Greater Fibroblast Proliferation on an Ultrasonicated ZnO/PVC Nanocomposite Material

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Statement of Purpose: There has been a significant and growing concern over nosocomial medical device infections. Previous studies have demonstrated that embedding nanoparticles alone (specifically, ZnO) in conventional polymers (such as polyvinyl chloride or PVC) can decrease *Staphylococcus aureus* growth after 24 hours by as much as 87% compared to non-nanoparticle controls, without the aid of pharmaceutical agents.[1] Other research has shown that nanomaterials may have the potential to prevent or disrupt bacterial processes that lead to infection, due to changes in surface energy which influence initial protein adsorption events important for mediating bacterial functions. However, little to no studies have been conducted to determine mammalian cell functions on such a nanocomposite material. Clearly, for certain medical device applications (like wound healing dressings, orthopedic soft tissue healing devices, etc.), maintaining healthy mammalian cell functions, while decreasing bacteria growth, is imperative. For this reason, in the presented study, ZnO nanoparticles of varying sizes (from 10nm to >200nm in diameter) and functionalization (including no functionalization to doping with aluminum oxide and functionalizing with silane coupling agent KH550) were incorporated into PVC either with or without ultrasonication. Results of this study provided the first evidence of greater fibroblast density after 18 hours of culture on the smallest ZnO nanoparticle incorporated into PVC samples with increased dispersion aided by ultrasonication.

Materials and Methods: In order to produce ZnO/PVC nanocomposites, medical grade PVC (taken from a commercial Sheridan® 6.0mm ID uncuffed endotracheal tube) was dissolved in cyclohexanone with the aid of mechanical stirring and heating to 80°C on a hot-plate. Once the PVC was completely dissolved, ZnO nanoparticles with diameters of 10-30 nm (labeled 10 nm), 35-45 nm (labeled 35 nm), 80-200 nm (labeled 80 nm), and <200 nm (labeled 200nm); 15 nm ZnO nanoparticles doped with 2wt% aluminum oxide (labeled AZO); and 20 nm ZnO nanoparticles coated with 1wt% KH550 (a silane coupling agent (labeled KH550) were added to the mixture at 15wt%. Control samples were prepared in the same manner, absent the addition of any nanoparticles. The mixtures were then stirred for 15 minutes until visibly homogenous. Then, some of the samples were placed in an ultrasonicator (BioLogics Ultrasonic Homogenizer Model 150VT) at 45-60 Watts at 20 kHz for about three minutes to ensure an even distribution of the nanoparticles. Samples (either ultrasonicated or not) were then pipetted onto glass coverslips to dry. To determine fibroblast proliferation on the samples of interest, human skin fibroblasts (American Type Culture Collection, line: Detroit 551) were seeded onto samples with 1 mL of growth media at a density of 10,000 cells/cm^2 and were cultured for 18 hours. The samples with adherent cells were transferred to a fresh well plate and washed with PBS. 1 mL of new growth media was added to each well and 150 µL of an MTT dye. The samples were incubated for four hours at 37°C and were stopped with the addition of 1mL of a stop solution. 200µL from each well were transferred to four wells of a 96 well plate and the plates were read by a spectrophotometer (Spectromax M3), from which the final cell density was calculated using a standard curve.

Results and Discussion: Results of this study provided the first evidence of greater fibroblast proliferation on PVC nanocomposites with ZnO functionalized with silane chemistry, ZnO with aluminum oxide, and samples of decreasing ZnO nanoparticle sizes (Figure 1). It is intriguing to ponder why fibroblast proliferation increased on ZnO/PVC nanocomposites with smaller nanoparticles, yet, bacterial adhesion and propagation decreased on such samples. Although requiring additional experiments for the present materials, we have observed greater wettability for nanomaterials which led to the enhanced adsorption of proteins (such as fibronectin and vitronectin) known to promote fibroblast and decrease bacterial adhesion, and suspect the same events are occurring here.[2]

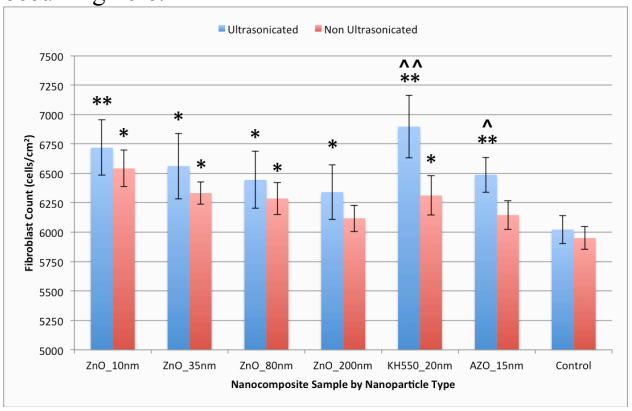

Figure 1: Greater fibroblast proliferation was observed at confidence levels of *p<0.05, **p<0.01 (compared to respective controls); ^p<0.05, ^^p<0.01 (compared to respective non ultrasonicated samples). Data represents the mean ± SD; N=3, n=3.

Conclusions: Our results have important implications. An inexpensive material that is simultaneously beneficial to human cells and detrimental to bacteria would be a wonderful material for medical devices. It is envisioned that this simple and inexpensive approach could lead to a reduction in nosocomial infections, longer medical device lifetimes, and decreased antibiotic usage and reliance. In summary, coupled with previous antibacterial studies, the present study demonstrated that highly dispersed ZnO/PVC nanocomposites should be further studied for numerous medical device applications.

References: [1] Geilich BM et al. *Int J Nanomedicine* 2013; 8: 1177. [2] Khang D et al. *Biomaterials* 2007; 28(32):4745-4768.