Abstract

An interesting topic of investigation in the domain of bio-nanotechnology is interaction between bacteria and nanomaterials. On one hand, bacteria is responsible for the most lethal infections caused to man, whereas on the other hand, it is the foremost contributor to the biodiversity on earth and plays a vital role in regulating the environment for sustaining life on earth. Thus, interaction of nanoparticles with bacteria follows a multifaceted approach-: one in which development of antibacterial nanoparticles is targeted for the killing of pathogenic bacteria -: while in another, interaction of nanoparticles with bacteria is studied in order to limit its harmful effects on bacterial ecology. In both cases, understanding the fundamental mechanism of interaction of bacteria with nanoparticles is vital in order to achieve a specific application. This study aims at the development of antibacterial nanoparticles and also emphasizes on the mechanism of interaction and modes of entry of nanoparticles into bacteria cells (gram positive and gram negative bacteria).

Comprehending the interplay of precisely defined particles with bacteria forms a rationale for understanding the in vitro and in vivo aspects of antibacterial nanomaterials. In this study, we have investigated the antibacterial efficacy of size, shape and composition controlled ZnO nanomaterials. ZnO nanoparticles of five different sizes (9 to 75 nm) were synthesized by varying the calcination temperature from 200ºC to 600ºC and the particles were designated based on the calcination temperature (Z200-Z600). An increase of particle size with increasing calcination temperature was evident from the TEM images. Shape controlled synthesis of ZnO (spheres, petals, rods) was achieved in this work by the use of PEG400, water and toluene as the solvent. The shape dependence of antibacterial activity of ZnO is still in the embryonic stage, and researchers have mostly focused on the mechanism of formation of nanostructures rather than antibacterial application. To increase the ROS generation and aid the antibacterial efficiency, ZnO nanoparticles were doped using Ag, Fe and Cu. There is almost no work reported on the study of the detailed mechanism of antibacterial action of Cu and Fe doped ZnO NPs. These nanomaterials have mostly been investigated in semiconductor and photovoltaic applications. Although Ag doped ZnO is under study for its antibacterial activity, the detailed mechanism of action is not clearly understood.

In this work, the nanoparticle toxicity was assessed against two model bacteria species: gram positive *S.aureus* and gram negative *E.coli* at different concentration. Disc diffusion assay, minimum inhibitory concentration (MIC), and time kill assay were used for the preliminary assessment of antibacterial activity of nanoparticles at different concentrations. The amount of protein leakage from the bacteria on exposure to nanoparticles at different time intervals was quantified by Bradford assay. It was noted that lower the size of nanoparticles, better the antibacterial activity. For different shapes of nanoparticles, the antibacterial activity followed the order: ZnO nanosphere>ZnO nanorods> ZnO nanopetals. Doping of nanoparticles with Ag, Cu and Fe significantly increased its activity in the order Ag-ZnO>Fe-ZnO>Cu-ZnO for both *S.aureus* and *E.coli* while Ag-ZnO was found to be most efficient. FTIR analysis was carried out to probe the molecular damage induced on the bacterial surface on exposure to nanomaterials and this enabled us to investigate the mechanism of action of nanoparticles on bacteria.

Cellular internalization of Z200 (lowest size) was observed for *E.coli* (from TEM images) but unlike *S.aureus*, it did not affect the cell wall structure. All other sizes of nanoparticles affected both the cell wall and protein structure. ZnO spheres and ZnO petals affected the cell wall structure of *S.aureus* but ZnO nanorods altered only the protein secondary structure leaving the cell wall almost unaffected. Ag-ZnO distorted the cell wall structure leading to cell death but the protein structure of *S.aureus* was unaltered. For *E.coli*, Cu-ZnO mostly affected the protein secondary structure while Ag-ZnO and Fe-ZnO also damaged the cell wall apart from protein distortion. Most of these observations were supported with TEM images. DNA damage was confirmed by imaging the intact and nanoparticle exposed bacteria in confocal microscope after staining with DAPI. Based on all these analysis, we propose that the bacteria damage could be mainly due to cell wall disruption and protein distortion due to physical contact or internalization, ROS generation or metal ion release. The cytotoxicity of nanoparticles against MG-63 osteoblast like immortalized cell line indicated that ZnO nanomaterials of all sizes and shapes under consideration had negligible cytotoxicity. No appreciable toxicity was observed on cells exposed to Ag-ZnO and Cu-ZnO nanostructures for concentration less than 20μg/ml while Fe-ZnO was toxic to the cells at concentrations higher than 20μg/ml.

Keywords: Bacteria, Reactive oxygen species, Morphology, Doping, Cytotoxicity, Protein leakage, Cell wall.