# Antibacterial activity of heterogeneous $TiO_2$ and ZnO nanoparticles against Gram-positive and Gram-negative bacterial pathogens

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Received 01 Apr 2018.
Revised 01 June 2018.
Accepted 25 July 2018.
Published Online 15 Aug 2018

*Abstract*—Hospital-acquired infections (HAIs) are responsible for over 40% of cases in acute-care hospitals and commonly associated with catheters-associated urinary tract infections (CAUTIs). Current nanotechnology approach focus on improving the aseptic procedures for medical devices and manage the HAIs risk. $TiO_2$ and ZnO nanoparticles (NPs) have been widely reported independently, to have a photocatalytic killing potential. The present study evaluates the antibacterial activity of heterojunction between $TiO_2$ and ZnO NPs on several types bacterial pathogens model including *Staphylococcus aureus*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*. The antibacterial screening test on $TiO_2$/ZnO nanoparticles (NPs) were done under dark and light conditions with different molar ratio 25T75Z, 50T50Z and 75T25Z according to Clinical Laboratory Standards Institute (CLSI) guidelines MO2-A11. ZnO and $TiO_2$/ZnO (25T75Z and 50T50Z) NPs at the highest concentration (1000µg/µL) showed mean diameters of the zones of inhibition (mm); (12.5 ± 0.58), (12.13 ± 0.85), and (7.25 ± 1.44) in dark condition. Increment in inhibition zones was obtained under light condition; (21.38 ± 0.48), (17.50 ± 1.0), and (12.38 ± 1.80). Findings from this study highlights the heterogeneous $TiO_2$ and ZnO NPs could become a promising bacteriostatic and/or bactericidal agent to combat against the HAIs.

***Keywords* — $TiO_2$/ZnO nanoparticles, Hospital-acquired infections, Antibacterial activity, Bacteriostatic agent, Bactericidal agent, Biomedical Product, Biomaterial, Nanomaterial**

## 1 INTRODUCTION

Healthcare-associated infections (HAIs) caused by Gram-positive and Gram-negative bacterial pathogens are significant economic and public health burdens globally, having an estimated level of 6.4% and 1,000,000 reported cases annually [1]. The most common HAI pathogens include Gram-positive bacteria, such as *Staphylococcus aureus, Staphylococcus epidermidis* and *Enterococcus faecalis*, and Gram-negative bacteria, including *Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis* and *Pseudomonas aeruginosa*, and yeasts, such as *Candida* sp. [2]. HAI is closely related to medical device contamination which poses high patient morbidity and mortality cases [3]. Rutala et al. (2013) studied a rational approach in cleaning, disinfection and sterilisation techniques for patient care items and medical devices developed by Earle H. Spaulding for the last four decades as one of the strategies to reduce HAIs [4]. Failure to provide a proper disinfection or sterilisation on medical devices may lead to the introduction of pathogenic infection, thereby risking patients' lives.

Advancement in nanotechnology can provide new approaches in controlling HAI risks. The physicochemical properties of metal oxides NPs, such as $TiO_2$ and ZnO, have substantial important uses in several pharmaceutical industries. A number of studies have indicated the widely uses of $TiO_2$ and ZnO in therapeutic applications such as biosensing [5], imaging labels [6,7] and drug delivery [8,9]. These NPs also present unique properties such as cytocompatibility, biocompatibility, chemical stability, environmental-friendliness, wide band gap and relatively low cost [10, 11]. Moreover, $TiO_2$ and Zn NPs have been widely studied as antibacterial agents owing to their photocatalytic activities [12-15]. In fact, several efforts have been successful in improving the photocatalytic capabilities and synergistic effects of metal oxide NPs, such as CuO/ZnO, cerium oxide/yttrium oxide and Ag orthophosphate ($Ag_3PO_4$/ $TiO_2/Fe_3O_4$) NPs [16,17]. Thus, the present study investigates the antibacterial activity of $TiO_2$/ZnO NPs with different molar ratios against the selected Gram-positive and -negative bacteria under both dark and visible light conditions.

