>	N.D.	N.D.	500	200	N.D.
<i>B. subtilis</i>	350–400	230–240	200	160	200

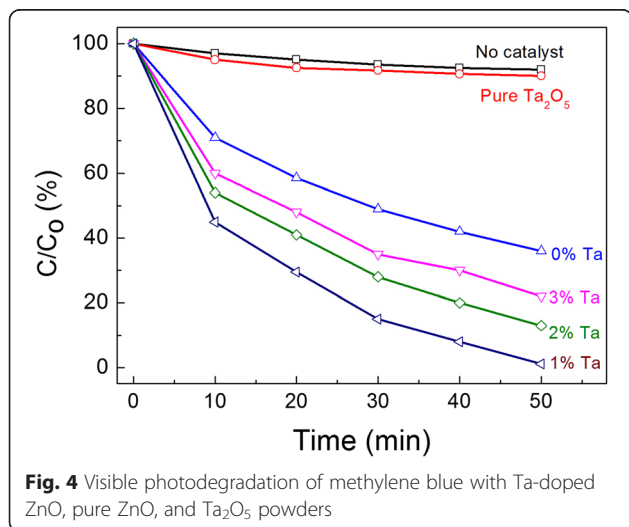
N.D. not determined (it means that no antibacterial effect is observed at the concentration of 1000 µg/ml)



160 $\mu\text{g/ml}$ concentration (see Fig. 3d). For samples containing pure ZnO and 3 % Ta-doped ZnO nanoparticles, the gradually enhanced OD with time indicates the worse microbial control on *B. subtilis*, similar to the previous MIC results. Five percent Ta-doped ZnO nanoparticles show also similar antibacterial activity of *B. subtilis* under visible light and in the absence of light. The only exception is that 1 % Ta-doped ZnO nanoparticles exhibit slightly enhanced biocidal effect on *B. subtilis*. Maybe

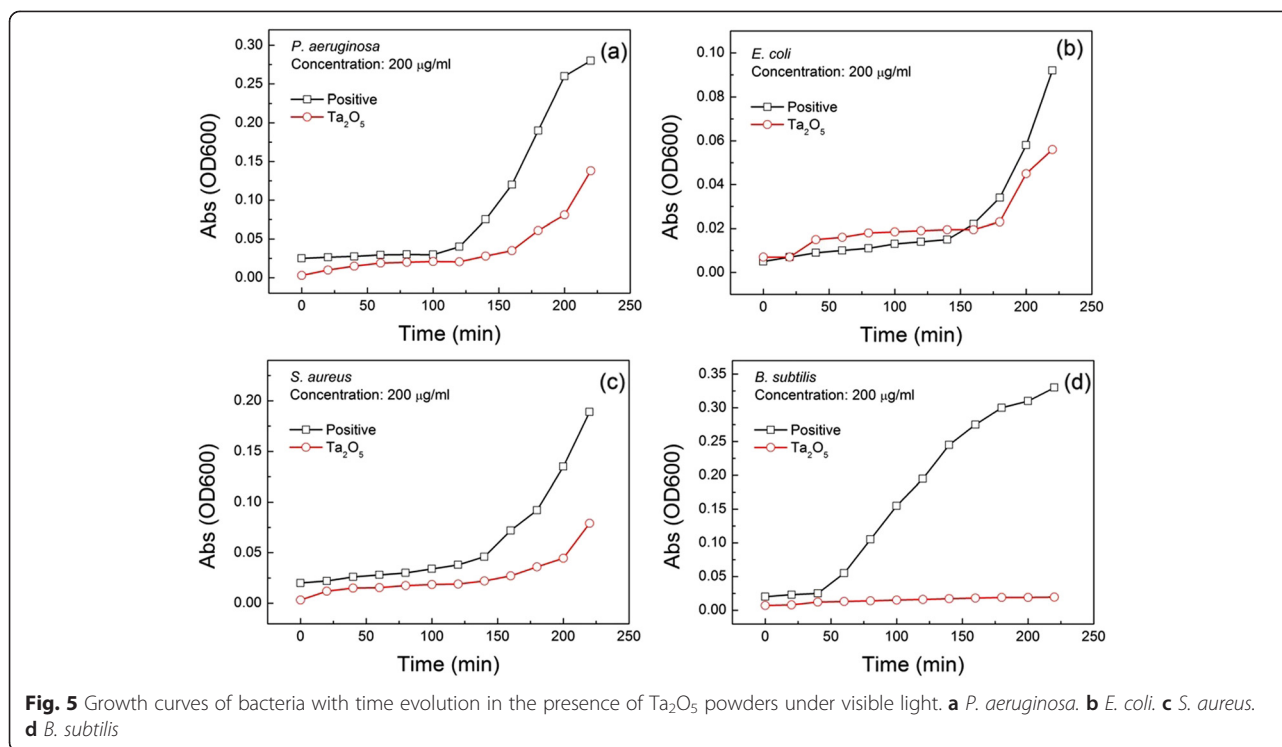
it is related to the fact that 1 % Ta-doped ZnO nanoparticles has optimal photocatalytic activity in Fig. 4 [28].

