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Effect of surface modification and UVA photoactivation on antibacterial bioactivity of zinc oxide powder

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ABSTRACT

The effects of surface modification of zinc oxide (ZnO) powder and UVA illumination on the powder towards *Escherichia coli* and *Staphylococcus aureus* were investigated. FESEM-EDS results showed that oxygen annealing increased the O:Zn ratio on the surface of ZnO-rod and ZnO-plate samples. Surface conductances of ZnO-rod and ZnO-plate pellets were reduced from 1.05 nS to 0.15 nS and 1.34 nS to 0.23 nS, respectively. Meanwhile, UVA illumination on the surface of the ZnO-rod and ZnO-plate samples was found to improve surface conductance to 7.08 nS and 6.51 nS, respectively, due to the release of charge carrier. Photoluminescence results revealed that oxygen annealing halved the UV emission intensity and green emission intensity, presumably caused by oxygen absorption in the ZnO lattice. The antibacterial results showed that oxygen-treated ZnO exhibited slightly higher growth inhibition on *E. coli* and *S. aureus* compared with unannealed ZnO. UVA illumination on ZnO causes the greatest inhibition toward *E. coli* and *S. aureus*. Under the UVA excitation, the inhibition of *E. coli* increased by 18% (ZnO-rod) and 13% (ZnO-plate) while the inhibition of *S. aureus* increased by 22% (ZnO-rod) and 21% (ZnO-plate). Release of reactive oxygen species were proposed in antibacterial mechanisms, which were aided by surface modification and UVA photoactivation. The reactive oxygen species disrupted the DNA and protein synthesis of the bacterial cell, causing bacteriostatic effects toward *E. coli* and *S. aureus*.

1. Introduction

Inorganic materials are important materials in physical, biological, biomedical, and pharmaceutical applications [1–3]. Crystalline powders have uniform sizes and shapes, with sizes ranging from nano to micron scale. Metal oxides exhibit numerous interesting and important properties (e.g., catalytic, electrical, optical). Particles of zinc oxide (ZnO), calcium oxide, and magnesium oxide also show remarkable antibacterial properties without the presence of light [4–6]. Humans can benefit from the use of these inorganic materials, which contain mineral elements that are essential to the human body and possess strong antibacterial properties [7,8]. Among these metal oxides, ZnO particles are outstanding candidates because of their unique properties and widespread application.

ZnO responds well to ultraviolet (UV) illumination, where its conductivity increases rapidly and persists long after the UV light is turned off. Surface modification correlates to the surface density of negatively charged adsorbed oxygen species (O_2^{-}) [9,10]. Both UV illumination and surface modification involve the alteration of surface properties, which may have a considerable impact on antibacterial activity. The different structural morphologies of ZnO powder may also possess different types of reactivity in the inhibition of bacterial growth [11,12]. Therefore, the affinity of morphologies (rod, plate, tripod, tetrapod, or irregularly shaped particles) in bacterial inhibition must be elucidated.

Several studies and observations have been conducted to quantify the mechanisms of antimicrobial purpose of ZnO particles. Most of these works emphasize on the interaction of reactive oxygen species (ROS) with the microbe cell, including the superoxide anion (O_2^{-}), hydrogen peroxide (H_2O_2), and hydroxyl radical (•OH). The role of ROS in antibacterial studies has been the subject of debate and no consensus has yet been reached. Oxidative stress (OS), which damages lipids, carbohydrates, proteins, and DNA, is

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believed to be the main mechanism of toxicity in nanoparticles [13–16]. Feris *et al.* [16] revealed that the bacterium and fungal lipid bilayer ruptures because of the cytotoxic behavior of ZnO nanoparticles, which results in the drainage of cytoplasmic contents. In this study, we studied the correlation of the surface properties and UV photoactivation of ZnO with the antibacterial interaction toward *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*). ZnO was subjected to UVA illumination and oxygen annealing in order to alter its surface potential. The effect of different ROS was discussed to reveal the formation and function of ROS against bacteria.

2. Experimental details

Two sets of commercial ZnO powders (purity > 99.7%) were the starting materials in this study. The powder was synthesized in a ZnO foundry (Approfit ZnO Manufacturing Co., Ltd.) using the French process at about 1300 °C combustion temperature with an added air supply of 1.5 bar pressure. Oxygen annealing was performed on the ZnO powders in an annealing tube for 1 h with the gas flow regulated at 2.4 L/min at 700 °C. The morphology and structure of the ZnO were investigated using FEI field emission scanning electron microscopy (FESEM, TLD detector, WD = 5.0–5.3 mm, 10 kV) and Philips CM12 transmission electron microscopy (TEM, 120 kV). For TEM samples, the ZnO powders were dispersed in ethanol through sonication and then captured on a carbon film-coated copper grid. The atomic percentage of ZnO powder was characterized using energy-dispersive X-ray spectroscopy (EDS, 10 kV). The structural crystalline phase of ZnO was studied using an X-ray diffractometer (XRD, PANalytical X'Pert PRO MED PW3040) with Cu-K_α radiation (λ = 1.5406 Å) for excitation, scan step 0.05°, divergence slit 0.38 mm, receiving slit 1.82 mm and tube current 30 mA. Besides, thermogravimetric analysis (TGA) was performed on ZnO pellet to determine changes in weight in relation to changes in temperature. It was conducted by TGA model Mettler Toledo with the heating rate at 10 °C/min.

