Applications of electrospun nanofibers in neural tissue engineering

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Abstract: Autografting and synthetic nerve grafts are options clinically to repair peripheral nerve defects which can occur due to trauma or a surgery. However, autografting suffers from limited donor site availability and synthetic nerve grafts have low efficiencies in repairing nerve defects. Recently electrospun nanofibers have emerged as an alternative approach to remedy these deficiencies. Electrospun nanofibers offer enhanced neurite outgrowth, ability to produce nanofiber based nerve guidance conduits and provides mechanical and biochemical cues to differentiate stem cells that have all shown promising results in neural tissue engineering.

Key words: Electrospun Nanofibers; Neuronal Growth; Tissue Engineering.

Introduction

Clinical research approaches to neural tissue diseases include radiosurgery (1,2) and gene transfection (3-6) to replace the damaged cell population. Autografting and synthetic nerve grafts are other options clinically to repair peripheral nerve defects which can occur due to trauma or a surgery. However, autografting suffers from limited donor site availability and synthetic nerve grafts have low efficiencies in repairing nerve defects (7,8). Recently, there has been great interest in electrospun polymer nanofibers to remedy these deficiencies (9-11). Electrospun nanofibers provide the fiber diameters to be at nanoscale and its fibrous morphology mimicking extracellular matrix (ECM) is a great advantage for neural tissue engineering applications. Electrospun nanofibers have high porosity and large surface area that provides efficient nutrient delivery and cellular communication. Nanofibers can be functionalized with native ECM proteins to enhance cellular attachment, differentiation, proliferation and migration. Electrospun nanofibers can be aligned in unidirectional to promote and enhance neuronal outgrowth. Being biodegradable and having good biocompatibility, synthetic polymers such as poly(3-caprolactone) (PCL), poly(L-lactic acid) (PLLA), poly(L-lactide-co-glycolide) (PLGA) and naturally occurring polymers such as gelatin have all been electrospun and used in neural tissue engineering applications successfully.

Electrospinning process

An electrospinning setup includes a metal capillary, a high voltage source and a grounded collector (Figure 1). Capillary is attached to a syringe which contains the polymer solution. A syringe pump is used to push solution inside the syringe. When applied high voltage exceeds surface tension of the pendant drop, it turns into a conical shape known as Taylor cone. Polymer solution then undergoes elongation and a bending instability which then freely spins onto the grounded collector. By adjusting parameters of this process such as capillary tip to collector distance, electric field strength and solution viscosity, one can then optimize the electrospun nanofibers morphology and diameter. It is also possible to obtain aligned nanofibers. In this case, the collector is a high speed rotating wheel that allows deposition of nanofibers unidirectionally (12-15).

