Novel Porous Polycaprolactone Fumarate (PCLF) Scaffold for Adipocyte Derived Mesenchymal Stem Cell Engineering and Platelet Lysate Enhanced Ligament Differentiation

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Statement of Purpose: Intra-articular ligament injuries can lead to abnormal articular loading and ultimately progress to degenerative changes of the joint. Anterior cruciate ligament (ACL) and scapholunate (SL) ligament injuries in the knee and wrist, respectively, have very poor regenerative potential and surgical reconstruction often does not relieve the patient’s pain and joint instability. A synthetic scaffold can provide the initial mechanical demands and provide an environment to promote appropriate cell proliferation and differentiation to aid in tissue regeneration. We aimed to create a composite “neoligament”, i.e., a polymeric, synthetic scaffold that is able to be seeded with progenitor cells and associated factors (growth factors, cytokines, etc) to restore native ligamentous tissue and architecture.

Methods: Polycaprolactone fumarate (PCLF) was synthesized as previously described. Scaffolds were designed to mimic rabbit ACL tendon size (4mm x 6mm) and to have large pores (500 or 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 the sacrificial molds and cured using UV crosslinking. Molds were removed using a mixture of methanol and acetone. Toxicity protocols included multiple hydrophobic solvents, including methylene chloride, ethanol, and acetone.

Cell culture and characterization: Adipocyte-derived human mesenchymal stem cells (aMSCs) were harvested as previously described and cultured in DMEM and 10% FBS. The analysis compared this media to media composed of DMEM with 5% platelet lysate (PL), a mixture of platelet release products. The cells were seeded onto the scaffolds with a dynamic bioreactor. Cellular proliferation and metabolic activity was assessed with MTS assays. aMSC viability was analyzed with Live/Dead immunostaining. Baseline cell characterization was performed using a GAG and ALP assay. Total collagen content assay and immunostaining for collagen I, tenascin-C and collagen III examined ligament differentiation.

Results: PCLF scaffolds were created by sacrificial molding. The scaffolds were designed with pore sizes of 500 or 750 μm and porosities of 45% and 60%, respectively. We compared multiple toxicity preparation protocols to determine the most effect method to remove toxic byproducts. At the conclusion of this regimen, the pores shrank by 10% and porosity increased to 70%. After dynamic cell seeding of the progenitor cells on the PCLF, the cells remained viable for 2 weeks when cultured on in vitro culture plates (Figure 1). The cell density significantly increased during culture, invading throughout the pores and distributed evenly along the scaffolds. The metabolic activity of the scaffolds increased as cell proliferation continued along the 3 dimensional PCLF scaffolds. Additionally, aMSC proliferation rates were increased when cultured in the presence of platelet lysate (PL) compared to FBS. The cells had a low baseline expression of ALP and GAG throughout their culture time-period.

Figure 1. Adipocyte-derived mesenchymal stem cells (aMSCs) seeded and cultured over 14 days on the PCLF scaffolds.

Figure 2. Total collagen production produced by aMSCs increased by FGF and FGF combined with platelet lysate (PL).

Figure 3. Tenascin-C and collagen I immunostaining after aMSCs were cultured for 4 weeks on the PCLF scaffolds under different conditions. (red signal is Tenascin-C or Collagen I, blue is DAPI)

The ligament and tenogenic growth factor FGF-2 induced ligament differentiation by increasing the expression of total collagen. This effect was significantly augmented when cultured in the presence of PL. Immunostaining for the ligament markers Tenascin-C and Collagen I demonstrated marked expression throughout the body of the scaffolds by cells cultured in FGF and PL, comparable to the expression of human fibroblasts grown on the PCLF scaffolds.

Conclusions: Our results demonstrate aMSCs are able to attach, proliferate and differentiate into ligamentous phenotypes along the porous PCLF scaffold. This novel scaffold has potential in stem cell engineering and ligament regeneration.


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