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Final/Annual Report

New Jersey Commission on Spinal Cord Research – One-Time Start-Up Grant
PI: Treena Arinzeh, PhD, New Jersey Institute of Technology, Department of Biomedical Engineering, 323 Martin Luther King Blvd., Newark, NJ 07102, 973-596-5269
Grant Title: Neuronal Differentiation of Stem Cells using Nanomeshes
Grant Number: 06-3057-SCR-E-0
Grant Period Covered by the Report: 12/2006 through 12/2008
Date of Submission: 3/1/2009

This final/annual report for the One-Time Start-Up Grant at the New Jersey Institute of Technology summarizes the final/annual status of the start-up efforts including any remaining equipment purchases, integration of the laboratory into the larger research community, future research plans and all publications.

"Start-up" Efforts

As detailed in the previous annual report summarizing efforts from 12/2005 to 12/2006, all construction and most of the major equipment purchases were completed during this year. The remaining equipment purchases were completed from 1/2007 to 12/2007. Items such as patch-clamp equipment and a real-time PCR machine were purchased during this time. The laboratory has been fully functional as of 1/2008.

Laboratory's Integration into the Larger Research Community

The laboratory continues to develop collaborations with colleagues in the Biomedical Engineering (BME) Department at the New Jersey Institute of Technology (NJIT). Bryan Pfister, PhD, assistant professor in BME at NJIT, has laboratories specializing in axonal regeneration using mechanical stretch and performs electrophysiology measurements of primary neurons. His laboratory is adjacent to the PI's laboratories on the third floor of the CHEN building. They also occupy a shared cell culture and biochemical/molecular biology facility. A formal collaboration between Drs. Pfister and Arinzeh (PI) has been established. Dr. Pfister is a member of the PhD thesis committee of Dr. Arinzeh's student, Yee-Shuan Lee where he provides advisement and training on dorsal root ganglion culture and electrophysiology measurements. They are also collaborators on two grant proposals, as detailed in the next section on research plans. The PI also continues to establish a collaboration with Dr. Mesut Sahin, an assistant professor in BME at NJIT. His interests lie in neural prosthetics.

Dr. Arinzeh has also established a formal collaboration with Robert Heary, MD, neurosurgeon at the University of Medicine and Dentistry of New Jersey (UMDNJ). Dr. Heary's research is in developing clinically relevant in vivo models for studying spinal cord injury. The PI is currently investigating the biomaterials developed in her laboratory in these animal models. Other collaborators at UMDNJ consist of Dr. Pranela Rameshwar, Department of Medicine, and Dr. Nicholas Ingolglia, Department of Neuroscience.

In the larger research community, the PI is beginning to establish relationships with the program directors at the National Institutes of Health, specifically the National Institute of Neurological Disorders and Stroke (NINDS) and other researchers in the spinal cord community. Drs. Arinzeh and Pfister are co-chairing a workshop at the upcoming national meeting of the Biomedical Engineering Society (BMES) in the fall of 2009 focusing on spinal cord injury. Invited speakers will include clinicians and scientists specializing in this area. They are working with the NIH to determine the group of invited speakers and panelists.

Research Plans – Current and Future

Our research in spinal cord injury has focused on a tissue engineering approach to axonal regeneration. We are currently investigating two possible cell sources, neural

stem/progenitor cells or mesenchymal stem cells in combination with an aligned fibrous substrate to facilitate axonal elongation uniaxially along the cord. We have developed a novel, nanofibrous substrate that consists of a piezoelectric material, which provides electrical stimulation in response to minute mechanical deformations. Electrospun fibrous scaffolds of piezoelectric polyvinylidene fluoride-trifluoroethylene (PVDF-TrFE) were shown to enhance neurite extension. An aligned fibrous piezoelectric scaffold was investigated in order to provide physical cues (via contact guidance) and local electrical activity to promote neural differentation and neurite extension. Rat pheochromocytoma (PC12) and dorsal root ganglion explants (DRGs) were cultured on random or aligned fibrous PVDF-TrFE scaffolds. Neurites of PC12 cells were observed to extend along the direction of the aligned fibers. Neurite extension of DRGs was also observed on both random and aligned electrospun PVDF-TrFE scaffolds suggesting the potential use of these scaffolds in spinal cord repair (Figure 1).