Differentiation of stem cells

Differentiation of stem cells is possible with chemical
and mechanical cues (16-20). Nanoscale morphologies and ability to be coated with ECM proteins allow electrospun nanofibers to provide a suitable microenvironment for differentiation of stem cells. Neural stem cells (NSC) in particular C17.2 is a multipotent self-renewing cell line that can be used as neuron precursors. Yang et al. electrospun PLLA nano (250-300 nm) and micro (1.25-1.5 µm) fibers and compared their differentiation efficiency by seeding C17.2 cells. It was found that cells seeded on nanofibers had 80% differentiation whereas on microfibers it had 40% (21). The cellular elongation was parallel to the aligned nanofiber direction in the aligned nanofiber samples. In another study, PLGA nanofibers were used. C17.2 cells attached and differentiation was demonstrated by Scanning Electron Microscopy (SEM) micrographs (22). When neural stem cells were cultured in a petri dish with 1 mM retinoic acid and 1% fetal bovine serum (FBS), the neural stem cell spreaded and differentiated into oligodendrocytes. However, when the cells were on 749 nm and 1452 nm fibers, they differentiated into neuronal lineage (23). Characteristic morphology of oligodendrocytes is that they obtain a stretched and radiant network of dendrites. The cellular response to 283 nm nanofibers was that a large population of cells obtained the oligodendrocyte phenotype. This was due to the fact that fiber diameter was smallest compared to other experimental groups. However, when the fiber diameter was enlarged to 749 nm, the Tuj1 (mature neuronal marker) immunostained population was highest and cellular elongation was along a preferred fiber axis demonstrating that the nanofiber diameter was able to modulate cellular differentiation. Xia et al. have (24) demonstrated that embryonic stem cells (ES) could differentiate into neural lineage when seeded onto aligned PCL nanofibers. Tuj1 immunostaining showed mature neurons that had long axonal extensions and GFAP immunostaining showed that ES cells differentiated into astrocytes with a characteristic flat morphology. O4 staining which is an antibody for oligodendrocyte specific glycolipids showed multipolar morphology on the nanofibers. PC-12 is a cell line from rat adrenal pheochromocytoma and is used as a model for studying neuronal differentiation (25). Alvarez-Perez at al.(25) studied PC-12 cell differentiation on Gelatin/PCL (Gel/PCL) electrospun nanofibers blend. The blended nanofibers offer the advantage of utilizing mechanical properties of synthetic polymers and better cellular affinity of natural polymers at the same time (26). Alvarez-Perez et al. reported that immunostaining against neuronal growth associated protein (GAP-43) in Gel/PCL blended nanofibers showed higher differentiation than PCL nanofibers alone demonstrating gelatin cues supported neuronal differentiation. Another study reported chitosan-PCL blended nanofibers (27). Chitosan, a natural polymer, is one major component of the ECM and favors cell adhesion and proliferation. The interaction was physical and possibly via the intermolecular hydrogen bonding between the carbonyl group of PCL and hydroxyl or ammonium ions of chitosan. After two weeks in cell culture, nanofibers did not swell and retained its structural integrity. Cooper et al. (27) have demonstrated that PC-12 cells seeded on aligned chitosan-PCL blend of nanofibers have significantly higher β-tubulin (Tuj1) expression than the cells seeded on chitosan-PCL film. Incorporation of ECM proteins into nanofibers has also been studied. Synthetic polymers by themselves can be hydrophobic and changing this surface property towards hydrophilicity requires surface modifications (28,29). RGD peptides that have cell recognition domains enhance cell-material interaction. Oxygen plasma treatment is another approach to change surface chemistry. Oxygen radicals induces OH groups on the nanofiber surfaces that become hydrophilic (41,42).

Koh et al.(30) have compared coupling of laminin onto PLLA nanofibers using covalent binding, physical adsorption and blending electrospinning techniques. Laminin activates signaling pathways via the interaction surface integrin receptors such as Schwan cell integrin receptors αβ1, αβ1, αβ4 (31).They found that when laminin is blended with PLLA (250:1, weight of PLLA:weight of Laminin) during electrospinning, the PC-12 cells had higher differentiation compared to other techniques. Laminin incorporation was verified by confocal fluorescence microscopy, X-ray photoelectron spectroscopy (XPS) and MicroBCA protein assay. Confocal images showed that fluorescein isothiocynate (FITC) conjugated laminin distribution was homogenous and XPS results showed N1s peak in the spectrum. Other than the surface, bulk of the laminin inclusion was verified by MicroBCA assay. Enhancing PC-12 cell differentiation via addition of Laminin also showed that ECM proteins will not be denatured by the electrospinning process. Malkoc et al. (32) also reported that coating collagen on the Gel/PCL nanofibers enhances differentiation of PC-12 cells compared to nanofibers that were not collagen coated. PC-12 cells that were in contact with collagen coated nanofibers had a higher probability of differentiation compared to controls which were more prone to cluster formation and thus preventing a contact with nanofibers.