In order to understand the Ta role in the photocatalyst, the similar photocatalysis test of Ta_2O_5 powders at 200 $\mu\text{g/ml}$ concentration under visible light was also performed, as seen in Fig. 5a–d. It is clearly seen that Ta_2O_5 powders have evident bactericidal efficacy on *B. subtilis* under visible light illumination (Fig. 5d), similar to the MIC results in the absence of light. Compared to positive controls, Ta_2O_5 powders show weak antibacterial effect on *P. aeruginosa*, *E. coli*, and *S. aureus* under visible light irradiation, as seen in Fig. 5a–c. The rapid increase of OD value after 200 min indicates that Ta_2O_5 powders do not effectively prevent *P. aeruginosa*, *E. coli*, and *S. aureus* from multiplying. Based on the photodegradation of methylene blue with Ta_2O_5 powders under visible light (Fig. 4), there is hardly a photocatalytic activity for Ta_2O_5 powders. Why Ta_2O_5 powders exhibit better biocidal action on only *B. subtilis* in dark ambient or light irradiation is unclear now. Maybe, it is related to certain special role of Ta^{5+} ion.



Discussion on the Antibacterial Mechanisms

To gain direct evidence of the antibacterial behavior of Ta-doped ZnO nanoparticles, SEM were used to characterize the bacterial morphology with and without anti-treatments. Figure 5 shows the four kinds of bacteria images of positive control samples, samples

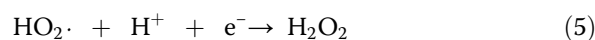
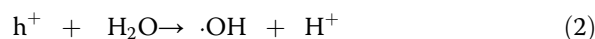
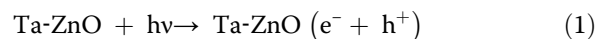


treated with Ta-doped ZnO nanoparticles under dark and visible light ambient. The difference of bacteria morphology between the positive control and anti-treated samples is easily observed. The former keeps better cell shape and cell state; however, the latter shows torn cell membrane, destruct tissue, and large areas of dead bacteria. The whole cell death process can be seen from Fig. 6. First the membrane hole appears, then the cell tissue outflows, and finally the bacteria are killed. It seems that the damage to the cell membrane directly leads to the leakage of minerals, proteins, and genetic materials, causing cell death.

Although the anti-treatments of the bacteria with Ta-doped ZnO nanoparticles under dark and visible light ambient lead to considerable damage to cell membranes, compared to the MIC under dark ambient, the antibacterial effect is significantly enhanced in visible light. The anti-treatment on *P. aeruginosa*, *E. coli*, *S. aureus*, and *B. subtilis* for 10 min under visible light illumination by photocatalysis produces similar injury effect to that for 48 h in the absence of light by MIC in Fig. 6. Evidently, the antibacterial mechanism of ZnO and Ta-doped ZnO under visible light is different from under dark ambient.

