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Antibacterial responses of zinc oxide structures against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Streptococcus pyogenes*

Ling Chuo Ann[a,*], Shahrom Mahmud[a], Siti Khadijah Mohd Bakhori[a], Amna Sirelkhatim[a], Dasmawati Mohamad[b], Habsah Hasan[c], Azman Seeni[d], Rosliza Abdul Rahman[c]

[a]*Nano-optoelectronic Research (NOR) Laboratory, School of Physics, Universiti Sains Malaysia, 11800 Pulau Pinang, Malaysia*
[b]*School of Dental Sciences, Universiti Sains Malaysia, Kubang Kerian, 16150 Kelantan, Malaysia*
[c]*School of Medical Sciences, Universiti Sains Malaysia, Kubang Kerian, 16150 Kelantan, Malaysia*
[d]*Advanced Medical and Dental Institute, Universiti Sains Malaysia, Bertam, 13200 Pulau Pinang, Malaysia*

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Abstract

The antibacterial responses of zinc oxide (ZnO) structures against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Streptococcus pyogenes* were investigated. Two ZnO powder samples, one with rod-like (ZnO-1) and the other with plate-like (ZnO-2) structures, were characterized for their morphological, structural, and optical properties. The rods were 30–120 nm in diameter, and the plates were 40–100 nm thick. XRD results revealed the wurtzite crystallinity of ZnO with average crystallite sizes of 33.72 (ZnO-1) and 39.25 (ZnO-2) nm. ZnO-2 possessed a relatively higher green photoluminescence than that of ZnO-1, suggesting a relatively higher amount of oxygen vacancies in ZnO-2 structures. Optical density measurements showed that both ZnO samples inhibited the growth of *S. aureus*, *P. aeruginosa*, and *S. pyogenes* by 29–98% after 24 h of treatment. The most dramatic growth inhibition was observed in *S. pyogenes* with 96% and 98% inhibition for ZnO-1 and ZnO-2, respectively, leading to a probable bactericidal phenomenon. The toxicological effect on *S. pyogenes* was probably due to the absence of catalase, making the bacteria vulnerable to the harmful reactive oxygen species (ROS) released by ZnO. ZnO-1 induced higher inhibition toward *S. aureus* and *P. aeruginosa* than that of ZnO-2 because of the smaller particle size of rod structures compared to plate and slab structures. The adhesion of ZnO particles on the membrane of bacteria could be the underlying cause of zinc toxicity effect towards the bacteria. ZnO-1 possessed larger surface area and provided higher amount of zinc atom, thereby inducing higher level of toxicity toward the bacteria. Two possible mechanisms were proposed to explain the inhibition of bacteria, namely, ROS toxicity toward cellular constituents and interaction of zinc with bacteria membrane through adhesion of ZnO particle. Several ZnO morphological-antibacterial correlations were presented in this work.

Keywords: D. ZnO; Antibacterial; ROS; Morphology

1. Introduction

Nanomaterials are gaining considerable interest from various fundamental and applied researchers in the fields of science, engineering, and medicine because of their unique properties and enhanced performance compared with macroscopic materials. The ongoing revolution and upgrading of nanotechnology dramatically affects the development of biomedical and engineering applications. In recent years, nanoparticles smaller than 100 nm in size have been extensively studied because of their wide application in science and technology [1–2].

Zinc oxide (ZnO) is an essential inorganic material with multiple applications in optoelectronics, pigments, cosmetics, pharmaceuticals, varistors, and biosensors [3–9]. ZnO is known to exhibit antimicrobial activity and has higher stability than organic materials. The release of reactive oxygen species (ROS) especially hydrogen peroxide, superoxide anion, hydroxyl radical, and hydroxyl ion [10–11] has been documented as possible mechanisms of the antimicrobial behavior of zinc oxide. With the advancements in nanotechnology, various zinc oxide nanostructures have been prepared, such as rods, plates, tetrapods, tripods, wires, mallets, combs, and flowers [12–16]. The nanosized particles of zinc oxide possess a large surface-to-volume ratio that may exhibit stronger antimicrobial activity. Different structural morphologies of ZnO can exhibit selective antimicrobial

*Corresponding author. Tel.: +60 164708973.
E-mail address: lingchuoann@hotmail.com (L.C. Ann).

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responses. Meanwhile, different bacteria exhibit various affinities toward ZnO structures depending on bioactivity and bacteria life processes. Therefore, further investigation is required to justify the performance of different ZnO structures toward the targeted microbe. The selective bioactivity of bacteria is due to the different biological systems of the bacteria that may render ZnO ineffective for antibacterial application. More studies are necessary to determine the impact of ZnO structures toward various bacteria.

Pathogenic skin bacteria cause many skin infections, including impetigo, folliculitis, furunculosis, carbunculosis, ecthyma, erysipelas, and cellulitis. ZnO is known to cure many kinds of skin diseases and is widely used in health products and cosmetics. Moreover, certain morphological structures of ZnO particularly at the nanoscale are believed to show a relatively greater impact in overcoming skin infections. In this work, three types of pathogenic skin bacteria were studied, namely, *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The effect of ZnO samples that have different structural morphologies on selected skin bacteria was investigated. The level of inhibition of each skin bacteria was also discussed to compare the biological reaction of relevant bacteria with ZnO structures.

