Evaluation of Two Osteochondral Constructs for Osteochondral Tissue Engineering

Scott Maxson*, Duong T. Nguyen**, Karen J.L. Burg*, Joel D. Bumgardner**
*Department of Bioengineering & Institute for Biological Interfaces of Engineering, Clemson University, Clemson SC
**Department of Biomedical Engineering, University of Memphis, Memphis TN

Statement of Purpose:

Osteochondral defects occur in articulation regions with damage to the bone and cartilage. Current osteochondral tissue engineering strategies are focused on developing composite grafts combining synthetic cartilage and bone with compatible interfaces. This study evaluated two composite osteochondral constructs fabricated from different biomaterials with different matrix architecture. *Construct 1*: The bone phase was made of composite chitosan nano-hydroxyapatite beads sintered together. The cartilage phase was sintered chitosan beads. *Construct 2*: The bone phase was composed of a porous copolymer of 75:25 poly-L-lactide/ polycaprolactone, produced by the solvent evaporation/ poragen leaching method, mixed with 10% hydroxyapatite (PL/PCL/HAP). The cartilage phase was a 2% alginate hydrogel. For both constructs, the two phases were separated by a thin film of PL/PCL.

Methods:

Six constructs of each type were fabricated and ethylene oxide sterilized for a 14-day perfusion culture. The constructs were presoaked in DMEM containing 10% FBS, 1% antibiotic/antimyocotic and 0.2% fungizone for 24 hrs to allow protein adsorption, then were seeded with mesenchymal stem cells (MSCs) and cultured for 3 days in well plates. Before constructs were transferred to a perfusion bioreactor, two constructs were assessed for cell attachment using Live/Dead® staining. The remaining four constructs of each type were placed in a dual-mode perfusion bioreactor[1]. The bone phases of each construct were cultured in osteogenic differentiation medium and the cartilage phases were cultured in chondrogenic differentiation medium. The perfusion rate was 0.7 ml/min for the duration of the 14-day study.

When the constructs were removed, the cartilage phases were separated from the bone phases and the cellular activity was analyzed for chondrogenic and osteogenic differentiation by quantifying sulfated glycosaminoglycans (sGAG) and DNA using dimethylmethylene blue (DMB) method and a DNA Quantification Kit, respectively.

Alkaline phosphatase (ALP) activity was assessed for the bone phase of each construct. The standard protocol for the BCA Total Protein Kit was used to measure protein content in each sample. The results from the ALP assay were normalized to the protein content.

Real time RT-PCR was performed for cells in both the cartilage phase and bone phase of each construct. RNA was isolated using the RNeasy mini kit. Presence of cartilage marker aggrecan and bone markers osteocalcin and bone sialoprotein was assessed. GAPDH was used as a housekeeping gene for both groups. Relative expression was determined using the ΔΔCt method with GAPDH as the reference gene.

Results/Discussion:

Live/Dead staining at Day 2 showed cell attachment throughout the constructs, which suggests a favorable bioreactor flow rate (data not shown). After 14 days of culture in the bioreactor, the cartilage phase for both constructs showed production of sGAG (normalized with DNA); however, cells on/in the alginate had significantly higher concentrations (Figure 1A). The PL/PCL/HAP construct appeared to promote higher levels of cellular growth than the chitosan construct possibly due to the difference in porous architecture of the constructs (Figure 1B). However, normalized ALP activity of the two constructs was not significantly different, suggesting similar bone development on both constructs (Figure 1C). Aggrecan expression, reported as relative to GAPDH, and osteocalcin expression were evident for both constructs at similar levels (Figure 1D). Both scaffolds expressed bone sialoprotein but the PLA/PCL/HAP bone phase had higher expression (Figure 1E).

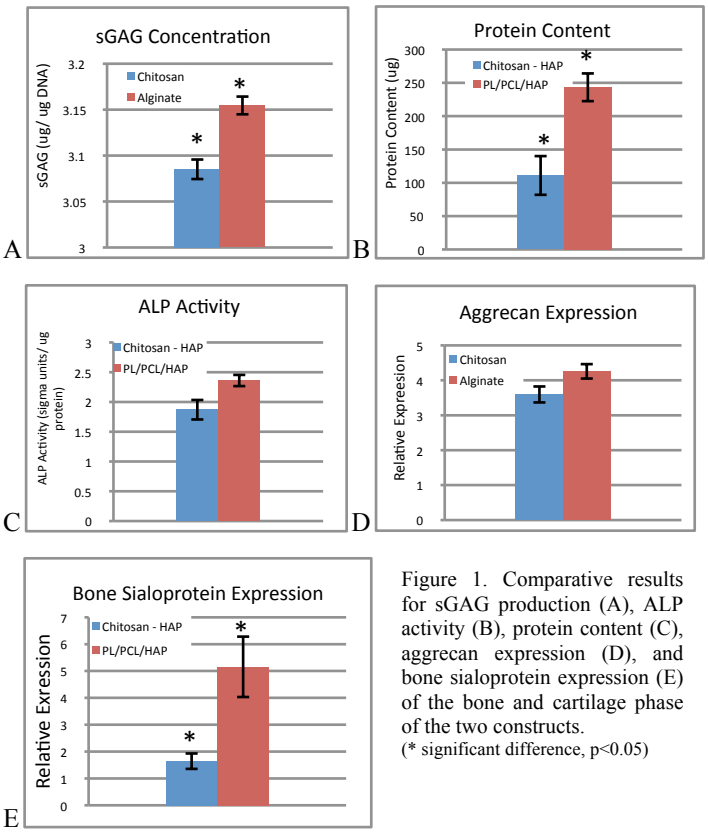

Figure 1. Comparative results for sGAG production (A), ALP activity (B), protein content (C), aggrecan expression (D), and bone sialoprotein expression (E) of the bone and cartilage phase of the two constructs.
(* significant difference, $p<0.05$)

Conclusion:

Both osteochondral constructs support chondrogenic and osteogenic differentiation of MSCs. The differences in sGAG content, protein content, and bone sialoprotein of the two constructs could be contributed to material and architectural differences. Future studies will focus on characterizing structural differences, degradation rates and mechanical properties of the constructs.

References: 1. DE Orr, KJL Burg, *Ann Biomed Eng*, 36(7):1228-1241, 2008.

Acknowledgements: Funding for this work was provided by the Clemson University Hunter Endowment. Support was also provided by the Biomaterials Applications of Memphis (BAM) research labs at the UoM - UTHSC