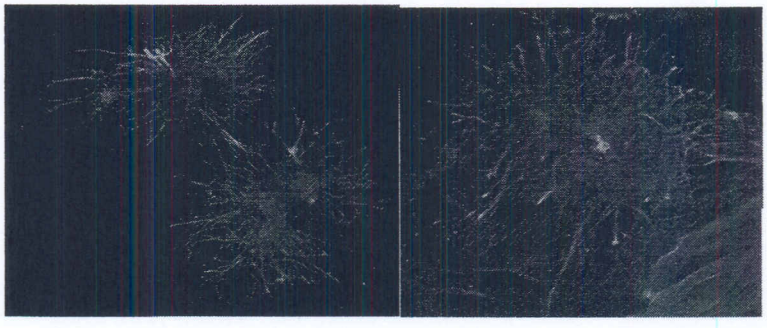

Figure 1: DRGs stained with Vybrant® CFDA SE cell tracker at day 3 on random (left) and aligned (right) PVDF-TrFE scaffolds (4x).

This work has served the basis for the current funding support for the PI's PhD student, Yee-Shuan Lee, under the NJSCR Fellowship. This provides stipend, travel and research support. Additional funding is being sought via the National Institutes of Health – R21 mechanism through the National Institute of Neurological Disorders and Stroke. The grant proposal (PI: Arinzeh, Co-PI: Heary) was submitted in June 2008. It was unscored. The reviewers' comments were addressable and is now being resubmitted in March 2009. Additional support is also being investigated through the NJSCR, PI is Bryan Pfister and Co-PIs are Arinzeh and Heary. This study investigates other compositions as suitable substrates for DRGs as an alternative cell source for spinal cord repair. The use of DRGs as a cell source was a concept developed by Dr. Pfister during his postdoctoral work. Arinzeh will contribute to the development of the construct including fabrication of the nanofiber scaffold and evaluate its suitability for implantation into the spinal cord.

List of publications

1. A provisional patent application was submitted in March 2008. U.S. Provisional Patent Application, 61/039,214: Electrospun electroactive polymer for regenerative medicine applications, Principal Inventor: T. Arinzeh, Co-inventors: N. Weber and

M. Jaffe. 3/2008. This provisional patent application has not been published. It can be made available upon request.

2. A manuscript is in preparation. Weber, N. Lee, Y-S., Jaffe, M., Arinzeh, T. Characterization and In Vitro Cytocompatibility of Piezoelectric Electrospun Scaffolds. Biomaterials (in preparation). 2009.
3. Attached is a conference proceeding that was submitted to the Northeast Bioengineering Conference to be held at the Massachusetts Institute of Technology in April 2009.. It was accepted for oral presentation and will be presented and published as a peer-reviewed conference proceeding.

An Electroactive Conduit for Spinal Cord Injury Repair

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Abstract— **Electrospun fibrous scaffolds of piezoelectric polyvinylidene fluoride-trifluoroethylene (PVDF-TrFE) were evaluated to enhance neurite extension for spinal cord injury (SCI) repair. An aligned fibrous piezoelectric scaffold was investigated in order to provide physical cues (via contact guidance) and local electrical activity to promote neuronal differentiation and neurite extension. Rat pheochromocytoma (PC12) and dorsal root ganglion explants (DRGs) were cultured on random or aligned fibrous PVDF-TrFE scaffolds. Neurites of PC12 cells were observed to extend and proliferated along the direction of the aligned fibers. Neurite extension of DRGs was observed on both random and aligned electrospun PVDF-TrFE scaffolds suggesting the potential use of these scaffolds in spinal cord repair.**

I. Introduction

Unidirectional aligned structure of the axons is disrupted [1] after SCI and restoring the original structure is necessary for functional recovery. A tissue-engineered bridging device is a promising method to guide axonal outgrowth. However, cell favors the implantation site more [1], thus appropriate topographic cues within the bridging device may be crucial in successfully guiding axons to extend out of the bridge and to enhance host-implant interaction.

Local electric fields have been measured during neural development or after nerve injury in various vertebrate systems [2]. Electric fields generated via electrodes have been shown to influence growth and orientation of neurons *in vitro* [3]. Piezoelectric polymers can induce transient change of surface charge without requiring additional energy sources or electrodes and have been shown to yield a higher level of neuronal differentiation and neurite outgrowth of mouse neuroblastoma cells [4]. The steric hindrance of the TrFe polymer in PVDF-TrFE forces the copolymer into an all-trans configuration and is considered piezoelectric [5].

Electrospinning is able to produce continuous fibers with high surface area to volume ratio. Random, aligned, and patterned nano-fibrous mesh and three-dimensional structures can be fabricated by altering collection methods. The topographic features of nano-aligned-fibrous scaffolds create contact guidance, which may further facilitate axonal extension. This study proposed a novel scaffold to be used to promote neuronal differentiation and neurite extension by incorporating the piezoelectricity property into the design.

