Biomimetic Charged Hydrogel Construct for Cartilage Regeneration

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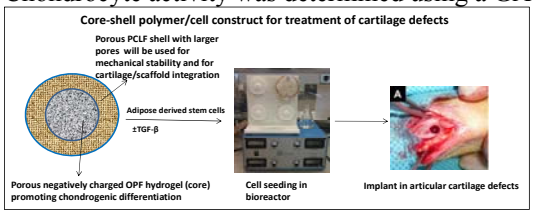
Statement of Purpose: Full-thickness articular cartilage defects are a major clinical problem and at present there is no treatment that is widely accepted to regeneratively repair these lesions. The biomaterial will offer biological reconstruction of the defect site by stimulating differentiation of chondrocyte precursor cells and cartilage formation in a sequential fashion. The novel biodegradable cell-polymer construct to be developed in this project will allow minimally invasive treatment of cartilage defects of various size and shape and provides mechanical stability at defect site. The degradable nature of the biomaterial will not require implant retrieval, saving patient's time, expense, and inconvenience. Our goal in this project is to develop a novel method to utilize the functional strength and stability of synthetic scaffolds seeded with the regenerative potential of progenitor cells and associated cytokines to restore native cartilaginous tissue and enhance stability at the bone to cartilage interface.

Methods: Negatively charged oligo (polyethylene glycol) fumarate (OPF) was used as a matrix for chondrocytes and adipose derived mesenchymal stem cell (ADSC) seeding. To incorporate negative charge into OPF hydrogel, sodium methacrylate (SMA) was copolymerized with OPF under UV light. We used salt leaching technique using NaCl particles with maximum diameter of 300μm for fabrication of porous hydrogels. Dynamic mechanical analysis (DMA), thermogravimetric analysis (TGA), and differential scanning calorimetry (DSC) were performed on hydrogel samples to examine mechanical and thermal stability of polymers. Toxicity of the material was assessed using an MTS assay. Rabbit ear chondrocytes (RECs) were seeded into the hydrogels using a rotating bioreactor spun at a rate of 16 rotations/minute for 24 hours. Cell attachment, activity, and proliferation were quantified through MTS assays, dsDNA measurements, and confocal microscopy. Chondrocyte activity was determined using a GAG assay.



Results: Live/Dead staining showed that the majority of chondrocytes seeded on porous negatively charged hydrogels remained viable and SMA was crucial in supporting cell attachment and proliferation. Cells visibly lined up in the pores of the hydrogel versus a clumped arrangement in unfavorable environments.

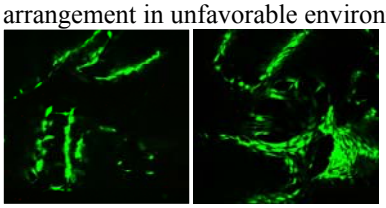


Figure 1. Confocal microscopy of live/dead staining of seeding chondrocytes at 14 days. SMA20% (left); SMA 30% (right).

Cell activity, using an MTS assay, showed increased cell metabolism typical of improved attachment and proliferation on hydrogels with 20% and 30% SMA.

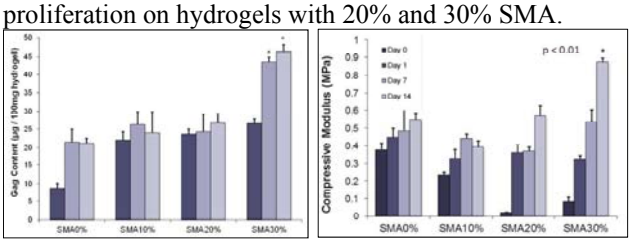


Figure 2. GAG content of each hydrogel sample. Asterisk (*) marked groups are statistically significant compared to all other samples at any time point with a p-value < 0.01 (left panel). The maximum compressive modulus of the same samples as above, compressive modulus increased significantly following cell attachment (right panel).

DMA showed a decrease in compressive modulus of samples with increasing concentration of SMA, however, chondrocytes allow for increased mechanical properties following attachment. Chondrocyte activity and GAG production increased of hydrogels with 30% SMA at days 7 and 14.

Conclusions: Porous OPF hydrogels are able to support chondrocyte activity and proliferation. A 3D matrix environment allows for additional support between cells. With the inclusion of SMA, these hydrogels can promote expression of cartilage markers. The inclusion of SMA changed some of the innate mechanical and thermal properties of the OPF material, but was significantly important for cell growth. Overall, the advantages of including SMA overwhelm the initial mechanical disadvantage. Future work: we are testing similar protocols on human adipose-derived mesenchymal stem cells to examine differentiation effects. Additionally, we are designing a PCLF outer shell to incorporate with these OPF inner cores to improve physical properties. Lastly, we will implant these materials into a cartilage defect model in the rabbit knee.

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