ZnO powders have been studied as inorganic antimicrobial agents due to their photocatalytic activity under UV light. Some mechanisms have been proposed. The exact mechanism of antibacterial activity of ZnO is still unclear and may involve generation of ROS, release of Zn²⁺ ions, and damage to cell membranes [19].

Tunney et al. thought that the photocatalytic generation of H₂O₂ might be one of the primary mechanisms [35]. The suspension of ZnO nanoparticles with defects can be activated by both UV and visible light and produce electron-hole pairs (e⁻h⁺). The holes split H₂O molecules into OH⁻ and H⁺. Dissolved oxygen molecules react with e⁻ and H⁺ in turn, and finally, molecules of H₂O₂ are obtained. For Ta-doped ZnO nanoparticles, similar reactions also exist under visible light [36].



Under visible light irradiation, an electron-hole pair forms, and then a conduction-band electron and a valence-band hole separate on the surface of Ta-doped ZnO (Eq. 1). The high oxidative potential of the holes can form very reactive hydroxyl groups through decomposing of water (Eq. 2). In the presence of the dissolved O₂, electrons from the photoexcited Ta-doped ZnO produce superoxide anion radicals ·O₂⁻ (Eq. 3), which subsequently could generate H₂O₂ by the intermediate HO₂²⁻ and HO₂· steps (Eqs. 4, 5). The free radicals ·O₂⁻ and ·OH produced in the reactions can react with organic