To study the electrical performance and optical property of the two sets of ZnO powders, they were turned into pellet form using a technique introduced in previous works [17,18]. Oxygen annealing was subsequently performed on the surface of the ZnO pellet using the same parameters as those in powder annealing. Current–voltage (I–V) measurement (Keithley 4200-SCS) was conducted on the ZnO pellets in dark conditions and under UVA exposure (390 nm, 1.8 mW/cm^2), respectively. The I–V test was conducted using sweep voltage as stimulus with size interval of 1 V. The gap between the probes on the ZnO pellet surface was about 3 mm. The ZnO pellets were subsequently examined for their luminescence properties via photoluminescence (PL) spectroscopy (Jobin Yvon HR 800 UV) at room temperature.

E. coli (ATCC 25922) and *S. aureus* (ATCC 25923) were used for the antibacterial test in this study. Initially, inoculums of bacterium 0.5 McFarlands (1×10^6 CFU/ml) were prepared and cultured in tryptone soy broth; 100 μl of ZnO suspension (10 mM) was mixed with 100 μL of bacterial suspension and incubated at 37 °C for 24 h. The final ZnO concentration in the mixture became 5 mM. Afterwards, the bacteria cloudiness in the mixture was assessed using a spectrophotometer (Versamax microplate reader) of visible light 600 nm. The optical density measurement of the bacteria was conducted for 7 h. A negative control (bacteria and tryptone

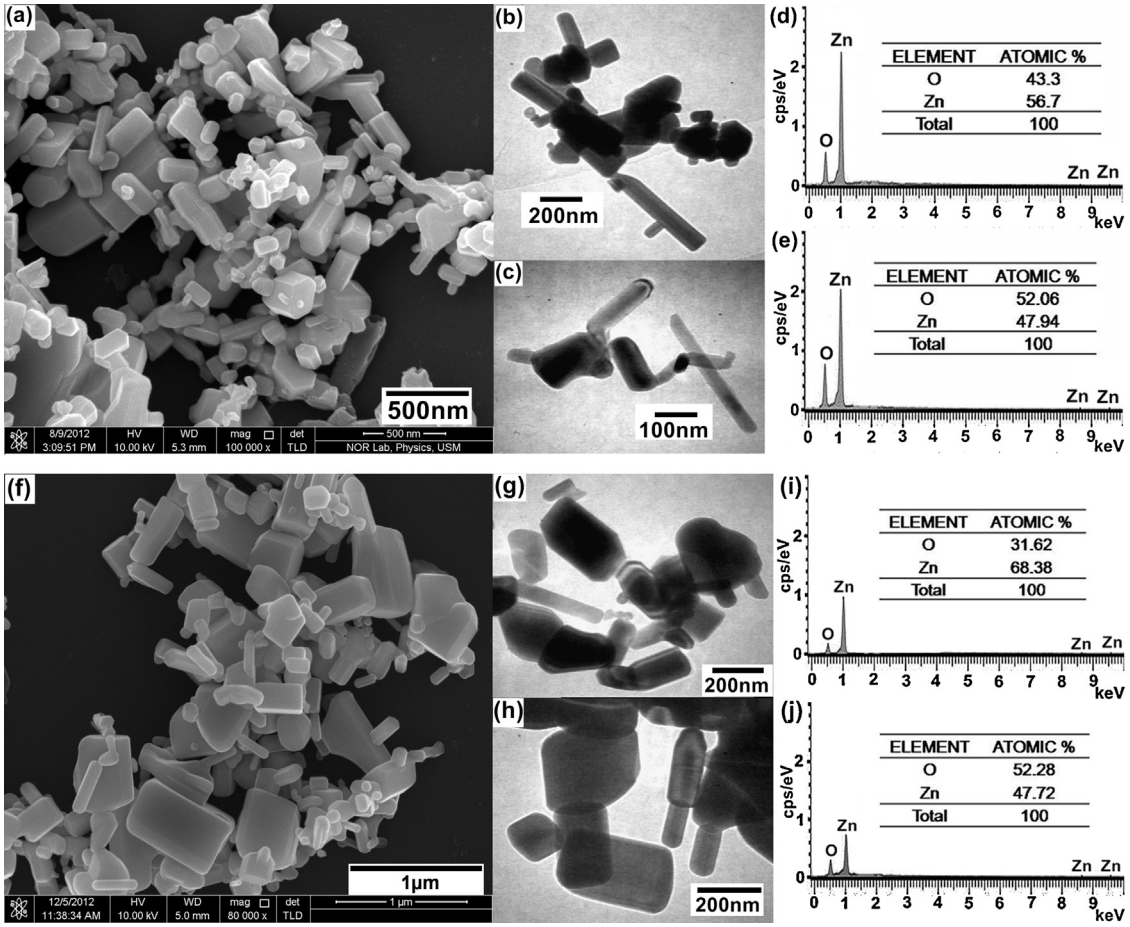

Fig. 1. Morphological study of ZnO-rod: (a) FESEM micrograph, (b and c) TEM images, (d) EDS patterns of ZnO-rod, (e) EDS patterns of annealed ZnO-rod. Morphological study of ZnO-plate: (f) FESEM micrograph, (g and h) TEM images, (i) EDS patterns of ZnO-plate, (j) EDS patterns of annealed ZnO-plate.