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Probing interaction of Gram-positive and Gram-negative bacterial cells with ZnO nanorods

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ABSTRACT

In the present work, the physiological effects of the ZnO nanorods on the Gram positive (*Staphylococcus aureus* and *Bacillus subtilis*) and Gram-negative (*Escherichia coli* and *Aerobacter aerogenes*) bacterial cells have been studied. The analysis of bacterial growth curves for various concentrations of ZnO nanorods indicates that Gram positive and Gram negative bacterial cells show inhibition at concentrations of ~64 and ~256 μg/mL respectively. The marked difference in susceptibility towards nanorods was also validated by spread plate and disk diffusion methods. In addition, the scanning electron micrographs show a clear damage to the cells via changed morphology of the cells from rod to coccoid etc. The confocal optical microscopy images of these cells also demonstrate the reduction in live cell count in the presence of ZnO nanorods. These, results clearly indicate that the antibacterial activity of ZnO nanorods is higher towards Gram positive bacterium than Gram negative bacterium which indicates that the structure of the cell wall might play a major role in the interaction with nanostructured materials and shows high sensitivity to the particle concentration.

1. Introduction

Interaction of microbial systems with surrounding environment has always fascinated researchers and it is full of surprises as microbial systems are quite intelligent, dynamic and heterogeneous systems [1]. The exposure to toxic environment of metal ions is known to induce an unpredictable stress response which can lead to the extracellular formation of metal and metal oxide nanoparticles inherently functionalized by biomolecules [2–4]. Similarly, the interaction of inorganic nanomaterials with microbes is non-trivial. Recently, our group reported a room temperature, microbial process to convert ternary oxide nanoparticles of size > 200 nm to nanoparticles of size less than 10 nm [5]. This interaction is further complicated by the presence of surface legends, type of the surface charge, its distribution apart from the size and shape of the nanomaterials. The study of the true mechanism of bacterial interaction in particular with functionalized metal oxide nanomaterials is becoming more demanding due to the increasing applications of these nanomaterials not only in plant/pathogen management but also a variety of other industrial applications [6,7]. In these consumer driven applications, the fate of these nanomaterials on the bacteria present in the water bodies such as rivers, marine environment is yet to be fully understood [8].

Among these metal oxides nanoparticles, it is especially lucrative to study the microbial interaction of ZnO nanoparticles in various shapes and sizes, due to the fact that ZnO is commonly used in various applications [9]. It has also been reported by various researchers that inorganic metal oxides such as ZnO can be used as an antibacterial agent because of its stability under high temperatures and pressure conditions when compared to organic antimicrobial agents [6]. Bulk ZnO is reported to be biocompatible, non-toxic as food additive, and is commonly used in skin products [10]. However, it also behaves as an antimicrobial agent and thus, it can be used in the food industry and agriculture as well [6]. These studies suggest that release of ZnO into the environment may not cause any harm to human beings and animals [10]. The antibacterial activities of ZnO nanoparticles in various forms have been investigated and it has been reported that ZnO nanoparticles show enhanced bactericidal effect in comparison of bulk form [11]. The exact mechanism is not clear but this might occur due to the damage of the cell membrane by its direct interaction with nanoparticles [11]. As we indicated earlier, the antibacterial activity is further complicated by the nature of surface [12]. However, for a particular concentration of nanoparticles in the medium, some of these nanoparticles might sediment and others may remain suspended in the medium. The suspended and sedimented nanoparticles might show different level of antimicrobial interaction [13]. Recently, cotton fabric deposited with ZnO nanoparticles was found to have antibacterial properties against *Escherichia coli* and *Staphylococcus aureus* cultures [14,15]. Nutrient conditions of growth media as well as the synthesis conditions of oxide nanoparticles might also play an important role in deciding the tolerance of microbes against

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nanoparticles [7,16]. In addition, there has been considerable attention towards studying the comparative effects of nanoparticles on the Gram-negative and Gram-positive bacterial cells due to the difference between their cell membrane structure [6,16–18]. The presence of lipopolysaccharide layer (LPS) layer in Gram-negative bacterial cells, may protect the damage of cell membrane from toxic molecules [16].

Considering the important role played by the aspect ratio of nanomaterials, in the present article, we have studied the effect of bare ZnO nanorods, on the Gram-positive and Gram-negative bacteria. Below, we present our experimental results on the effect of ZnO nanorods towards two Gram-positive and two Gram-negative bacterial strains.

2. Materials and method

2.1. Synthesis of ZnO nanorods

For the synthesis of ZnO nanorods, zinc acetate dihydrate ($Zn(CH_3COO)_2 \cdot 2H_2O$), and sodium hydroxide (NaOH) were dissolved in deionized water in stoichiometric ratio and stirred for 45 min at room temperature followed by the hydrothermal reaction in a stainless steel vessel with Teflon liner at 120 °C for 24 h. The resultant material was washed several times with ethanol and water to remove the ionic impurities and dried in a vacuum oven for 6 h at 60 °C to result in a white powder [19]. Material characterization and detailed methodology have been discussed in supporting information.