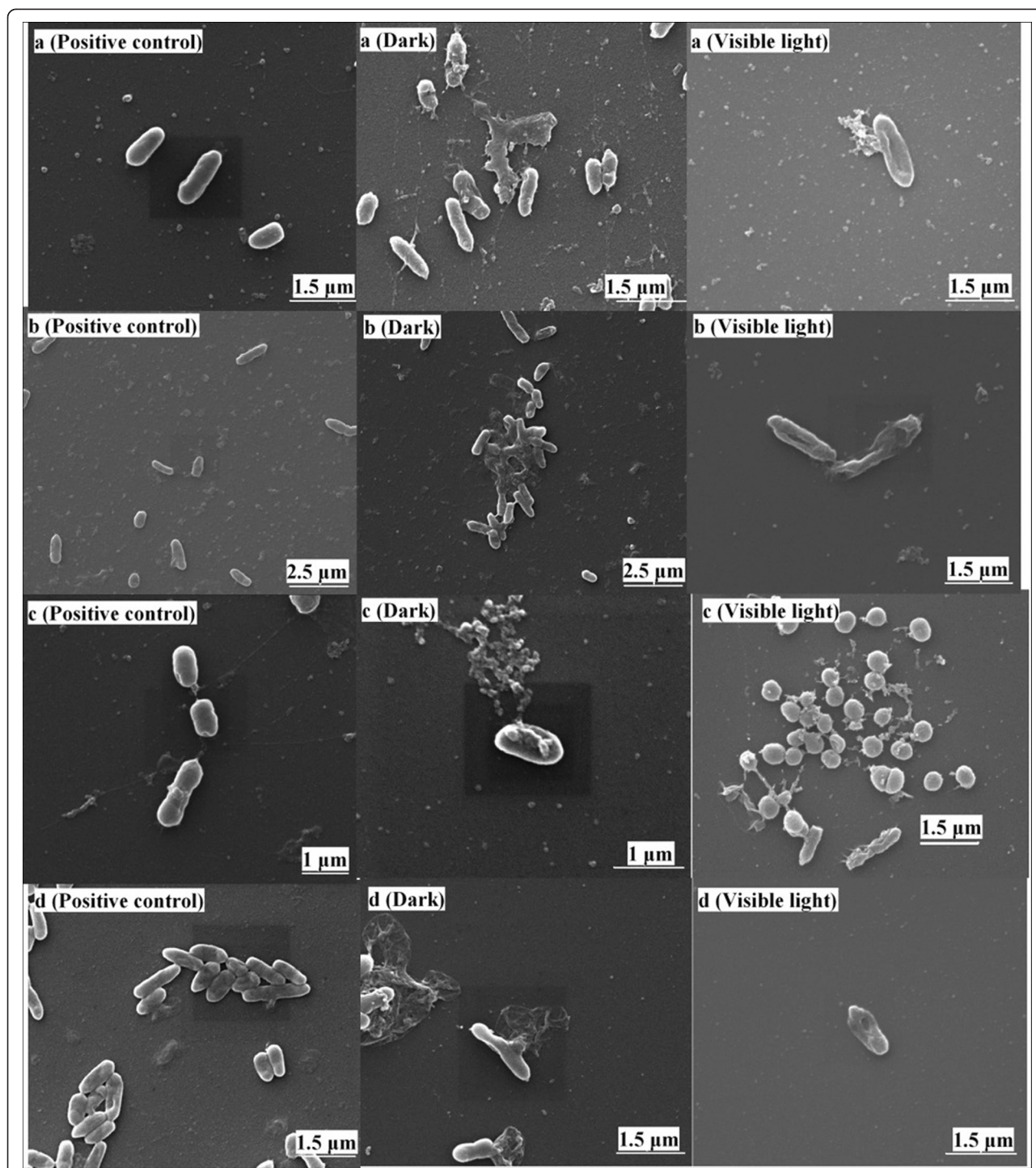


Fig. 6 SEM images of four kinds of bacteria with and without anti-treatments. **a** *P. aeruginosa* positive control and treated with 200 $\mu\text{g/ml}$ of 3 % Ta-ZnO by MIC under dark ambient and with 160 $\mu\text{g/ml}$ of 1 % Ta-ZnO under visible light for 10 min. **b** *E. coli* positive control and treated with 300 $\mu\text{g/ml}$ of 3 % Ta-ZnO by MIC under dark ambient and with 160 $\mu\text{g/ml}$ of 1 % Ta-ZnO under visible light for 10 min. **c** *S. aureus* positive control and treated with 200 $\mu\text{g/ml}$ of 1 % Ta-ZnO by MIC under dark ambient and with 160 $\mu\text{g/ml}$ of 1 % Ta-ZnO under visible light for 10 min. **d** *B. subtilis* positive control and treated with 160 $\mu\text{g/ml}$ of 1 % Ta-ZnO by MIC under dark ambient and with 160 $\mu\text{g/ml}$ of 1 % Ta-ZnO under visible light for 10 min

substance inside bacterial cells to produce bacterial toxins, leading to death of the bacteria. Especially, the generated H_2O_2 easily penetrates into the cell membrane and kills the bacteria [37].

H_2O_2 produced by visible light catalytic process might play an important role in antibacterial test, so we measured the H_2O_2 content of Ta-doped ZnO, pure ZnO, and Ta_2O_5 solution under visible light (concentration of 200 $\mu\text{g/ml}$) at 0 and 40 min, as shown in Table 2. H_2O_2 has been detected in all samples, even if in solution containing Ta_2O_5 powders. Obviously 1 % Ta-doped ZnO produces the highest H_2O_2 content and Ta_2O_5 with lowest H_2O_2 content after 40 min visible light irradiation; however, it is difficult to establish the clear relationship between antibacterial activity and generated H_2O_2 under visible light because except Ta_2O_5 , pure ZnO and Ta-doped ZnO with 1, 3, and 5 % contents exhibit better antibacterial activity on *P. aeruginosa*, *E. coli*, and *S. aureus* under visible light illumination. Although now we cannot determine which factor is the most important in antibacterial mechanism, without doubt the photocatalytic activity of Ta-doped ZnO and pure ZnO under visible light plays a key role.

