Characterization of Oligodendrocyte Progenitor Cell Differentiation on Co-electrospun Nanofibers of PCL with Gelatin

Bikesh Shrestha[1]*, Muhammet Ceylan[2], Alex Moon[1]
Faculty: Li Yao[1] and Ramazan Asmatulu[2]

1 Department of Biological Sciences, Wichita State University,
2 Department of Mechanical Engineering, Wichita State University.

Abstract. Axonal re-myelination in injured central nervous system (CNS) has been a scientific challenge. Focal delivery of Oligodendrocyte Progenitor Cells (OPCs) provides promising approach. We used a novel bioengineering technique to create co-electrospun nanofibers of Gelatin and Poly-ε-caprolactone (PCL) to be used as a scaffold for neural tissue regeneration. Growth and differentiation of Oligodendrocyte progenitor cells (OPCs) (from neonatal rat brain) on the scaffold were studied. Our study revealed that Gelatin/PCL nanofibers provide a hospitable substrate for OPC growth and differentiation. Our long term goal is to develop a scaffold that bridges the injured spinal cord (SC) and aid its functional recovery

1. Introduction

Although shielded by a protective barrier formed by the vertebral column, the spinal cord is susceptible to many disease related and injury associated damage. Any mechanical injury to the vertebral column that causes it to pinch, bruise or tear can potentially render the part of the spinal column down from the injury site useless. Despite the severity of Spinal Cord Injury (SCI), no effective treatment has been formulated yet. Therapy consisting of stabilization and application of high doses of methylprednisolone is clinically practiced for post SCI rehabilitation. However, some studies have shown that methylprednisolone should not represent a standard treatment as its impact is rather weak and may represent random events and recommends future trials before application [1]. Novel techniques that capitalize on the regenerative capacity of the central nervous system hold the key to SCI treatment. Central Nervous System (CNS) and Peripheral Nervous System (PNS) differ greatly in regeneration after an injury. In PNS, the nerve tissues are more likely to regenerate and regain functionality. The CNS offers bigger challenges when it comes to nerve regeneration across the injured site. Glial scar formation hinders axonal regeneration to their synaptic target. Oligodendrocytes (OLs) undergo both necrosis and apoptosis shortly after spinal cord injury. Loss of OLs causes demyelination and thereby impairs axon function and survival. Endogenous oligogenic response is insufficient against OL loss and demyelination post-SCI. Novel techniques involve the use of a biomaterial scaffold that not just bridge the gap between two injured sites to act as a guidance medium for axonal regeneration but also as a vehicle to deliver cells and biomolecules to favorably modify the microenvironment at the injured site to allow remyelination and functional recovery.

In our attempt to identify a suitable biomaterial scaffold to direct axonal projection into the lesion of the neural tissue and to deliver Oligodendrocyte Progenitor Cells (OPCs), we used a bioengineering technique to create co-electrospun nanofibers of Gelatin and Poly-ε-caprolactone (PCL). The nanofibers were then characterized while we investigated OPC growth and differentiation on their surface.

2. Experiment, Results, Discussion, and Significance

Postnatal day P1-2 rats were sacrificed and cerebral cortices were isolated from the brain. The isolated cortices tissues were triturated twice sequentially through needles with a 1 ml syringe. The tissue suspension was passed through a 70 mm nylon cell strainer placed on a 50 ml conical tube and the flow-through was collected. The isolated cells were cultured for about 10 days. The OPCs were then isolated from the mixed cell culture layer by mechanically shaking the cell culture flasks. The collected OPCs were cultured for additional 3-4 days and grown on electrospun fibers. The cultured OPCs were induced to form differentiated oligodendrocytes.

Nanofibers were co-electrospun using a varying concentration of gelatin and PCL (mixing ratio: 0, 20,

50, 80 and 100 wt % of gelatin to PCL) in a mixture of acetic acid and acetonitrile (50:50 (v/v)). Fibers were collected on a stationary collector placed 20 cm from the infusion syringe at an infusion speed of 0.5 ml/hr at 25kV.