II. Methods

A. Electrospinning

Polymer solution was prepared by mixing PVDF-TrFE powder in methyl-ethyl-ketone (MEK). Random and aligned scaffolds were collected on a plate and a rotating drum, respectively. All samples were vacuum dried for two days prior to using in culture. Scanning electron microscopy (SEM) images were taken to evaluate fiber diameter and alignment.

B. Thermal and Piezoelectric Properties Evaluation

Thermal and piezoelectric properties were evaluated using differential scanning calorimetry (DSC) and thermal stimulated depolarization current (TSDC) on both unprocessed powder and electrospun PVDF-TrFE. A heat-cool-heat cycle from -60°C to 200°C with heating and cooling ramp of 7°C/min was used on DSC to evaluate thermally active transition such as crystallization, melting, and phase transition. Electrospun PVDF-TrFE or the powder was sandwiched between the two Teflon films and heated from -60 to 140 °C for TSDC experiments [6].

C. Electric Response

Electrodes (10mm x 10mm) were attached to the ends of the scaffold using silver conductive epoxy. The scaffold was mechanically deformed at the rate of 10mm/min using Instron. The electrodes were connected to a custom-made amplifier circuit and the signals were recorded using Matlab.

D. PC12 and DRG culture

The scaffolds were pre-conditioned in cell culture media for one day prior to seeding. PC12 cells were seeded at 0.18e6 cells/cm^2 on to the scaffolds and collagen coated plates (control) and were cultured in either control media or induction media containing neural growth factor (NGF, 250ng/mL) a day after. PC12 cells were stained with Phalloidin (cytoskeletal stain, Invitrogen) and proliferation was evaluated by MTT cell proliferation assay (Invitrogen) at day 10 and 14.

DRGs isolated from E15 embryonic rat pup were plated on the scaffolds and stained with Vybrant® CFDA SE cell tracker (Invitrogen, Carlsbad, CA) at day 3.

III. Results

Average fiber diameter of electrospun PVDF-TrFE was 0.75μm±0.08. Directional fiber orientation was observed in the aligned scaffolds (Fig. 1). Crystallization (data not shown) and melting point of electrospun PVDF-TrFE (148.1°C) were shifted to a higher temperature as compared to the unprocessed powder (146.1 °C) (Fig. 2). Current movement occurred just before melting in the unprocessed powder (Fig. 2a). Current movement started at 65°C and continued before the melting in temperature of electrospun PVDF-TrFE (Fig. 2b). The 35°C peak is the spontaneous relaxation of the Teflon sheets. When the mechanical deformation started, an increase in electric response occurred

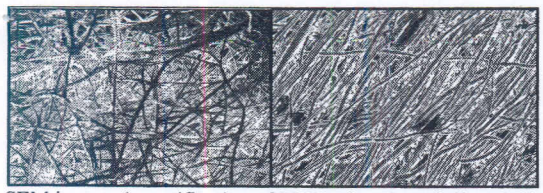

Fig. 1. SEM images (magnification of 3500) of random (left) and aligned (right) electrospun PVDF-TrFE scaffolds.

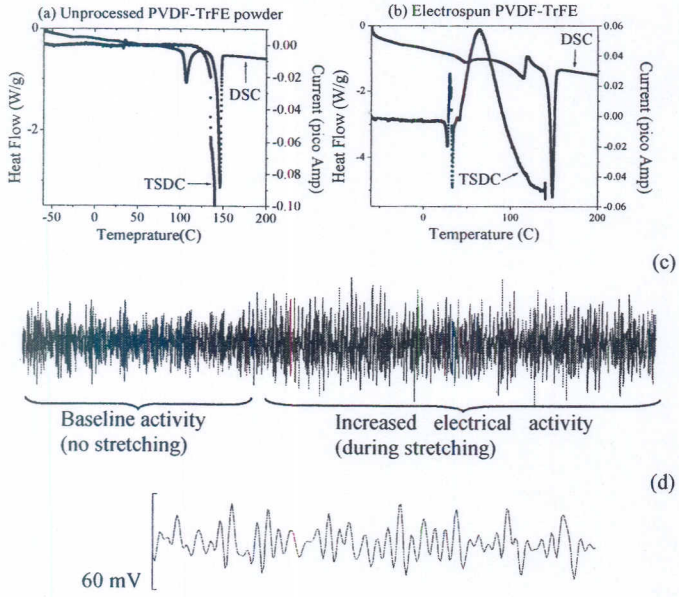

Fig. 2. DSC (heat flow) and TSDC (current) results for unprocessed powder (a) and electrospun PVDF-TrFE (b). Electric response of PVDF-TrFE scaffold (c) when initializing deformation and (d) 25ms duration while deforming.