Recently, Yin group reported the photogenerated charge carriers and reactive oxygen species in ZnO/Au hybrid nanostructures with enhanced photocatalytic and antibacterial activity by electron spin resonance (ESR) spectroscopy [19]. Maybe next, we may try ESR measurements to determine the generation of ROS and photoinduced charge carriers (electron or hole) of Ta-doped ZnO under visible light so as to better understand the antibacterial mechanism and clarify the correlation between charge carrier formation, generation of reactive intermediates, and antibacterial activity, especially, the role of individual ROS in antibacterial activity of photoexcited Ta-doped ZnO.

Our previous work shows the introduction of Ta^{5+} ions into ZnO can cause a series of changes in structure, morphology, and photocatalytic degradation of methylene blue, such as larger lattice parameter, smaller grain size, and more active defect sites and hydroxyl groups [28, 29]. Based on the antimicrobial study on Ta-doped ZnO nanoparticles, the incorporation of Ta^{5+} ions indeed improves the biological activity in dark ambient in most cases. The MIC results show that except the *P. aeruginosa* samples, the Ta-doping significantly enhances the bacteriostasis effect of ZnO nanoparticles on *E. coli*,

S. aureus, and *B. subtilis* in the absence of light. Usually, ZnO particles directly adsorbed to the cell walls may inhibit bacterial growth by penetration of the cell envelope and disorganization of bacterial membrane. The cell wall rupture is related to the surface activity of ZnO in contact with the bacteria.

Ta^{5+} as a high valence metal ion doped into ZnO lattice may generate zinc vacancy or oxygen interstitial in order to keep the electric neutral equilibrium, leading to the deviation of O/Zn ratio from stoichiometry. Table 3 lists the XPS results of pure ZnO and 3 % Ta-doped ZnO before and after Ar-ion etching. The Ar-ion etching was utilized to remove the surface adsorption oxygen of nanoparticles. The O/Zn ratio of 1.20 for 3 % Ta-doped ZnO is higher than 0.97 for pure ZnO after Ar-ion etching, implying that the introduction of Ta^{5+} ions leads to the formation of oxygen interstitial defect in 3 % Ta-doped ZnO. Evidently, the Ta-doped ZnO undoubtedly contains more defects with reduced grain size, improving the surface activity of ZnO. On the other hand, Stojmenov et al. ever reported that the electrostatic forces may strength the binding of the metal oxide nanoparticles on the bacteria surface, facilitating the bactericidal action [38]. The incorporation of high valence Ta^{5+} ion into ZnO lattice also enlarges the electrostatic force, producing stronger adsorption and binding of nanoparticles on bacteria. Ta-doped ZnO nanoparticles exhibit more effective bactericidal efficacy than pure ZnO in dark ambient, which can be attributed to the synergistic effect of enhanced surface bioactivity and increased electrostatic force due to the incorporation of Ta^{5+} ions into ZnO.

Additionally, for various bacteria, the antibacterial action of Ta-doped ZnO nanoparticles under visible light and dark ambient shows some differences. Under dark environment, Ta-doped ZnO nanoparticles exhibit very weak bacteriostasis effect on *P. aeruginosa*, but in visible light irradiation, strong bactericidal efficacy on *P. aeruginosa* is observed. For *B. subtilis*, the action of the photocatalysis of Ta-doped ZnO seems to be limited. These phenomena might be related to various structures and functions of several bacteria. Further in-depth work is needed.

Above all, according to our antibacterial tests, 5 % Ta-doped ZnO is a more effective antimicrobial agent than pure ZnO.

