

PIEZOELECTRIC NANOCOMPOSITES FOR IMPROVED NEUROLOGICAL APPLICATIONS

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Introduction

A common treatment for critical defects in the peripheral nervous system, which may also be applicable to the central nervous system, is the implantation of a nerve guidance channel (NGC). Currently, commercially available NGCs are fabricated from inert, biodegradable polymers. However, using a biomaterial with stimulatory cues to enhance nerve tissue regeneration could accelerate or permit healing that would not otherwise occur.

Along this line, recently, nanomaterials have been shown to enhance neural cell function while minimizing astrocyte activity. Reducing astrocyte activity has been a significant impediment to central nervous system tissue engineering [1]. Considering that piezoelectric materials have also been shown to promote neural cell function, the objective of this study was to combine such approaches and develop piezoelectric nanomaterials for neural tissue engineering applications. Such materials could produce a desirable electrical response, a known stimulus for axonal outgrowth and neural tissue regeneration, when mechanically deformed (through such external means as ultrasound).

There are a number of fundamental reasons why nanomaterials are well suited for tissue engineering applications. First, nanomaterials are biomimetic. In other words, materials with nanoscale surface features more accurately mimic the nanoscale proteins which compose biological tissue. Second, engineering a material to exhibit nano-roughness provides a greater functional surface area for the interaction with surrounding cells and tissue. This increased surface area coupled with greater surface energy allows for increased select protein adsorption, a critical intermediate step in the adhesion and function of cells [2]. Third, due to an increase in grain boundaries, nanomaterials have greater surface energy than their conventional counterparts. Not only can this promote greater protein adsorption, it also may provide an ideal environment for protein conformation, thus, bioactivity [3].

Experimental

Sample preparation

Composite materials composed of ZnO nanoparticles

(~60 nm diameter, Nanophase Technologies, Romeoville, IL) and polyurethane (PU; Tecoflex medical grade SG80A, 90-140×10³ MW, Thermedics, Wilmington, MA) 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 appropriate amounts of PU and ZnO nanoparticle stock solutions. The PU stock solution was prepared by dissolving 0.1 g PU in 13 ml chloroform (Sigma-Aldrich, St. Louis, MO) during sonication (Sonicator 3000, Misonix, Farmingdale, NY). The ZnO nanoparticle stock solution was prepared by dispersing 0.1 g ZnO nanoparticles in 13 ml 1, 2-dichloroethane (Sigma-Aldrich, St. Louis, MO) during sonication. A range of solutions were mixed to provide the appropriate weight ratios and were sonicated for 2 h at a power of 3 W to ensure nanoparticle dispersion. Solutions were then immediately placed onto 18 mm glass coverslips (Fisher Scientific, Pittsburgh, PA) and dried in a vacuum oven. To ensure the evaporation of solvents, samples were left in an oven overnight at 50° C in a vacuum of -10" Hg. All samples were rinsed with ethanol and exposed to UV light prior to characterization or cell culture experiments.

Cell culture studies

Rat astrocytes (CRL-2005, American Type Culture Collection, Manassas, VA) were cultured in Dulbecco's Modified Eagle Medium (DMEM; ATCC, Manassas, VA) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT) and 1% penicillin/streptomycin (PS; Hyclone, Logan, UT) at 37° C in a humidified environment of 5% CO₂ /95% air. Cells were passaged 4-5 times before seeding on the substrates.

Astrocyte adhesion

Astrocytes were detached from the culture flasks with trypsin and collected in a conical tube for centrifugation. Cell pellets were resuspended in 1 ml DMEM and counted using a hemocytometer. The number of cells needed to seed samples at a density of 5000 cells/cm² was resuspended in 400 µl of cell media per sample. Cell-seeded samples in a 12-well plate were carefully transferred to an incubator and left for 4 h. After 4 h, the media was aspirated from the wells and the