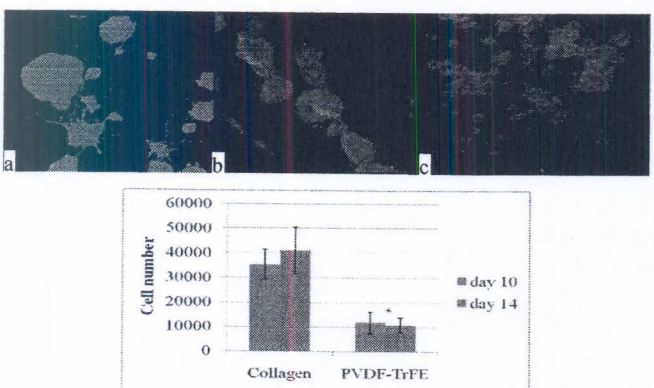

Fig 3. PC12 cells stained with Phalloidin on random (a) or aligned (b) PVDF-TrFE and collagen (c) in induction media (20x). (g) MTT viability assay for PC12 cells on random PVDF-TrFE and collagen in induction media. Cell proliferation on collagen was higher than PVDF-TrFE($p>0.05$).

(Fig. 2c). Amplitude of response varied between -30mV to 30mV (Fig. 2d).

PC12 neurite extension was observed on both random and aligned scaffolds (Fig. 3b,d). Neurite extension both occurred along the direction of alignment (Fig. 3d) and on the collagen coated plates (Fig. 3f). PC12 proliferation was higher on collagen in the induction group on both days 10 and 14 (Fig.

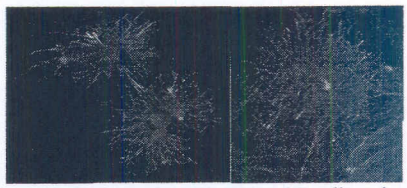

Fig. 4. DRGs stained with Vybrant® CFDA SE cell tracker at day 3 on random (left) and aligned (right) PVDF-TrFE scaffolds (4x).

3g). Proliferation in the control media was similar for both materials at both time points (data not shown). Neurite extension of DRGs was observed on both random and aligned PVDF-TrFE scaffolds (Fig. 4).

IV. Discussion

DSC results of unprocessed and electrospun PVDF-TrFE indicated no significant alternation occurred during the electrospinning process, as indicated by similar melting temperatures. Shifting of melting and crystallization temperature suggested extended chain crystallization during the electrospinning process. The piezoelectric phenomenon is characterized by the presence of dipole crystal structure. Dipole movement would occur upon heating and could be observed as the current movement on TSDC. DSC and TSDC results of electrospun PVDF-TrFE (Fig. 2b) suggested a phase transform allowing dipole movement. Crystal structure movement upon melting contributed to the current movement in the unprocessed PVDF-TrFE powder (Fig. 2a). The electrical activity detected while deforming the electrospun PVDF-TrFE (Fig. 2c,d) corresponded to the observation of its piezoelectric properties.

PC12 cell proliferated and extended neurites along the direction of fiber alignment indicated the influence of contact guidance. No difference in cell proliferation was observed in control media on both days suggesting it may due to the differentiation process. Neurite extension of both PC12 cells and DRGs was observed on both random and aligned electrospun PVDF-TrFE scaffold suggesting its potential use as a scaffold for spinal cord repair.

Acknowledgement

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References

[1] N. Zhang, H. Yan, X. Wen, "Tissue-engineering approaches for axonal guidance," *Brain Res Brain Res Rev*, vol 49, pp. 48-64, 2005.

[2] R.B. Borgens, "Electric Fields in Vertebrate Repair" in *Natural and Applied Voltage in Vertebrate Regeneration and Healing*, Wiley-Liss, 1989.

[3] N.B. Patel, M.M. Poo, "Perturbation of the direction of neurite growth by pulsed and focal electric fields," *Journal of Neurosci*, vol 4, pp 2939-47, 1984.

[4] R. Valentini, "Electrically charged polymeric substrates enhance nerve-fiber outgrowth in vitro," *Biomaterials*, vol 13, pp. 183-90, 1992.

[5] A.J. Lovinger, "Ferroelectric Polymers," *Science*, vol 220, pp 1115-21, 1983.

[6] L.H. Catalani, G. Collins, M. Jaffe, "Evidence for molecular orientation and residual charge in the electrospinning of poly (butylenes terephthalate) nanofibers," *Macromolecules*, vol 40, pp. 1693-7, 2007.