Table 2 The H_2O_2 content of different samples of 200 $\mu\text{g/ml}$ at 0 and 40 min under visible light

Samples ($\mu\text{g/ml}$)	ZnO	1 % Ta	3 % Ta	5 % Ta	Ta_2O_5
0 min	28.7	86.3	64.7	64.7	14.3
40 min	39.4	100.7	50.3	43.1	21.5

Table 3 The XPS results of ZnO and 3 % Ta-doped ZnO before and after Ar-ion etching

Samples	Ar-ion etching	O	Zn	O/Zn ratio
ZnO	No	44.09	32.01	1.38
ZnO	Yes	45.52	46.96	0.97
3 % Ta-doped ZnO	No	37.94	28.04	1.35
3 % Ta-doped ZnO	Yes	50.43	42.06	1.20

Conclusions

In summary, novel photocatalyst of Ta-doped ZnO nanoparticles was prepared by a modified Pechini-type method. The antibacterial activity of Ta-doped ZnO nanoparticles on several bacteria of *P. aeruginosa*, *S. aureus*, *E. coli*, and *B. subtilis* were investigated using a standard microbial method. The Ta-doping concentration effect on MIC of various bacteria under dark ambient has been evaluated. The photocatalytic biocidal behavior of Ta-doped ZnO nanoparticles under visible light irradiation was also characterized. The MIC results indicate that the incorporation of Ta⁵⁺ ions into ZnO significantly improve the bacteriostasis effect of ZnO nanoparticles on *E. coli*, *S. aureus*, and *B. subtilis* in the absence of light. Compared to MIC results without light irradiation, Ta-doped ZnO and pure ZnO nanoparticles show much stronger bactericidal efficacy on *P. aeruginosa*, *E. coli*, and *S. aureus* under visible light illumination. The possible antibacterial mechanisms in Ta-doped ZnO systems under visible light and dark conditions have been proposed. Ta-doped ZnO nanoparticles exhibit more effective bactericidal efficacy than pure ZnO in dark ambient, which can be attributed to the synergistic effect of enhanced surface bioactivity and increased electrostatic force due to the incorporation of Ta⁵⁺ ions into ZnO. Based the antibacterial tests, 5 % Ta-doped ZnO is a more effective antimicrobial agent than pure ZnO.

Competing Interests

The authors declare that they have no competing interests.

Authors' Contributions

BLG carried out the sample fabrication and device measurements. BLG and PH drafted the manuscript. BLG and YQC did the data analysis and interpreted the results. LCG and YQC helped to finish SEM observation. ADL, DW, JZK, and HFZ participated in the discussion of results. ADL supervised the whole work and revised the manuscript. All authors critically read and commented on the manuscript. All authors read and approved the final manuscript.

Acknowledgements

This project is supported by the Natural Science Foundation of China (51202107), a grant from the State Key Program for Basic Research of China (2015CB921203 and 2011CB922104). Ai-Dong Li also thanks the support from Open Project of National Laboratory of Solid State Microstructures (M27001), PAPD in Jiangsu Province, and Doctoral Fund of Ministry of Education of China (20120091110049).

Author details

¹National Laboratory of Solid State Microstructures, Materials Science and Engineering Department, College of Engineering and Applied Sciences, Collaborative Innovation Center of Advanced Microstructures, Nanjing University, Nanjing 210093, People's Republic of China. ²College of Life Science, Nanjing University, Nanjing 210093, People's Republic of China.

Received: 12 May 2015 Accepted: 10 August 2015

Published online: 21 August 2015

References

- Wang ZL. Nanostructures of zinc oxide. *Mater Today*. 2004;7:26.
- Xu F, Zhang P, Navrotsky A, Yuan ZY, Ren TZ, Halasa M, et al. Hierarchically assembled porous ZnO nanoparticles: synthesis, surface energy, and photocatalytic activity. *Chem Mater*. 2007;19:5680.

