

# Antibacterial Zinc Oxide Nanoparticles in Polymer Biomaterial Composites

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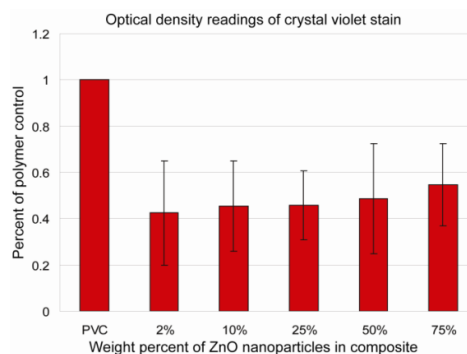
**Statement of Purpose:** Particulate zinc oxide (ZnO) is a known antibacterial agent. Studies have shown that reducing the size of ZnO particles to the nanoscale dimensions further enhances their antibacterial properties. Polymers, like all biomaterials, run the risk of harboring bacteria which may produce an antibiotic-resistant biofilm. The addition of ZnO nanoparticles, to form a polymer composite, may reduce undesirable bacteria activity. The purpose of the present *in vitro* study was to investigate the antibacterial properties of ZnO nanoparticles when incorporated into a polymer biomaterial. Specifically, *Staphylococcus aureus* were seeded at a known cell density (optical density = 0.52) onto coverslips coated with a film of polyvinyl chloride (PVC) with varying concentrations of ZnO nanoparticles (2%, 10%, 25%, 50% or 75% by weight). Samples were cultured for 24 or 72 h. Crystal violet staining indicated a reduced presence of a biofilm on ZnO nanoparticle polymer composites compared to polymer controls. Live/dead assays provided images to confirm the reduced presence of active bacteria on samples with zinc oxide nanoparticles. Development of this technology may improve biomaterial effectiveness for applications, such as endotracheal tubes and implanted biomaterials, which are prone to bacterial infection.

**Materials and Methods:** Samples cultured for 72 h, with a seeding density of  $3 \times 10^6$  bacteria at  $t = 0$  h and a TSB media change every 24 h, were evaluated for biofilm formation. At 72 h, samples were rinsed twice with phosphate buffered saline (PBS) and soaked in 0.1% crystal violet in H<sub>2</sub>O for 15 min. Samples were then rinsed and dried overnight. After 24 h, samples were soaked in ethanol for 15 min. The optical density of the resulting solution was read in a 96-well plate at 560 nm.

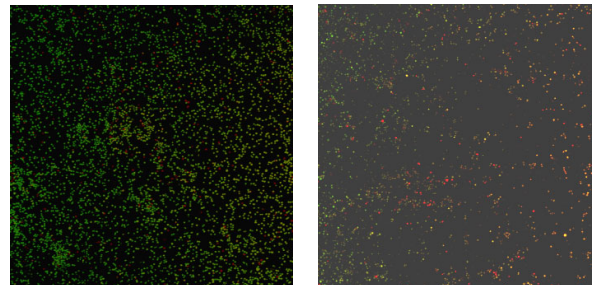
Samples incubated with bacteria suspensions for 24 h were evaluated for cell viability using a BacLight Live/Dead stain kit (Invitrogen, Carlsbad, CA). Cell culture media was aspirated from well plates containing composite samples and the samples were rinsed twice with a 0.85% NaCl buffer. A live/dead stain solution was prepared by mixing equal parts of Syto 9 and propidium iodide and then adding the combined solution to 0.85% NaCl buffer at a concentration of 3  $\mu$ l/ml. After adding 1 ml of staining solution to each well, samples were then incubated for 15 min in the dark at room temperature. Samples were imaged with a fluorescence microscope (DM5500B, Leica Microsystems, Wetzlar, Germany).

**Results:** Crystal violet staining measured the reduction of biofilm formation directly on the composite surface (Figure 1). For all concentrations of ZnO nanoparticles in composites, biofilm formation was significantly reduced compared to pure polymer controls. Perhaps due in part to the large standard error from sample to sample, biofilm

formation was not reduced significantly more on high ZnO weight percent composites compared to low ZnO weight percent composites. Live/dead staining visually confirmed a reduced live bacteria count and indicated an increased number of dead bacteria (Figure 2). For all weight percent composites, the live/dead cell images were generally indistinguishable from each other at the magnification used for imaging.



**Figure 1.** Optical density readings (as a percentage of polymer control) of crystal violet stain for composite samples at various concentrations of ZnO exposed to bacteria cultures for 72 h. All composite samples showed a significant reduction compared to pure PVC ( $p < 0.05$ ).



**Figure 2.** Fluorescence images of live/dead staining at 24 h of a polymer control (left) and a composite containing 25% ZnO nanoparticles (right). Fluorescence images (63X mag.) of composites appear to show a reduced total number of bacteria, fewer live bacteria (green), and a greater number of dead bacteria (red).

**Conclusion:** In this study, ZnO nanoparticles reduced the activity of *Staph. aureus* when added to PVC/ZnO composites. Results indicated that all weight percentages of ZnO nanoparticles in PVC significantly reduced bacteria activity compared to pure polymer. This suggests that polymer and nanoparticle composites could be used for biomaterial applications prone to excessive bacterial growth, such as orthopedic implants and endotracheal tubes, due to their ability to reduce biofilm formation.

**Acknowledgements:** The authors would like to acknowledge the Hermann Foundation for funding.