**Neurite outgrowth**

Electrospun nanofibers offer enhanced neurite outgrowth when it is blended with natural polymers, is aligned or coated with ECM proteins (Table 1). Xie at al. (43) have studied embryonic chick dorsal root ganglion (DRG) cell behavior on PCL nanofibers and laminin coated PCL nanofibers for both random and aligned morphologies. Neurite length increased from 857 µm for laminin coated random fibers to 1085 µm for aligned fibers that had no coating. When the aligned nanofibers were coated with laminin, the neurite length was even more enhanced to 1542 µm demonstrating the directionality and biochemical cues effect on neurite outgrowth. It was shown that when two metal collectors were separated by an air gap, the electrospun nanofibers were freely deposited on the metal collectors whereas in between the electrodes authors obtained aligned nanofibers. DRG cells seeded on the border and neurite extensions falling on the random nanofiber region were random whereas on aligned nanofiber region were unidirectional demonstrating the contact guidance effect of aligned nanofibers (9, 33, 34). The average neurite length of the aligned nanofibers in (21) was 100 µm compared to control and micron sized fibers which were in the range of 75-80 µm. This suggests that when the cells are aligned in the nanofiber direction, the probability of neurite outgrowth becomes higher compared to control groups. Gertz et al. (35) have electrospun...
random and aligned nanofibers (Figure 2) and compared their performance to glass and PLLA film controls. Motor neurons seeded on PLLA nanofibers were shown to have accelerated neuritogenesis compared to controls. 14 hrs after plating, motor neurons were able to extend neurites whereas controls had no extensions. In the aligned nanofibers, motor neurons’ neurite outgrowth was aligned in the direction of nanofibers (Figure 3). After 24 hrs, neurite outgrowth on aligned nanofibers were higher compared to glass controls as well (35.5 µm vs. 26.3 µm).

Schnell et al. (36) have shown with NF200 immuno- staining that isolated sensory neurons from DRG explants had significantly better axonal guidance on the collagen/PLA nanofibers than PCL alone.

Electrospun nanofibers based nerve conduits

While nerve guidance conduits (NGC) made of synthetic materials are encouraging, they suffer from the material failure, swelling and cytotoxicity. Therefore they do not have the structural stability and pliability. They can cause inflammation and occlusion in the implanted site (37). Instead of these, recently nanofiber based nerve guidance conduits have been giving promising results. Nanofiber based conduits offer the advantage of adjusting electrospun nanofiber diameter, porosity and blended structure which result in different morphologies and porosities that are required for nutrient delivery and altered chemical composition that meets certain conditions in microenvironments such as mechanical durability and biocompatibility. Bhattarai et al. (38) have found that electrospun nano-

### Table 1. Neurite outgrowth in blended, aligned or coated electrospun nanofibers compared to controls.

<table>
<thead>
<tr>
<th>Material</th>
<th>Coating</th>
<th>Random NF</th>
<th>Aligned NF</th>
<th>Film</th>
<th>Neurite Length (µm)</th>
<th>Cell Type</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCL (43)</td>
<td>Laminin</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>857</td>
<td>DRG</td>
</tr>
<tr>
<td>PCL (43)</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>1057</td>
<td>DRG</td>
</tr>
<tr>
<td>PCL (43)</td>
<td>Laminin</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>1542</td>
<td>DRG</td>
</tr>
<tr>
<td>PLLA (21)</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>-</td>
<td>85</td>
<td>NSC</td>
</tr>
<tr>
<td>PLLA (21)</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>100</td>
<td>NSC</td>
</tr>
<tr>
<td>PLLA (35)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>26.3</td>
<td>Motor Neurons</td>
</tr>
<tr>
<td>PLLA (35)</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>35.5</td>
<td>Motor Neurons</td>
</tr>
<tr>
<td>PCL (36)</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>400</td>
<td>DRG</td>
</tr>
<tr>
<td>Collagen/PCL(36)</td>
<td>-</td>
<td>-</td>
<td>X</td>
<td>-</td>
<td>600</td>
<td>DRG</td>
</tr>
</tbody>
</table>

