Differentiation of Mesenchymal Stem Cells on Polymeric Ligament Fascicle Substitute

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Statement of Purpose: There occur over 200,000 anterior cruciate ligament (ACL) ruptures in the United States every year [1]. The ACL is one of the intra-articular ligaments in the knee that stabilizes the joint during movement. Ligament tissue is avascular and intrinsically does not heal well after injury; if left untreated, the injury could lead to the development of osteoarthritis in the joint. Partial tears can often be surgically sutured but complete ruptures require a replacement; this is usually achieved through the use of an autograft or allograft. Autografts have limitations associated with donor site morbidity and are limited in supply. Using allograft tissue is associated with disease transfer because in order to keep the tissue viable, it cannot be sterilized. There is increase interest in using biodegradable scaffolds that have similar mechanical properties of native tissue. This study aims to investigate polycaprolactone (PCL) and poly-L-lactide (PLA) materials for the development of a biomimicing polymeric ligament replacement. This scaffold will be designed to increase cell adhesion and allow for cell infiltration as the scaffold degrades. Electrospun nanofibers are used as an outer layer on extruder fibers to form a composite ligament fascicle substitute. Results of tensile testing have shown that there is no significant difference between core fibers with and without the electrospun nanofiber layer. The aim of this study is to investigate the effects of the electrospun nanofiber polymer layer on mesenchymal stem cell (MSC) differentiation.

Methods: 80,000 MW PCL was extruded at 115°C to create fibers with 265±15μm diameters. Samples with the electrospun nanofiber layer were created by electrospinning for 20 minutes, at 20 keV, with a 12wt% polycaprolactone polymer solution in 3:1 chloroform :methanol at 12μl/minute. There was a 20 cm working distance between the dispensed polymer solution and the 1000 rpm rotating fiber. The same parameters for electrospinning were used for 80,000 MW PLA but used a 12.5 wt% polymer solution in a 29:3 chloroform :dimethlyformamide solution. Tensile testing was performed on a Mach-1 (Biomomentum, Laval, QC, Canada). Scanning electron micrographs were taken with a Supra 55 (Zeiss, Germany). MSC growth will be monitored over 28 days in culture by using an AlamarBlue proliferation assay, SEM and staining will be performed to observe cell morphology and matrix formation while RT-PCR will be used to quantify differentiation. A variety of markers will be used to investigate the effects that the presence of electrospun nanofibers have on MSC differentiation.

Results: Tensile testing revealed that both PCL and PLA core and composite fibers had no significant difference in elastic modulus or yield strength (Table 1). SEM shows that cells grown on electrospun nanofibers have a different morphology than those grown on the core fiber (Figure 1).

Table 1: Tensile testing results of PCL and PLA core fibers, and their composite fibers.

	Elastic Modulus (MPa)	Yield Strength (MPa)
Polycaprolactone (n=5)		
Core Fiber	202.51 ±42.13	12.91 ± 2.96
Composite Fiber	202.86 ±31.63	16.04 ± 0.28
Poly-L-lactide (n=6)		
Core Fiber	1571.93 ± 319	45.69 ± 9.09
Composite Fiber	1466.47 ± 290.8	36.40 ± 6.75

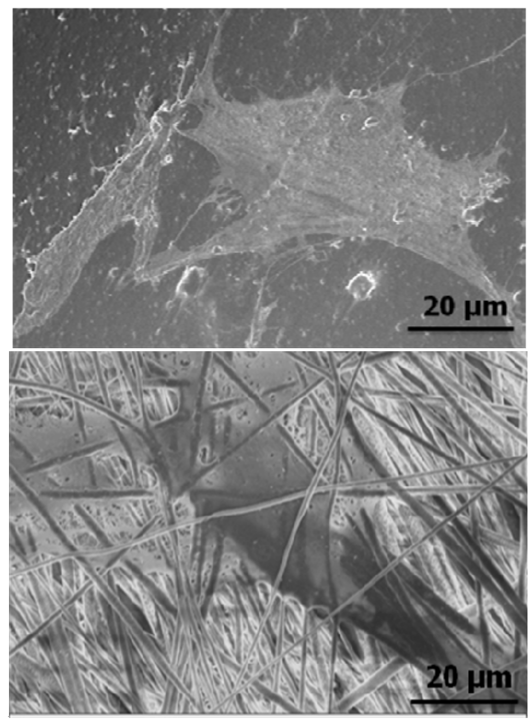

Figure 1: Scanning electron micrographs of MSCs grown on (top) core fibers and (bottom) composite fibers with electrospun nanofiber coating.

Conclusions: Tensile testing demonstrated that the mechanical strength of the fibers comes from the core fiber and is not affected by the presence of electrospun nanofibers. It was observed that there was more cell adhesion on the polymer fascicle substitutes with the presence of nanofibers. This basis will allow for the development of a scaffold that promotes cell adhesion and mechanical function similar to native ligament tissue. RT-PCR data will show the effects of the surface morphology on the differentiation of MSCs.

References: [1] Iobst, C.A. and C.L. Stanitski, *Acute knee injuries.* Clinics in sports medicine, 2000. **19**(4).