- Hariharan C. Photocatalytic degradation of organic contaminants in water by ZnO nanoparticles. *Appl Catal A: Gen*. 2006;304:55.
- Lu F, Cai WP, Zhang YG. ZnO hierarchical micro/nanoarchitectures: solvothermal synthesis and structurally enhanced photocatalytic performance. *Adv Func Mater*. 2008;18:047.
- Sawai J, Igarashi H, Hashimoto A, Kokugan T, Shimizu M. Effect of ceramic powder slurry on spores of *Bacillus subtilis*. *J Chem Eng Jap*. 1995;28:288.
- Sawai J, Igarashi H, Hashimoto A, Kokugan T, Shimizu M. Detection of active oxygen generated from ceramic powders having antibacterial activity. *J Chem Eng Jap*. 1996;29:251.
- Sawai J, Yoshikawa T. Quantitative evaluation of antifungal activity of metallic oxide powders (MgO, CaO and ZnO) by an indirect conductimetric assay. *J Appl Microb*. 2004;96:803.
- Adams LK, Lyon DY, Alvares PJJ. Comparative eco-toxicity of nanoscale TiO₂, SiO₂, and ZnO water suspensions. *Water Res*. 2006;40:3527.
- Jones N, Ray B, Ranjit KT, Manna AC. Antibacterial activity of ZnO nanoparticle suspensions on broad spectrum of microorganisms. *EMS Microb Lett*. 2008;279:71.
- Huang ZB, Zheng X, Yan DH, Yin GF, Liao XM, Kang YQ, et al. Toxicological effect of ZnO nanoparticles based on bacteria. *Langmuir*. 2008;24:4140.
- Sawai J. Quantitative evaluation of antibacterial activities of metallic oxide powders (ZnO, MgO and CaO) by conductimetric assay. *J Microb Methods*. 2003;54:17782.
- Xu Y, Yang ZX, Li ZH, Qian JM, Shen ZW. Study on antibacterial property of TiO₂ composite powder. *J Func Mater*. 2002;33:682.
- Wang YL, Wan YZ, Dong XH, Cheng GX, Tao HM, Wen TY. Preparation and characterization of antibacterial viscose-based activated carbon fiber supporting silver. *Carbon*. 1998;36:1567.
- Makhluf S, Dror R, Nitzan Y, Abramovich Y, Jelinek R, Gedanken A. Microwave-assisted synthesis of nanocrystalline MgO and its use as a bactericide. *Adv Func Mater*. 2005;15:1708.
- Fortuny A, Bengoa C, Font J, Fabregat A. Bimetallic catalysts for continuous catalytic wet air oxidation of phenol. *J Hazard Mater*. 1999;64:181.
- Rana S, Rawat J, Sorenson MM, Misra RDK. Antimicrobial function of Nd³⁺-doped anatase titania-coated nickel ferrite composite nanoparticles: a biomaterial system. *Acta Biomater*. 2006;2:421.
- Li QL, Mahendra S, Lyon DY, Brunet L, Liga MV, Li D, et al. Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. *Water Res*. 2008;42:4591.
- Yamamoto O. Influence of particle size on the antibacterial activity of zinc oxide. *Inter J Inorgan Mater*. 2001;3:643.
- He WW, Kim HK, Wamer WG, Melka D, Callahan JH, Yin JJ. Photogenerated charge carriers and reactive oxygen species in ZnO/Au hybrid nanostructures with enhanced photocatalytic and antibacterial activity. *J Amer Chem Soc*. 2014;136:750–7.
- Atmaca S, Gul K, Clek R. The effect of zinc on microbial growth. *Turkish J Med Sci*. 1998;28:595.
- Lin H-F, Liao S-C, Hung S-W. The dc thermal plasma synthesis of ZnO nanoparticles for visible-light photocatalyst. *J Photochem Photobio A: Chem*. 2005;174:82.
- Ullah R, Dutta J. Photocatalytic degradation of organic dyes with manganese-doped ZnO nanoparticles. *J Hazard Mater*. 2008;156:194.
- Rekha K, Nirmala M, Nair MG, Anukaliani A. Structural, optical, photocatalytic and antibacterial activity of zinc oxide and manganese doped zinc oxide nanoparticles. *Phys B: Condens Matter*. 2010;405:3180.
- Mahata P, Madras G, Natarajan S. Novel photocatalysts for the decomposition of organic dyes based on metal-organic framework compounds. *J Phys Chem B*. 2006;110:13759.
- Zhang Q, Fan W, Gao L. Anatase TiO₂ nanoparticles immobilized on ZnO tetrapods as a highly efficient and easily recyclable photocatalyst. *Appl Catal B: Environ*. 2007;76:168.
- Anandan S, Vinu A, Lovely KLPS, Gokulakrishnan N, Srinivasu P, Mori T, et al. Photocatalytic activity of La-doped ZnO for the degradation of monocrotophos in aqueous suspension. *J Molecu Catal A: Chem*. 2007;266:149.
- Li D, Haneda H. Photocatalysis of sprayed nitrogen-containing Fe₂O₃-ZnO and WO₃-ZnO composite powders in gas-phase acetaldehyde decomposition. *J Photochem Photobio A: Chem*. 2003;160:203.
- Kong JZ, Li AD, Zhai HF, Gong YP, Li H, Wu D. Preparation, characterization of the Ta-doped ZnO nanoparticles and their photocatalytic activity under visible-light illumination. *J Solid State Chem*. 2009;182:2061–7.