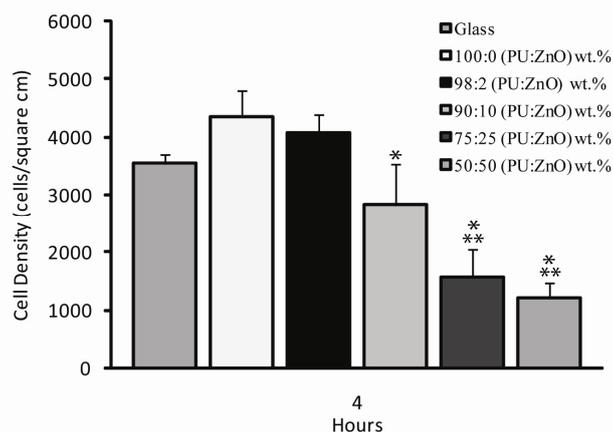


Figure 1. Results of astrocyte 4 h adhesion assay. 5000 cells/cm² were seeded on each sample and incubated for 4 h. Cell density was determined from images taken at five random locations on each sample. Values are mean±SEM; n=3; *p<0.05 (compared to 100:0 [PU:ZnO] wt. %), **p<0.05 (compared to 90:10 [PU:ZnO] wt. %).

samples were rinsed with phosphate buffered saline (PBS) to remove all non-adherent cells. Calcein-AM (BD Biosciences, San Jose, CA) was added to each sample at a concentration of 0.5 μM in PBS. After 30 min, samples were rinsed again with PBS before imaging on a fluorescence microscope. Five images were taken at random locations on each sample. Images were analyzed with Image J software (NIH, Bethesda, MA) to determine cell density.

Astrocyte proliferation

Astrocytes were collected and counted following the same procedures described for the adhesion assays. Specifically, 2500 cell/cm² were resuspended in 400 μl of cell media per sample and placed on each substrate. Seeded substrates in a 12-well plate were carefully transferred to an incubator and left for 24, 48, or 72 h. Media was changed daily for samples incubated for 48 and 72 h. After incubation, cells were stained with Calcein-AM (BD Biosciences) following the same protocol as reported for the adhesion assays.

Results and Discussion

For the first time, results of this study showed a reduced ability of astrocytes to adhere and proliferate on ZnO nanoparticle PU composites with higher nanoparticle concentrations (Figures 1 and 2). Specifically, after 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.

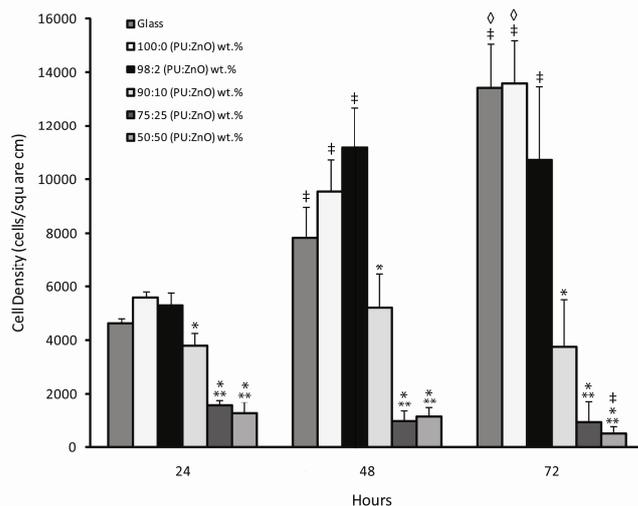


Figure 2. Results of 1, 2, and 3 day astrocyte proliferation assay. Cells were seeded at t=0 with 2500 cells/cm². Values are mean±SEM; n=3; *p<0.05 (compared to 100:0 [PU:ZnO] wt.% at the same time point), **p<0.05 (compared to 90:10 [PU:ZnO] wt.% at the same time point), ‡p<0.05 (compared to same sample composition at 24 h time point), †p<0.05 (compared to same sample composition at 48 h time point).

Conclusions

Importantly, this study showed for the first time that ZnO nanoparticles and polymer composites reduced the adhesion and proliferation of astroglial cells. 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 NGCs can benefit from the incorporation of nanomaterials that provide a superior environment for protein adsorption and cell activity.

Acknowledgments

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