## 2 METHODOLOGY

### 2.1 $TiO_2$ / ZnO NPs synthesis and characterization

Titanium (IV) isopropoxide 97% and Zinc acetate dehydrate 98% were supplied by Sigma Aldrich. The $TiO_2$/ZnO NPs photocatalyst of different molar ratio of $TiO_2$ and ZnO was synthesis by sol-gel process with molar ratio at 25T75Z, 50T50Z and 75T25Z. Commercial TiO2 P25, 100T and 100Z represent as control counterpart. The ZnO NPs were prepared as follows. ZnO sol was prepared by adding 0.114 mole Zinc acetate dehydrate (ZAD) into ethanol. The solution was stirred for 5 min in heated (70 °C) water bath to obtain the precursor solution. Next, continuous stirring for 5 h were carried out until transparent solution is obtained. Then, deionized water was added drop wise and stirred for 10 min. On the other hand, the $TiO_2$ NPs were prepared by dissolving Titanium Isopropoxide (TTIP) in ethanol with the volume ratio of 1:4 and stirred for 30 min. Then, deionised water was added dropwise and stirred for 3 h. After $TiO_2$ and ZnO sols are synthesized separately, the sols are mixed to different ratios and stirred continuously for 1h. The milky white suspension was centrifuged, washed with ethanol until white sediment was observed. The resultant sediment was oven dried overnight at 80 °C. The dried white precipitate was pulverized with pestle and mortar and calcined in air at 500 °C for 2h. The morphology of the $TiO_2$/ZnO NPs photocatalyst was further characterized using Field Emission Scanning Electron Microscope (FESEM) model of LEO GEMINI.

### 2.2 Photocatalytic activity

The photocatalytic activity $TiO_2$/ZnO NPs photocatalyst were studied by degradation of methylene blue (MB) solution under sunlight. 10 mg of each catalyst was suspended into 40 mL MB aqueous solution (3 ppm). The mixture was poured into a glass petri dish and kept in a dark environment for 30 min to allow it to attain an equilibrium adsorption state. It was then put under sunlight for a set of irradiation time. After the degradation, the mixture was withdrawn and the catalyst was separated from the suspensions by a centrifuge. The determination of MB concentration was performed using a Cary50 UV-vis spectrophotometer (Varian Corp) by measuring the absorption of MB at 644 nm.

### 2.3 Antibacterial Disc Susceptibility Tests

*S*. *aureus* ATCC 25923, *E*. *faecalis* ATCC 29212, *E*. *coli* ATCC 25922, *K*. *pneumoniae* ATCC 700603 and *P*. *aeruginosa* ATCC 27853 were tested. They were cultured aerobically at 37 °C on Luria–Bertani agar plates (Merck, Germany) for 24 h. Positive control was represented by the standard antibiotic and negative control discs (sample diluent) from 10% dimethyl sulfoxide (DMSO) (Sigma-Aldrich). Test samples were prepared in 10% DMSO as diluent with different concentrations (100, 200, 500, 800 and 1000 µg/µl). The disc diffusion assay of $TiO_2$/ZnO NPs was conducted according to the guidelines of CLSI MO2-A11 (Clinical and Laboratory Standard Institute MO2-A11) under dark and light conditions with visible light intensity of 5.70 w/m$^2$ [18]. All tests were done in triplicate.

## 3 RESULTS AND DISCUSSION

FESEM images of NPs are shown in Figure 1a–d. Most of the NPs photocatalyst were spherical in shape. Nevertheless, in certain regions of 25T75Z (Figure 1c) and 100Z (Figure 1d), rod structures were visible (marked in red square). The average particle size of the spherical shape was 91.35 nm, while the nanorod diameter and length were 66.13 and 409.03 nm, respectively.

The photocatalytic activity of 100T, 25T75Z, 50T50Z, 75T25Z and 100Z were evaluated by the photocatalytic decolourisation of methylene blue (MB) under sunlight irradiation (Figure 2). MB concentration was decreases with the increase in irradiation time. After 180 min of sunlight exposure, 100T, 25T75Z and 100Z displayed ~98% degradation, whilst 75T25Z and 50T50Z showed only 88% and 50% degradation, respectively. The results suggested that the improvement in photocatalytic activity in bare ZnO was contributed by less efficient radioactive recombination at UV emission. Meanwhile, for $TiO_2$/ZnO NPs photocatalyst, especially 25T75Z, enhancement was related to intrinsic defects and charge separation due to improved interface between ZnO and $TiO_2$.

