Human Foreskin Fibroblast Collagen Deposited Electrospun Polycaprolactone Scaffolds for Ligament Reconstruction

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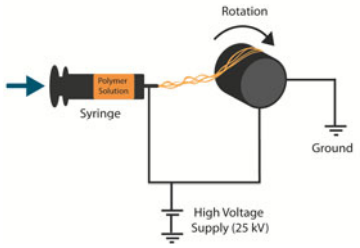
Statement of Purpose:
Rupture of the anterior cruciate ligament (ACL) can lead to knee instability and deterioration of the knee. Non-degradable synthetic ligaments were proposed in order to avoid the complications and disadvantages of biologic grafts. Human foreskin fibroblasts (HFFs) on electrospun polycaprolactone (PCL) polymers were co-stimulated with growth factors (basic fibroblast growth factor and transforming growth factor [bFGF and TGF-beta]) and uniaxial cyclic strain in a bioreactor to induce aligned collagen deposition. Thus, we aimed to construct a ligamentous scaffold by chemically and mechanically facilitating the HFF to deposit organized ECM onto the degradable synthetic fibers along the direction of the stress axis.

Methods: Polymers of varying Monocryl™ to PCL ratios will be dissolved in 1,1,1,3,3,3-hexafluoro-2-propanol (HFP), to create a 10% w/w solution. A separate solution of solution of growth factor(s) in chloroform was electrospun intermittently into the PCL/Monocryl layers. Electrospinning was carried out as previously described[1]. Briefly, the PCL/Monocryl solutions were delivered by a syringe pump (KDS 100, KD Scientific) at a constant flow rate (3 ml/h). The blunt-ended 18-gauge needle was clamped to the positive electrode of a high voltage supply (25 kV; Glassman High Voltage Inc., NJ, USA), and the negative electrode was connected to a stainless steel rotating mandrel at 2,500 rpm with an air distance gap of 15 cm. The mechanical properties of the electrospun PCL/Monocryl scaffolds were analyzed by a uniaxial testing machine with a custom symmetric stainless steel bar system to uniaxially pull electrospun scaffold rings at 10mm/min (Instron 5567). From the stress-strain curves, Young's modulus, tensile strength, and elongation at break were obtained. The morphology and the structure of the electrospun fibers (pore structure, fiber diameter, fiber angles) of the electrospun nanofibers were determined by scanning electron microscopy. For SEM analysis, the electrospun fibers were analyzed with a variable pressure SEM (Nova 230, FEI) at 50 Pa with an acceleration voltage of 10kV. The electrospun scaffolds were plasma treated and seeded with HFFs at 10^6 cells/ml, and then placed in a bioreactor with a removable center (16.5 x 5 x 5 mm) and subjected to uniaxial cyclic mechanical 0.6% strain at 0.125 Hz frequency. At 4 and 30 days, cell adhesion proliferation was analyzed via DAPI-positive nuclei staining, and microscopic analysis. Collagen content (incorporated ^{3}H-proline radiolabel) was also measured at those time points using a scintillation counter. For material degradation analysis, scaffolds were incubated in PBS at 37°C for up to 50 days and the glass transition temperature were observed through the differential scanning calorimeter and the degradation products were analyzed with gel permeation chromatography.



Results: Electrospun PCL/Monocryl scaffolds exhibited high mechanical strength with a Young's modulus comparable to the native human ACL (110MPa) in the case of electrospun Monocryl, as shown in Table 1[2].

Table I. Various electrospun polymers and mechanical properties

Scaffold	Max Load (N)	Young's Modulus (Mpa)	Ultimate Tensile Strength (Mpa)
PCL	11 ± 1	20 ± 3	3 ± 0
5:5 (M:PCL)	14 ± 3	28 ± 3	5 ± 1
Monocryl (M)	32 ± 5	61 ± 4	20 ± 5

At day 4, aligned PCL fibers of the electrospun scaffold were shown to promote HFF proliferation and alignment along the direction of the PCL fibers (Figure 1).

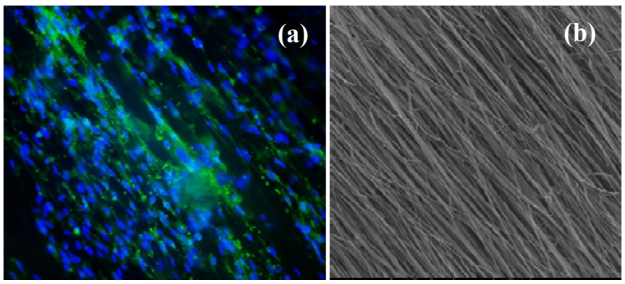


Figure 1. (a) Nuclear staining showed nuclei (blue, DAPI) alignment in the direction of the fibers. Immunostaining also revealed aligned actin (green, FITC) along the direction of the PCL fibers (200x). (b) SEM images at 500X reveal aligned fibers 1-10 microns in diameter.

Conclusions: PCL and Monocryl polymers could be successfully electrospun to yield scaffolds with high tensile strength and Young's modulus, comparable to the native human ACL. In addition, the electrospun PCL constructs supported HFF attachment and proliferation along the direction of the fibers. By providing HFFs contact guidance through organized electrospun scaffolds and co-stimulating the cells with growth factors and mechanical stimulation in a bioreactor, a neoligament tissue engineered scaffold can be constructed for the ultimate goal of implantation for ACL reconstruction.

References:
1. Heydarkhan-Hagvall, S. Biomaterials. 2008; 29:2907-2914.
2. Ge, Z. J Biomed Mater Res.2006; 77A:639-652.

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