

## The Evaluation of Neural Differentiation of Human Neural Stem/Progenitor Cells on Piezoelectric Scaffolds

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**Statement of Purpose:** Electric fields can influence neural growth and orientation *in vitro* (Patel NB. J Neurosci. 1984; 4:2939-47) and have been applied for the treatment of spinal cord injuries in recent clinical trials (Duffell LD. Mus Nerve. 2008; 38: 1304-11). Poly vinylidene fluoride-trifluoropolyethylene (PVDF-TrFE), a piezoelectric polymer, can induce a transient surface charge in the absence of additional energy sources or electrodes. The uniqueness of PVDF-TrFE occurs as a result of the steric hindrance of TrFE which forces the PVDF into an all-trans crystal configuration, thus, rendering the polymer piezoelectric (Lovinger AJ. Science. 1983; 220:1115-21). This study investigates the neural differentiation of human neural stem/progenitor cells (hNPCs) on fibrous, PVDF-TrFE to determine its potential use as a scaffold in nerve repair. The piezoelectric properties of PVDF-TrFE were enhanced by annealing to increase crystal organization. Comparisons were made with laminin coated tissue culture plastic (control).

**Methods: Scaffold Fabrication:** Polymer solutions for electrospinning were prepared with PVDF-TrFE in methyl-ethyl-ketone (MEK) Random and aligned electrospun scaffolds were collected on a plate and a rotating drum, respectively. Annealed samples were kept at 135°C for 96 hours and quenched with ice water.

**Characterization:** Scanning electron microscopy (SEM) images were taken to evaluate the fiber diameter and orientation. Differential scanning calorimetry (DSC) was used to evaluate thermally active transition such as melting temperature. X-ray diffraction (XRD) was performed to evaluate crystal structure of as-spun and annealed PVDF-TrFE. Thermally-stimulated current (TSC) was used to confirm piezoelectricity by measuring the current indicating the dipole movement in response to an increase in temperature.

**In vitro study:** hNPCs (Lonza), which are cryopreserved neurospheres obtained from fetal brain tissue (20 weeks), were seeded at 45,000 cells/cm<sup>2</sup> and cultured in differentiation media (Lonza) with 25 ng/mL brain-derived nerve growth factor (BDNF) or standard growth media for 9 days. Comparisons were made with laminin coated plates. The cells were fixed and stained with anti-Nestin (NPCs), glial fibrillary acidic protein (GFAP) (astrocytes), and neuron-specific beta-III tubulin (neuron), followed by DAPI as counter stain. 4 images were taken for each sample (n=6 per group) and positive stain was manually counted to obtain percentage of differentiation. One-way analysis of variance (ANOVA) and Tukey-Kramer test were used to determine the statistical significance between the groups (p<0.05).

**Results and Discussion:** The average fiber diameter of micron- (L) and sub-micron- (S) PVDF-TrFE were 3.32±0.2 μm and 0.75±0.08 μm, respectively. The melting point of as-spun of PVDF-TrFE (L) and (S)

increased from 147.9°C and 147.8°C to 152.4°C and 154.5°C after annealing, respectively. The increase in melting temperature suggested an increase in crystallinity due to annealing. XRD results indicated an increase in the intensity of the piezoelectric beta phase at 20.4° and the loss of the non-piezoelectric alpha phase around 18.5° on the annealed in comparison to the as-spun samples. The annealing process induced crystal organization hence, enhancing the piezoelectric properties.

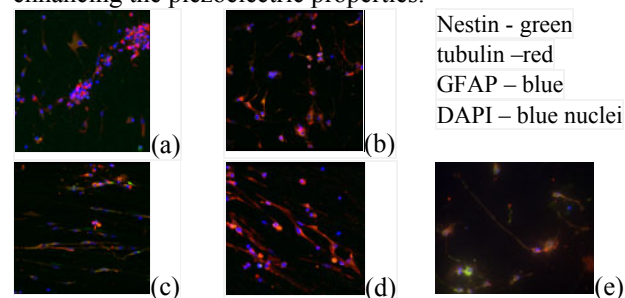


Figure 1. Confocal microscopy images of hNPCs on PVDF-TrFE-L a) random, b) random-annealed, c) aligned, d) aligned-annealed (all at 40x obj), and e) laminin (20x obj.).

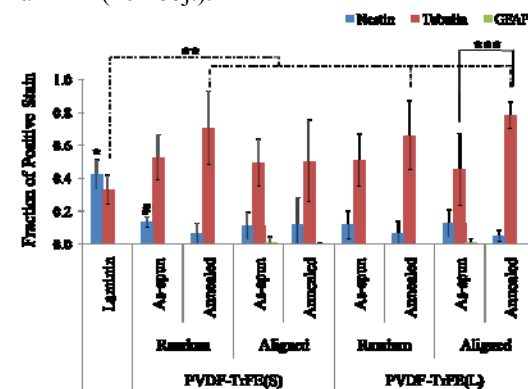


Figure 2. Fraction of positive nestin, tubulin, and GFAP on various scaffolds. \*Nestin positive cells were the highest on laminin. \*\*Tubulin positive cells were the lowest on laminin in comparison to annealed, random and aligned PVDF-TrFE(L) and annealed random PVDF-TrFE(S). \*\*\*Tubulin positive cells on annealed, aligned PVDF-TrFE(L) was significantly greater than as-spun aligned PVDF-TrFE(L). Very little GFAP positive cells could be detected in all of the samples.

**Conclusions:** This study demonstrated the potential use of this scaffold for nervous tissue repair. The scaffolds enhanced neural differentiation, as indicated by a lower level of nestin positive cells on scaffolds in comparison to laminin surfaces. Neuronal differentiation may be enhanced on annealed scaffolds, which display higher piezoelectricity, as indicated by the higher fraction of cells expressing beta-III tubulin.

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