Additionally, the photocatalytic degradation of MB for the entire photocatalyst was quantitatively investigated through a pseudo-first-order kinetic model, and the observed first-order rate constant (k) was determined. The values of the calculated k constants are presented in Figure 3. Kinetic data revealed that the degradation rate constants for 100T, 100Z, 25T75Z, 50Z50T and 75T25Z under sunlight irradiation were $1.55 \times 10^{-2}$, $1.71 \times 10^{-2}$, $1.80 \times 10^{-2}$, $0.4 \times 10^{-2}$ and $1.13 \times 10^{-2}$ $min^{-1}$, respectively. Thus, the degradation rate showed in an increasing order, that is, 50T50Z < 75T25Z < 100T < 100Z < 25T75Z. This result indicated that the presence of $TiO_2$ and ZnO (25T75Z) and the coexistence of zincite phase resulted in highest activity for NPs.

Meanwhile, the antibacterial profiles of $TiO_2$/ZnO NPs in terms of the differential molar ratios, concentrations and conditions against *S. aureus* are shown in Figure 4. The effect of $TiO_2$/ZnO NPs on *S. aureus* showed large inhibition zones at 1000 µg/µl with the presence of visible light, that is, 21.38 ± 0.48, 17.50 ± 1.0 and 12.38 ± 1.80, for 100Z, 25T75Z and 50T50Z NPs, respectively. *S. aureus* also showed inhibition zones even under dark condition with the maximum inhibition zones of 12.5 ± 0.58, 12.13 ± 0.85 and 7.25 ± 1.44 for 100Z, 25T75Z and 50T50Z NPs, respectively. Jesline et al. (2014) reported similar finding on the antibacterial activities of $TiO_2$ and ZnO NPs against *S. aureus* [19]. The ability of ZnO NPs and the heterogeneity of ZnO-based compounds are well-known excellent antibacterial agents. Thus, high ZnO content in the heterogeneous $TiO_2$/ZnO samples resulted in large inhibition zones in both conditions. The result indicated the bacteriostatic capability of 25T75Z and 50T50Z NPs against *S. aureus* in both conditions.

The bacteriostatic activity of ZnO and $TiO_2$ NPs photocatalyst under both conditions may be attributed to photocatalytic activity, which produced superoxide anion radicals. These superoxide anion radicals reacted with $H^+$ ions to form $HO_2^-$ radicals, which in turn reacted with electrons and $H^+$-yielding $H_2O_2$ molecules. Subsequently, $H_2O_2$ molecules penetrated the cell membrane and caused the death of *S. aureus*. The generation of O species, such as $H_2O_2$, $O_2^-$ and OH*, disrupted the bacterial membrane structure and killed the bacteria [20]. Several studies also reported ROS generation activity under dark conditions [21,22]. Therefore, our study confirmed that ROS generation under dark and visible light conditions resulted in phototoxic effects that inhibited or killed *S. aureus*. In the *S.aureus* bacterial cell wall, it consisting several layers of peptidoglycan. The porous structure of the interconnected peptidoglycan layer and interaction between positively charged NPs with negatively charged teichoic acid of *S. aureus* allowing diffusion of NPs into the bacterial membrane [26]. However, no inhibition zones were observed in the Gram-negative bacteria tested in this study under both (light and dark) conditions. This phenomenon explain due to the greater complexity of the double membranes of the Gram-negative bacteria which composed of asymmetric bilayers of phospholipid and lipopolysaccharides. It is apparent that the outer membrane serve as selective barriers that provide an extra layer of protection for diffusion or penetration of NPs [23-25].

## 4 CONCLUSION

The bactericidal activity of heterogeneous $TiO_2$/ZnO NPs is not only dependent on the concentration and type of bacteria but also on the photocatalytic activity via the generation of reactive oxygen species, such as superoxide anion radical, hydroxyl radical and $H_2O_2$. The potential bacteriostatic agents of $TiO_2$/ZnO NPs with molar ratios of 25T75Z and 50T50Z against HAIs requires further study.

## ACKNOWLEDGEMENT

The authors are thankful to the Ministry of Education (MOE) Malaysia for funding this work under Transdisciplinary Research Grant Scheme (TRGS) grant no. 6769003 and Universiti Sains Malaysia (USM) for providing the necessary facilities to carry out the research work.

## CONFLICTS OF INTEREST

The authors report no conflicts of interest. The authors are responsible for the content and writing of the paper.

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