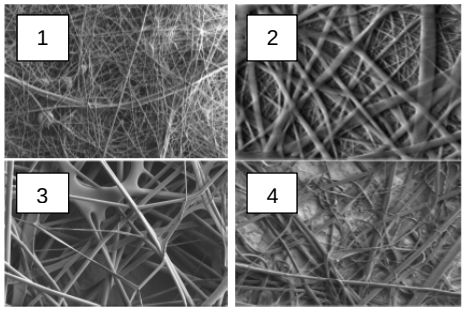

Figure 1: SEM image of co-polymer nanofiber: 1) PCL only 2) 20% Gelatin 3) 50% Gelatin 4) Gelatin only

SEM images of the co-polymer nanofibers were taken (Figure 1) and used to measure mean fiber diameter (Table 1) using ImageJ, an image analysis software. 50 measurements were taken from each SEM image out of 5-8 SEM images of each co-polymer fiber type. Contact angles of the fiber surface were measured to estimate hydrophilicity. We found the increase of gelatin in the co-polymer increased fiber diameter and decreased fiber contact angle.

Table 1:
Diameter and contact angle measurements of co-polymer nanofibers.

Co-polymer	Diameter (nm)	Contact Angle
PCL only	**200**	**112**
20% Gelatin	**600**	**100**
50% Gelatin	**1250**	**43**
Gelatin Only	**1100**	**-**

Cells were cultured on co-polymer nanofiber using OPC differentiation medium. The OPC differentiation media included the following components: DMEM (48 mL), SATO (500 µl), Glutamine (500 µl), NAC (50 µl), Na Pyruvate (500 µl), Pen/Strep (500 µl), Trace Elements B (50 µl), d-Biotin (40uM) (12 µl), Insulin (50 µl), T3 (50 µl), CNTF (50 µl).

The OPCs phenotype was studied by OPCs specific markers. These markers included anti-A2B5 monoclonal antibody, anti-PDGFα monoclonal antibody and anti-O4 antibody. Fluorescent images were taken to observe growth and differentiation. (Figure 2). We showed that OPCs grew and differentiated on gelatin and PCL co-electrospun fibers.

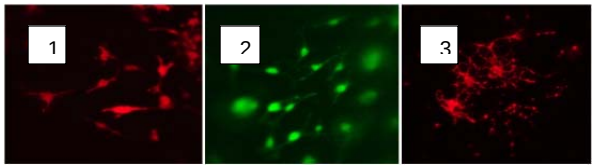

Figure 2: Fluorescent image of OPCs on 50% Gelatin nanofibers using 1) A2B5, 2) PDFGα and 3) O4 monoclonal antibody.

Discussion

Electrospinning enables us to fabricate nanofibers for various biomedical applications such as drug delivery or tissue engineering. The fiber parameters can be controlled and functionalized with biomolecules to elicit certain responses. They can also be aligned to construct micro-structured units. Gelatin was chosen due to its biocompatibility and it is derived from extra cellular matrix. PCL is a synthetic polymer popular for its biocompatibility. A co-polymer of PCL and gelatin generates a biocompatible nanofiber scaffold with enhanced mechanical strength. SEM image of nanofiber consistent with the gelatin only compared to the co-polymer combination shows better fiber formation with more distinct physical structures. Increase in gelatin percentage with PCL increased fiber diameters and decreased in contact angles. A decrease in contact angle indicates the surface to be hydrophilic. Hydrophilic properties are essential for cell attachment to the surface. Nanofiber characterization shows that PCL is vital for the integrity and mechanical strength of the fiber. By balancing the amount of PCL and gelatin, we can deliver an optimum co-polymer combination that would generate a perfect biomaterial scaffold for nerve cells. Fluorescent images of cells cultured on top of the fibers reveal distinct cell growth and differentiation.

Conclusion

Electrospun gelatin and PCL co-polymer nanofibers provides a hospitable substrate for OPC cell growth and differentiation. Further studies are being carried on to generate aligned PCL/gelatin nanofibers and construct neural conduits. The next step in our research is to investigate axon myelination with OPCs on these nanofibers with the ultimate goal being OPC delivery using electrospun nanofibers for an in vivo spinal cord regeneration and functional recovery.

Acknowledgement

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Reference:

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