Neural Cell and Protein Interactions with Zinc Oxide Nanoparticle Polyurethane Composites

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Statement of Purpose: Nanomaterials offer a number of properties that are of interest to the field of neural tissue engineering. Specifically, materials that exhibit nanoscale surface dimensions have been shown to promote neuron function while simultaneously minimizing the activity of cells such as astrocytes that inhibit central nervous system regeneration [1]. Studies demonstrating enhanced neural tissue regeneration in electrical fields through the use of conductive materials have led to interest in piezoelectric materials (or those materials which generate a transient electrical potential when mechanically deformed) such as zinc oxide (ZnO) [2, 3]. It has been speculated that ZnO nanoparticles possess increased piezoelectric properties over ZnO micron particles [4]. Due to this promise in neural applications, the objective of the present *in vitro* student was, for the first time, to assess protein adsorption and subsequent astroglial cell activity on ZnO nanoparticle polymer composites. The successful production of ZnO nanoparticle composite scaffolds suitable for decreasing astroglial cell density demonstrates their potential as a nerve guidance channel material with greater efficiency than what may be available today.

Methods: Composite materials composed of ZnO nanoparticles and PU were produced with a range of weight ratios. Specifically, 50:50, 75:25, 90:10, 98:2, and 100:0 (PU:ZnO) wt.% were produced by mixing PU and ZnO nanoparticles dissolved or dispersed in chloroform or 1,2-dichloroethane, respectively. After sonication, solutions were evaporated on glass coverslips and dried overnight in a vacuum oven. Samples were characterized with scanning electron microscopy and X-ray photoelectron spectroscopy according to standard operating procedures. Total protein adsorption on each composite sample was analyzed via a BCA protein assay after a 4 h incubation with fetal bovine serum. Astroglial cells were seeded on composite surfaces at a concentration of 2500 cells/cm² and 5000 cells/cm² for proliferation and adhesion studies, respectively. After 4 h for adhesion or 24, 48, or 72 h for proliferation, cell density was determined from fluorescence microscopy images of cells stained with Calcein-AM. Numerical data were analyzed for significance using the student's *t*-test. Values are reported as the mean±SEM. The threshold for significance was set at $p<0.05$.

Results: Sample surface characterization confirmed nanosurface roughness and ZnO nanoparticle exposure at the surface of composites. Protein adsorption data showed an increased protein adsorption from FBS onto sample surfaces with a nanoparticle weight ratio of 50:50 (ZnO:PU) wt.% compared to pure polymer. Additional results of this study showed a reduced ability of astrocytes to adhere and proliferate on ZnO nanoparticle and PU composites with higher nanoparticle concentrations. Cell images captured after a 4 h adhesion assay illustrated the reduced astrocyte cell density as ZnO nanoparticle concentration increased. At 4 h, cell adhesion was significantly reduced on samples with weight ratios of 50:50 (PU:ZnO) wt.%, 75:25 (PU:ZnO) wt. %, and 90:10 (PU:ZnO) wt.% compared to the pure polymer. Increased concentrations of nanoparticles in ZnO/PU composites also reduced cell proliferation. At 24, 48, and 72 h, cell density was reduced on samples with weight ratios of 50:50 (PU:ZnO) wt.%, 75:25 (PU:ZnO) wt.%, and 90:10 (PU:ZnO) wt.% compared to the pure polymer at the same time point. At all time points, cell density of samples with weight ratios of 50:50 (PU:ZnO) wt.% and 75:25 (PU:ZnO) wt.%, was further reduced compared to composites with weight ratios of 90:10 (PU:ZnO) wt.%. At 72 h, cell density on samples with weight ratios of 50:50 (PU:ZnO) wt.% was reduced compared to cell density on samples of the same composition at the 24 h time point.

Conclusions: ZnO nanoparticle and polymer composites incorporated elements from a number of current strategies for neural tissue repair such as nanotopographies and piezoelectric properties to create a new approach to promote nervous tissue regeneration. The next generation of nerve guidance channels can benefit from the incorporation of nanomaterials that provide a superior environment for protein adsorption and cell activity. Sample characterization revealed that ZnO/PU composites can be fabricated to create substrates with increased surface energy and nanoroughness at the sample surface. Importantly, this study showed for the first time that these samples enhanced total protein adsorption and reduced the adhesion and proliferation of astroglial cells. The piezoelectric activity of these samples must be investigated to evaluate the electrical stimulus that could be created by mechanically deforming the scaffold. These particular nanorough piezoelectric samples may combine a number of known regeneration-stimulating cues, making them superior to non-piezoelectric nanomaterials, conductive materials, or conventional piezoelectric materials alone. Future studies with these samples will evaluate neural cell activity and the piezoelectric response to mechanical deformation of the composites.

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References:

[1] Webster TJ. Nanotechnology. 2004;15:48-54.
[2] Aebischer P. Brain Research. 1987;436:165-8.
[3] Schmidt CE. Proc Natl Acad Sci USA. 1997;94:8948-53.
[4] Xiang HJ. Appl Phys Lett. 2006;89:223111-3.