Development of a Tissue Engineered Poly(Caprolactone Fumurate) (PCLF) Scaffold for Ligament Regeneration

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Statement of Purpose: Ligament injuries are becoming more common, and when unrepaired lead to eventual joint instability. Even with surgical repair or reconstruction, ligaments never fully regain their native, healthy strength. Specifically, even after 8 weeks of healing, allograft repairs in rabbit knee possess only half the strength of native tissue and do not exhibit native morphology. The lack of appropriate tissue organization is particularly apparent at the bone-ligament enthesis. We propose the use of a PCLF porous scaffold, seeded with adipose-derived mesenchymal stem cells (aMSCs), and subjected to cyclic mechanical stimulation. We hypothesize that this tissue engineered construct will provide the necessary mechanical strength to stabilize the repaired joint, while allowing the differentiated stem cells to regenerate native-like ligament tissue.

Methods: Polycaprolactone fumarate (PCLF) was synthesized as previously described[2]. Scaffolds were designed to mimic rabbit anterior cruciate ligament (ACL) tendon size (3 mm diameter) and to have large pores (750 µm) to allow cell-cell communication and nutrient flow. Porous scaffold molds were designed using SolidWorks CAD software and printed using a SolidScape 3D printer. PCLF was injected over these sacrificial molds with threaded Ethibon 0 sutures and cured using UV crosslinking. Molds were then removed using a mixture of methanol and acetone.

Scaffolds were then implanted in an *ex vivo* rabbit knee using a standard protocol. Scaffolds were mechanically tested *in situ* both with and without a fatiguing treatment. Some knees were exposed to 70° knee flexion for 5,000 cycles. All knees were then anchored in PMA and stretched to failure in an MTS testing machine. Load and displacement to failure are reported.

Additionally, aMSCs were cultured at 37°C in advanced DMEM media with 5% platelet lysate, 1000 units heparin, 1% glutamax, and 1% antibiotic (penicillin and streptomycin)[1]. Cells were expanded and used at passage 6 or earlier. Cells were loaded onto scaffolds (described above) using a Synthecon rotating wall bioreactor overnight. Scaffolds were then either placed in static culture or exposed to cyclic uniaxial tension in a custom built machine (Figure 1) for 1, 3, or 5 days. At each time point, both static and tensioned scaffolds were tested for metabolic activity via MTS assay and stained with a live/dead staining assay. Additionally, scaffolds were stained using immunohistochemistry for collagen I, collagen III, and tenascin-C. Finally, the alignment of the cells when stained for collagen was compared between the static and tensioned groups.

Multiple different sets of stimulation parameters were tested and a basic growth model was used to model the rate of cell growth and production of ligamentous proteins. The optimal tension parameters can then be determined by using the results from the growth model.

Results: We were able to reliably produce scaffolds with a 3 mm diameter and 750 µm pores. The strength of the scaffold is closely related to the strength of the suture. It failed at 75 N as compared to 325 N of the native ACL. After 5,000 cycles, the strength of the scaffolds was not reduced. While the strength of the scaffold was less than the strength of native ligament, it was greater than the strength of a semitendinosus autograft repair.

Cells were able to grow and proliferate to confluence on the scaffolds. The cells were stained for collage I, collagen III, and tenascin-C. The results of immunohistochemistry staining in static culture are shown in figure 2.

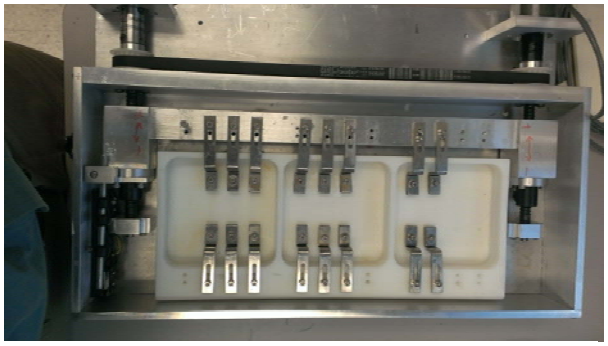

Figure 1: The bioreactor to apply uniaxial tension

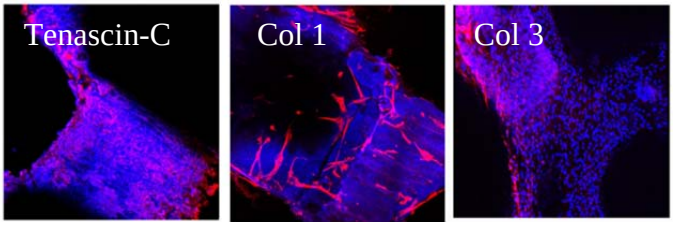

Figure 2: IHC staining for Tenascin, Col 1, and Col 3 in static culture. Blue DAPI and Red Marker of interest

The cells as seen by blue DAPI staining completely cover the surface of the scaffold. All three markers are expressed in static culture; however, only at low levels, suggesting that no inherent differentiation is occurring by the scaffold. Also, there is no alignment of the collagen fibers without applied tension. The alignment of the fibers following the application of tension will be quantified.

Conclusions: The PCLF scaffold, while not as strong as native ACL, is stronger than the semitendinosus autograft repair. The scaffold is capable of maintaining cell growth. The cells, without any stimulation, produce ligamentous markers. We hypothesize that the addition of mechanical stimulation will improve the production of ligamentous markers and fiber aligmnment and an optimal stimulation regiment can be determined.

References: 1. Kolle S, et al. *Cytotherapy*, 15(9): 1086-1097, 2013. 2. Runge B, et al. *Acta biomaterialia*, 8(1): 133-43, 2012.

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