Antibacterial Properties of Sonicated Piezoelectric Zinc Oxide Nanoparticles

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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 nanoscale dimensions further enhances their antibacterial properties. ZnO is also piezoelectric and can produce a transient electrical charge when mechanically deformed. The purpose of the present *in vitro* study was to investigate the antibacterial properties of ultrasonically stimulated ZnO nanoparticles. *Staphylococcus epidermidis* were seeded at a known cell density into 96-well plates and observed for 48 h with a 10 min ultrasonication period at 24 h. Optical density data was collected every 12 h, with an additional reading taken immediately after the ultrasonication period. Bacteria activity was significantly reduced in experimental groups exposed to nanoparticles and ultrasonication compared to groups exposed to nanoparticles or ultrasonication alone. Development of this technology may lead to novel antibacterial strategies.

Introduction: Bacterial infection is one of the most common complications associated with implants. The development of materials resistant to bacteria activity and the investigation into methods for reducing bacteria is of great interest. Zinc oxide has been shown to reduce activity of bacteria [1-3]. Reducing particle size, and thus increasing the specific surface area of a dose of particles, can further enhance antibacterial activity. Yet, the mechanism of bacteriocidal activity is not fully understood. At the same time, the piezoelectric characteristics of ZnO allow for the generation of an electrical stimulus upon mechanical deformation of the material, which can be generated through ultrasonic deformation [4]. Electrical stimulation is known to have an antibacterial effect on *Staphyloccocus epidermidis* [5].

Methods: Cells were plated at 3×10^6 cells per well. ZnO nanoparticles (~60 nm diameter, Nanophase Technologies, Romeoville, IL) were added to wells to provide final concentrations of 0.1 g/ml and 0.01 g/ml for a total cell culture volume of 200 µL. Bacteria density at select time points was determined by optical density at t = 0 h, t = 12 h, and t = 24 h. A 10 min sonication period at 90 W in a 2 L ultrasonic cleaner (VWR, West Chester, PA) followed immediately after the t=24 h optical density reading. Another optical density reading was taken immediately after sonication and then again at t = 36 h and t = 48 h.

Results: The effect of the combination of antibacterial chemistry and piezoelectrically induced electrical stimulus was investigated. ZnO nanoparticles alone reduced bacteria activity (Fig 1 and Fig 2). The high concentration (0.1 g/ml) of ZnO nanoparticles reduced bacteria activity further than low concentrations (0.01 g/ml) of ZnO nanoparticles. Sonication alone also reduced bacteria activity. After 48 h, the combination of a high concentration of ZnO nanoparticles and sonication reduced bacteria activity greater than exposure to nanoparticles or sonication alone (Fig 1).

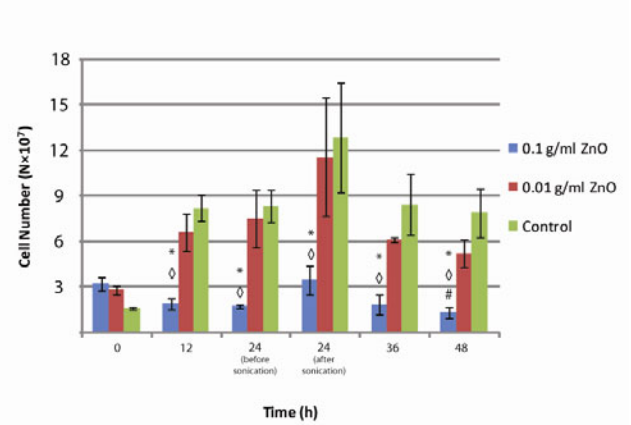

Fig 1. Bacteria activity for groups exposed to nanoparticles with ultrasonication. * = significant ($p<0.05$) compared to 0.01 g/ml at same time point. ◊ = significant ($p<0.05$) compared to control (0.0 g/ml ZnO) at same time point. # = significant ($p<0.05$) compared to 0.1 g/ml cultured under static conditions at same time point.

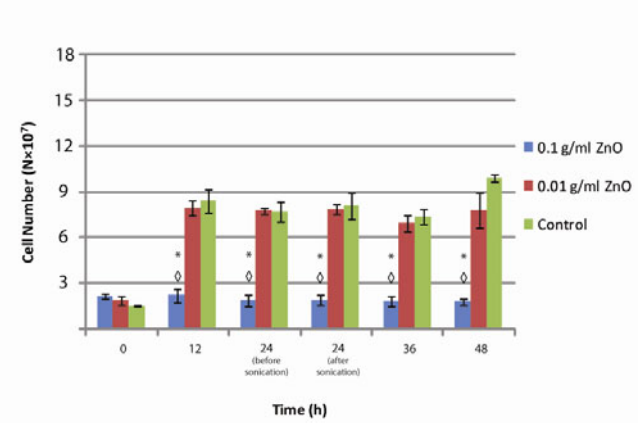

Fig 2. Bacteria activity for groups cultured in static conditions. * = significant ($p<0.05$) compared to 0.01 g/ml at same time point. ◊ = significant ($p<0.05$) compared to control (0.0 g/ml ZnO) at same time point.

Conclusion: In this study, ZnO nanoparticles coupled with ultrasonication had a greater antibacterial effect than ZnO nanoparticle or ultrasonication exposure alone and, thus, should be further studied for novel antibacterial applications.

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