3. Results and discussion

3.1. Physical properties of ZnO nanorods

Fig. 1(a) shows the XRD pattern where all the peaks could be indexed to the hexagonal wurtzite ZnO structure (JCPDS no. 810590) [20,21]. The sharp and intense peaks show highly crystalline nature of these rods. Fig. 1(b) shows the bright field TEM image confirming the rod shaped particle formation with average diameter ~45 nm and length ~250 nm. From the high resolution TEM (HRTEM) image [Fig. S1], the lattice fringes with d-spacing of 0.24 nm were observed which corresponds to (110) plane of ZnO.

Fig. 1 (c, d) shows the PL and XPS spectra. PL spectra show emission around 385 nm along with a broad emission around 558 nm. The small emission peak at 385 nm is close to the band-gap of ZnO 1*s*–1*s* electron transition (3.3 eV) [22]. Whereas, the broad green emission centered at ~558 nm is usually considered to be related to various intrinsic defects [23]. This suppression may occur due to the non-stoichiometric nature between Zn and O [24]. The XPS spectra of ZnO nanorods show the binding energy of O 1*s*, Zn $2p_{3/2}$ and Zn $2p_{1/2}$ at 530.2, 1021.5 and 1044.4 eV respectively. A shift towards lower binding energy was observed for Zn $2p_{3/2}$ and Zn $2p_{1/2}$ peaks. In the hexagonal ZnO crystal, zinc cation is coordinated with four oxygen atoms tetrahedrally and shows a peak at 1022.0 eV. However, a shift towards lower binding energy may arise due to the oxygen deficient Zn atoms in tetrahedrally coordinated ZnO structure [25,26]. The peak positions of Zn $2p_{3/2}$ and Zn $2p_{1/2}$ states match closely with the peak positions reported earlier [25], which further indicates that Zn atoms are present in the +2 oxidation state. The surface area of ZnO nanorods was measured using BET method, and it was found to be ~97.29 m^2/g.

3.2. Response of Gram-positive and Gram-negative bacterial strains in the presence of ZnO nanorods

S. aureus and *B. subtilis* were chosen as model systems for Gram-positive bacteria whereas *E. coli* and *A. aerogenes* were studied for Gram-negative bacteria. To see the effect of ZnO nanorods on the

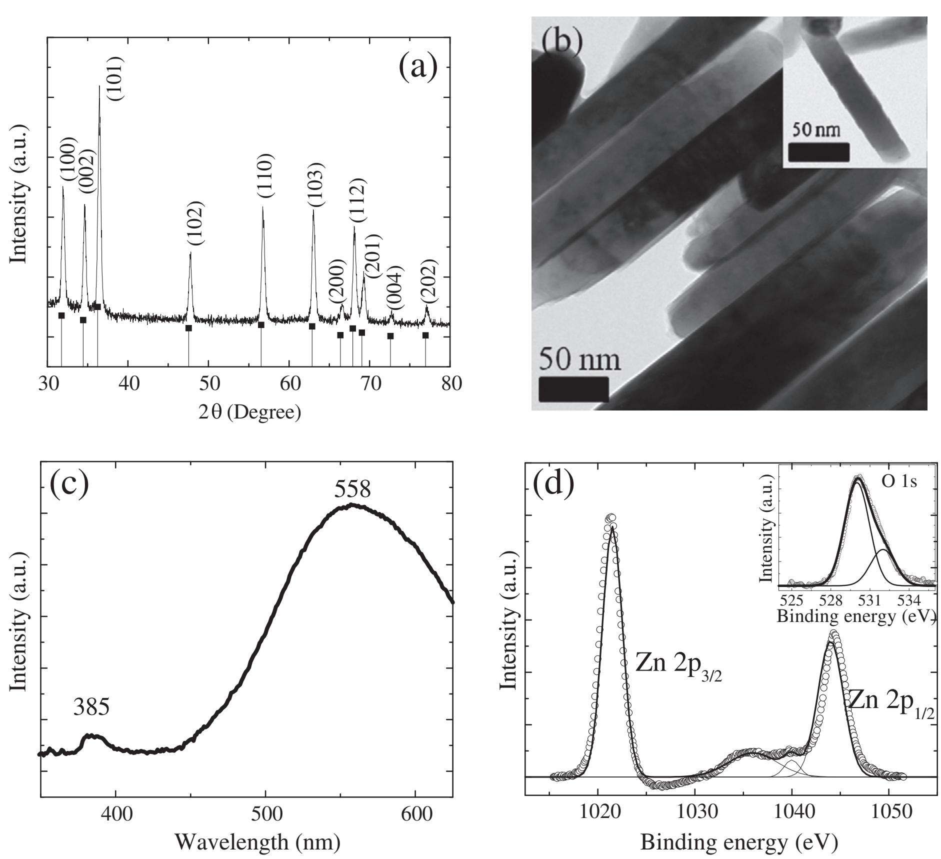

Fig. 1. (a) XRD, (b) TEM [inset shows single rod], (c), PL of the ZnO, and (d) XPS of zinc [inset shows XPS of oxygen].