Polycaprolactone Fumarate (PCLF) as a Backbone for Chondrocyte Attachment and Proliferation Augmented by Platelet Lysate

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Statement of Purpose: Cartilage has a very low regenerative potential once injured and as such is one of the most common sources of pain and morbidity in healthcare, accounting for billions of dollars annually. Repetitive joint loading or instability leads to progressive cartilage degeneration and eventual arthritis. Patients with advanced joint degeneration or large chondral defects have limited options to salvage their native cartilage, requiring total joint arthroplasty. Tissue engineering has the potential to be used as an innovative method to generate a scaffold able to sustain chondrocyte growth and differentiation, as well as native cartilaginous tissue production. Currently, there is no good option for patients with large chondral injuries. Our goals are 1) to create a biodegradable polymer scaffold with the capabilities of sustaining chondrocyte growth and proliferation, 2) to enable cell-cell communication and tissue regeneration via large pores, 3) assess the biological augmentation of the scaffold capabilities using a cocktail composed of platelet secretion products known as platelet lysate.

Methods: Polycaprolactone fumarate (PCLF) was synthesized as previously described[2]. Scaffolds were designed to allow cell communication via interconnected pores. Molds were designed using SolidWorks CAD software and printed on a SolidScape 3D printer. Scaffolds were created by sacrificial molding and UV crosslinking to create desired geometry and improve mechanical properties of PCLF. Organic solvents were used to dissolve mold and remove toxic compounds.

Cell culture, Viability, and Proliferation: Chondrocytes were isolated from the ear of adult rabbits and cultured in media composed of DMEM, 10% FBS. The chondrocytes were then seeded on to the scaffolds within 3 passages via a dynamic bioreactor. Cellular toxicity analysis was performed for 4 different polymer preparation protocols. The health of the cells was assessed with both viability and toxicity assays which were carried out via Live/Dead stain. Cellular proliferation was measured via cell counting and mitochondrial activity with a MTS kit. Platelet lysate (PL) was harvested from our institution's platelet biobank as previously described[1], then compared to fetal bovine serum (FBS) in proliferation assays. GAG and ALP assay kits were used to assess chondrogenic differentiation.

Results: PCLF scaffolds were designed to have 500 and 750 micrometer pores. The large interconnected pores enable cell-cell communication and eventual cartilage regeneration (**Figure** 1**,** left). We performed 3 toxicity and sterilization preparation regimens, using ethanol alone, acetone alone, or acetone and methylene chloride (A+MC). When compared to the control, the acetone and A+MC regimens enabled the cells to possess the same viability as the control (chondrocytes on the culture dish) (**Figure 1,** right). The pores decreased by approximately 10% after processing, however retained up to 70% porosity as demonstrated on microCT analysis.

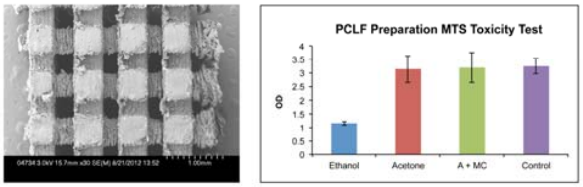

Figure 1. The large interconnected pores of the PCLF scaffolds. The toxicity analysis of 3 common toxicity and sterilization protocols.

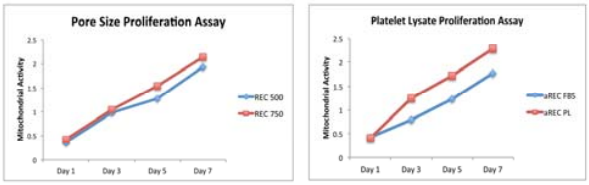

Figure 2. MTS proliferation assays comparing 500 and 750 micrometer pore sizes (left) and platelet lysate vs fetal bovine serum culture (right).

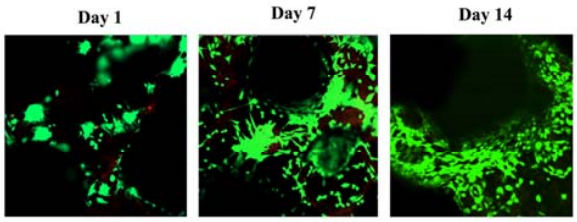

Figure 3. The cellular viability over 14 days demonstrated via Live/Dead stain based upon esterase activity.

After dynamic seeding and cellular attachment, the pore size did not seem to have an effect on the proliferation rates of the chondrocytes (**Figure 2,** left). However, chondrocytes seeded on scaffolds cultured in platelet lysate had a significant increase in cellular proliferation when compared to those cultured in FBS (**Figure 2,** right). Using the Live/Dead immunostain, it can be noted that the chondrocytes attached and were distributed throughout the body of the scaffold after dynamic seeding. They remained viable through 14 days in cell culture, appearing to completely cover the scaffolds and invade throughout the pores (**Figure 3**). The chondrogenic markers glycosaminoglycan (GAG) and total collagen contents increased over 2 weeks, while the osteogenic marker alkaline phosphatase (ALP) decreased.

Conclusions: Our results show that the PCLF polymer scaffold enables chondrocytes to attach, proliferate and retain their chondrogenic phenotypes. This novel scaffold and material has promise in chondrocyte engineering and cartilage regeneration.

Reference: 1. Crespo-Diaz, R., et al. *Cell transplantation*, 20(6): 797-811, 2011. 2. Runge B, et al. *Acta biomaterialia*, 8(1): 133-43, 2012.
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