fibers from a blended solution of 40:60 (chitosan:PCL) contains the optimum fibrous and mechanical properties. Satisfactory phase miscibility of chitosan-PCL blend was confirmed with Transmission Electron Microscopy (TEM) studies that blended structure was not partitioned. Degradation tests were done in lysozyme rich Phosphate Buffered Saline (PBS) at 37°C for one month. Material stability was confirmed with SEM/TEM and FTIR analysis. Cytotoxicity studies were done with Schwann cells and PC-12 cells. Schwann cells spread and obtained large polar morphology on nanofibers compared to chitosan-PCL films. PC-12 cells favored the surface and after immunostaining against Nerve Growth Factor (NGF), confocal fluorescence images showed significantly higher neurite extensions on chitosan-PCL nanofibers compared to film controls. Nanofiber based NGCs were produced by electrospinning chitosan-PCL solution onto a stainless steel rod collector. Variations in diameter of the rod, produced thicknesses of 0.2 -1.0 mm. The modulus for the chitosan-PCL nanofiber was found to be (110 ± 10) MPa. Schwann cells and PC-12 cells attached and spread on inner and outer surface of the conduits. Authors also showed that the nanofibers based conduit was implanted into a critical size defect of a rat sciatic nerve and after one month, regeneration of the nerve was observed. Panseri et al. (39) electrospun blend of PLGA/PCL micro/nano fibers to repair a 10 mm sciatic nerve defect. On a rotating wire, micron sized fibers were spun first where followed by spinning nanofibers on top. Nanofibers provided a high surface area for cell attachment and efficient nutrient delivery whereas first layer provided mecha-
nical strength. After four months of surgery, there was no tube breakage or inflammation in the implanted site. There was regenerated tissue lining inside the conduit and bridging the 10 mm nerve gap. Collagen and myelination were observed in the regenerated tissue. Confocal fluorescence images of β-tubulin staining showed myelinated fascicles. 70% of the treated animals showed compound Motor Action Potential (cAMP) after stimulation above the sciatic nerve. Wang et al. (40) have reported electrospun blended silk fibroin (SF) and poly(L-lactic-co-e-caprolactone) (P(LLA-CL)) nanofibers used for repairing a 10 mm nerve defect. Silk is a natural protein that enhances cell affinity when blended into synthetic counterpart. Blended solution was electrospun onto a rotating drum with 4000 RPM to obtain aligned nanofibers. Aligned nanofibers were then reeled onto a bar and sealed with nylon sutures to obtain conduit. The alignment of the nanofibers was parallel to the axis of the bar. The addition of silk fibroin caused a decrease in nanofiber diameter due to presumably the higher conductivity of silk fibroin. SF is an amphiphilic macromolecule electrolyte, composed of hydrophobic and hydrophilic blocks with charged amino acids. Increased ionic concentration led to increased conductivity. At 8 weeks after implantation, electrophysiological recording showed that nerve conduction velocity of SF/(P(LLA-CL)) was 29.25±3.27 ms\(^{-1}\) compared to (P(LLA-CL)) which was 21.23± 2.50 ms\(^{-1}\). The number of myelinated axons was significantly higher in silk blended nanofibers (6638 ± 166) than in control (4973 ± 118).

Conclusions

Electrospun nanofibers have potential and can serve as an alternative approach to repair peripheral nerve defects that are caused by a trauma or a surgery. They enhance neurite outgrowth, have the ability to produce nanofiber based nerve guidance conduits and provides mechanical and biochemical cues to differentiate stem cells. Nanofibers can be modified with ECM proteins to enhance its effects. Nanofiber diameter and morphologies can be controlled by electrospinning parameters and can therefore respond to behaviors of different cellular types in microenvironment.

References

91–100.