29. Kong JZ, Li AD, Li XY, Zhai HF, Gong YP, Li H, et al. Preparation, characterization of the Ta-doped ZnO nanoparticles and their photocatalytic activity under visible-light illumination. *J Solid State Chem.* 2010;183:1359–64.
30. Li AD, Kong JZ, Zhai HF, Chen JB, Wu D. Synthesis, characterization, and applications of water-soluble tantalum carboxylate precursors via a flux method. *J Amer Ceram Soc.* 2009;92:1959–65.
31. Ramnath M, Beukes M, Tamura K, Hastings JW. Absence of a putative mannose-specific phosphotransferase system enzyme iiaB component in a leucocin a-resistant strain of *listeria monocytogenes*, as shown by two-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis. *Appl Environ Microbio.* 2000;66:3098.
32. Padmavathy N, Vijayaraghavan R. Enhanced bioactivity of ZnO nanoparticles—an antimicrobial study. *Sci Techno. Adv Mater.* 2008;9:035004.
33. Meletiadis J, Mouton JW, Meis JFG, Bouman BA, Donnelly PJ, Venweij PE, et al. Comparison of spectrophotometric and visual readings of NCCLS method and evaluation of a colorimetric method based on reduction of a soluble tetrazolium salt, 2,3-bis-2-methoxy-4-nitro-5-2H-tetrazolium-hydroxide, 2,3-bis 2-methoxy-4-nitro-5-2H-tetrazolium-hydroxide, for antifungal susceptibility testing of *Aspergillus* species. *J Clin Microbio.* 2001;39:4256.
34. Rodriguez-Tudela JL, Cuenca-Estrella M, Diaz-Guerra TM, Mellado E. Standardization of antifungal susceptibility variables for a semiautomated methodology. *J Clin Microb.* 2001;39:2513.
35. Tunney MM, Ramage G, Field TR, Moriarty TF, Storey DG. Rapid colorimetric assay for antimicrobial susceptibility testing of *pseudomonas aeruginosa*. *Antimicrob Agents Chemother.* 2004;48:1879.
36. Fang MF, Chen JH, Xu XL, Yang PH, Hildebrand H. Antibacterial activities of inorganic agents on six bacteria associated with oral infections by two susceptibility tests. *Inter J Antimicrob Agents.* 2006;27:513.
37. Blake DM, Maness PC, Huang Z, Wolfrum EJ, Huang J, Jacoby WA. Application of the photocatalytic chemistry of titanium dioxide to disinfection and the killing of cancer cells. *Separat Purif Methods.* 1999;28:1.
38. Stoimenov PK, Klinger RL, Marchin GL, Klabunde KJ. Metal oxide nanoparticles as bactericidal agents. *Langmuir.* 2002;18:6679.

Submit your manuscript to a SpringerOpen[®] journal and benefit from:

- Convenient online submission
- Rigorous peer review
- Immediate publication on acceptance
- Open access: articles freely available online
- High visibility within the field
- Retaining the copyright to your article

Submit your next manuscript at ► springeropen.com