2. Experimental details

Two types of ZnO powder were used in this study. Both types were produced via French process described in our previous work [17]. Both samples possessed high purity ($>99.97\%$) and were labeled as ZnO-1 and ZnO-2. The structural morphologies of the both ZnO samples were characterized by field-emission scanning electron microscopy (FESEM; FEI NovaNanoSEM 450) and transmission electron microscopy (TEM; Philips CM12). Electron spectroscopy imaging (ESI) was conducted by energy-filtered transmission electron microscopy (EFTEM; Zeiss Libra 120) to investigate elemental distribution of ZnO particle surfaces. The distribution of zinc atoms and oxygen atoms were localized and are illustrated using different colors (green for Zn; blue for O).

The structural crystallinity of the ZnO powder were investigated using an X-ray diffractometer (model PANalytical X'Pert PRO MED PW3040) with Cu-K_α radiation ($\lambda = 1.5406$ Å) for excitation. The crystallite sizes were calculated from the XRD data and compared with the particle sizes observed from TEM images. Then, ZnO pellets were prepared using a technique introduced in our previous work [17]. The ZnO pellets were characterized for their optical properties using photoluminescence (PL) spectroscopy (Jobin Yvon Horiba HR800UV).

Three types of skin bacteria were chosen in this antibacterial study: two Gram-positive bacteria (*S. pyogenes* ATCC 19615 and *S. aureus* ATCC25923) and one Gram-negative bacteria (*P. aeruginosa* ATCC 27853). Microdilution technique was used to culture and treat the bacteria in a 96-well plate. Initially, bacteria 0.5 Mcfarlands were inoculated and diluted in tryptone soy broth (TSB), producing bacterial colony of about 1×10^6 CFU/ml. Four concentrations of ZnO suspensions were prepared: 1, 2, 3, and 4 mM. 150 µl of adjusted inocula (1×10^6 CFUml) was mixed with 150 µl of ZnO suspension in a 96-well microplate. The final ZnO concentration was halved: 0.5, 1, 1.5, and 2 mM. The mixtures of ZnO and bacteria in TSB were incubated at 37 °C for 24 h.

The growth of bacteria was measured using a spectrophotometer (Versamax microplate reader). Using a light source wavelength of 600 nm, optical density (OD) assessment indicated the level of light scattering caused by the bacteria turbidity. A higher number of bacterial colony in the TSB corresponds to a higher OD reading. OD measurements of *S. aureus* and *P. aeruginosa* were conducted hourly up to 8 h. Given the typically slow growth of *S. pyogenes*, bacterial growth was monitored up to 10 h. The percentage inhibition of bacterial growth was determined from the OD after 24 h of incubation. The antibacterial test was done in triplicate, and the average of three ODs was calculated. A negative control (bacteria and TSB) was also prepared for the measurement.

To observe the morphological structures of the bacteria, a mixture of bacteria and TSB was fixed and processed. The bacteria were washed with phosphate buffer 0.2 M and fixed with McDowell–Trump fixatives. Subsequently, the samples were post-fixed in 1% osmium tetroxide for 1 h, washed with distilled water, dehydrated with ethanol, and treated with hexamethyldisilazane. Lastly, the samples were air dried at room temperature. The dried cells (powder form) were mounted on a FESEM specimen stub with a double-sided carbon tape and coated with platinum. The morphologies of the bacteria were observed under FESEM (FEI).

Numerical data of OD measurements were analyzed for significance using Sigma-plot's *t*-test ($n = 3$). Values are reported as mean ± standard deviation. The threshold for significance was set at $p < 0.05$.

3. Result and discussions

3.1. Morphological and atomic mapping analyses

Figs. 1 and 2 show the morphology and structural details of the two types of ZnO, namely, ZnO-1 and ZnO-2. Fig. 1(a) shows that the morphologies of ZnO-1 are made of several structures: micro/nanorods and irregular-shaped particles. Most structures (about 70%) possess rod-shaped structures. The dimensions of the rods range from 100 nm to 500 nm for the length and from 30 nm to 120 nm for the diameter, respectively. The rod structures are observed to have rounded and tapered tips. The average width sizes were calculated from 100 particles based on many TEM images such as in Fig. 1(b–c). The nanoscale widths have the highest percentage (26%) in the 51–60 nm range [Fig. 1(d)]. The microscopic particles are mostly irregular-shaped particles that are not desired, and their formations were due to the non-uniform crystallization condition during Zn oxidation [17]. Fig. 1(e) shows the elemental mapping of Zn atom and O atom in the ZnO structures using EFTEM (ESI). Results reveal the non-stoichiometric distribution of Zn and O atoms on the surface of ZnO rod structures, whereby the rod structures possess a relatively higher content of O atom on the surface than Zn atom [18].