Comparison of Acellular Biomatrices for Cartilage Engineering

Jason Dragoo,[1,2] Hadeel Khammash,[1] Kaitlyn Whitney,[1] Ruth McCarrick-Walmsley[1]
[1]University of Colorado School of Medicine, Anschutz Medical Campus, Department of Orthopedics, Denver, CO
[2]UCHealth Steadman Hawkins Clinic, Denver, CO
*corresponding author: j.dragoo@cuanschutz.edu

Disclosures: Jason Dragoo (N), Hadeel Khammash (N), Kaitlyn Whitney (N), Ruth McCarrick-Walmsley (N)

INTRODUCTION: Articular cartilage has a lack of vascularity, impairing its capacity for healing [1]. Strategies that aid in cartilage restoration using four classes of biomatrices [2,3] can help address this challenge. Most recent research efforts have been aimed at determining the appropriate source of MSCs, such as adipose derived stem cells (ADSCs), bone marrow derived stem cells (BMSCs), or human chondrocytes (HCs), that can proliferate and differentiate into functional chondrocytes. However, the type of scaffold employed in these biologic techniques has not been well studied. The goal of this study is to characterize ADSCs, BMSCs, and HCs when seeded onto three different acellular biomatrices.

METHODS: HCs and BMSCs were cultured to passage 4 while ADSCs were used at passage 2. Cells were seeded at 10^6 cells/5mm^2 scaffolds. A total of nine culture conditions were used for each cell type. 1. Cells only; 2. SurgiMend 2 (S2); 3. SurgiMend 3 (S3); 4. SurgiMend 4 (S4); 5. Allopatch (A); 6. SurgiMend 2/TGF-β1 (S2/T); 7. SurgiMend 3/TGF- β1 (S3/T); 8. SurgiMend/TGF-β1 (S4/t); 9. Allopatch/ TGF- β1 (A/T). Total RNA from biomatrix constructs was extracted and RT-PCR for AGG, COL1, COL2, SOX9, and GAPDH were performed. Based on these results, optimal cell types and conditions were selected for histological analysis: HCs on A/T, ADSCs on A/T, and BMSCs on S2/T. After 6 weeks of culture, cells were fixed and counted. Immunohistochemistry staining was performed on a total of 9 sections per cell type.

RESULTS: RT-PCR results indicated that HCs, ADSCs, and BMSCs expressed chondrocyte specific mRNA (AGG, COL2, and SOX9), dependent on scaffold type, when cultured in the presence of chondrocyte differentiation medium and TGF- β1. HCs showed increases in AGG and Col II in the A/T group and increases in SOX9 across all scaffold types with the addition of TGF-B1. Col I, a fibroblast marker, was increased 3X over cells alone in S3 and S4 scaffolds without TGF-B1. For ADSCs, Col II and SOX9 expression was significantly higher in the A/T group. BMSCs in the S2/T group showed significant increases in AGG and Col II, increases in SOX9 across all groups, and a decrease in COL I expression in S2/T, indicating successful chondrocyte differentiation for that scaffold/media combination. When the most favorable condition was chosen for each cell type and histological analysis was performed, it was found that there was no difference in cell count between HCs (71.7+/-8.7) and ADSCs (76.6+/-2.4), both grown in A/T conditions, while BMSCs grown on S2/T has significantly lower cell count (29.2+/-12.0). All three cell types grew on the surface and within the pores of acellular matrices. ADSCs on A/T matrix and BMSCs on S2/T matrix stained positive for type II collagen, both on the surface and within the pores. Both BMSCs and ADSCs were able to proliferate and differentiate into functional chondrocytes with chondrocyte specific extracellular matrix production.

DISCUSSION: This in vitro study served to characterize the use of various biomatrices for chondrogenic differentiation of both BMSCs and ADSCs. Results from this study indicate that for ADSCs there was optimal differentiation on Allopatch scaffolds supplemented with TGF- β1 and for BMSCs on the SurgiMend2 supplemented with TGF- β1. Results from RT-PCR exhibited that all three cell types can differentiate into cartilage when placed on acellular matrices. It was also concluded that TGF- β1 allowed for optimized growth and proliferation. Histology demonstrated physical evidence of growth on both the surface and within the matrix pores. Immunochemistry staining supported the combination of biomatrices and growth factors to promote proliferation and differentiation of ADSCs and BMSCs into functional chondrocytes.

SIGNIFICANCE/CLINICAL RELEVANCE: The biomatrices used in this research are natural, decellularized human or animal tissues, already FDA approved for soft tissue and wound repair. Cell implantation requires utilization of scaffolds to retain seeded cells and to provide mechanical support in the intra-articular environment [2]. This study supports the hypothesis that readily available biomatrices may help support the differentiation of progenitor cells into functional chondrocytes.

REFERENCES:

1. Safran, M.R., H. Kim, and S. Zaffagnini, *The use of scaffolds in the management of articular cartilage injury.* J Am Acad Orthop Surg, 2008. **16**(6): p. 306-11.
2. Tuan, R.S., A.F. Chen, and B.A. Klatt, *Cartilage regeneration.* J Am Acad Orthop Surg, 2013. **21**(5): p. 303-11.
3. Bentley, G., et al., *Repair of osteochondral defects in joints--how to achieve success.* Injury, 2013. **44 Suppl 1**: p. S3-10.
4. Locke, M., J. Windsor, and P.R. Dunbar, *Human adipose-derived stem cells: isolation, characterization and applications in surgery.* ANZ J Surg, 2009. **79**(4): p. 235-44.
5. Ahmed, T.A. and M.T. Hincke, *Mesenchymal stem cell-based tissue engineering strategies for repair of articular cartilage.* Histol Histopathol, 2014. **29**(6): p. 669-89.
6. Liu, Q., et al., *Chondroprotective effects of taurine in primary cultures of human articular chondrocytes.* Tohoku J Exp Med, 2015. **235**(3): p. 201-13.

IMAGES AND TABLES:

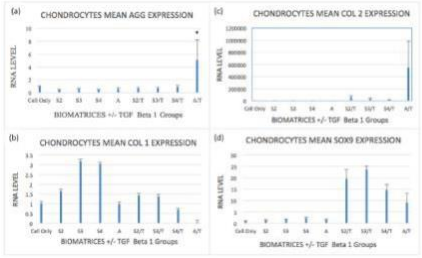

Figure 1(a-d). Human Chondrocytes mean mRNA expression after normalization with glyceraldehyde 3-phosphate (GAPDH). Data were shown as mean ± standard deviation. Key: S=SurgiMend, A=Allopatch, T=TGF-β1 added. Roman numeral 2, 3, 4 means thickness in millimeter of SurgiMend biomatrix. *Indicates ($p < 0.05$).

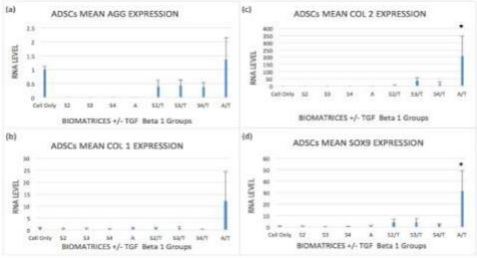

Figure 2(a-d). Adult adipose derived stem cell (ADSCs) mean mRNA expression after normalization with glyceraldehyde 3-phosphate (GAPDH). Data were shown as mean ± standard deviation. Key: S=SurgiMend, A=Allopatch, T=TGF-β1 added. Roman numeral 2, 3, 4 means thickness in millimeter of SurgiMend biomatrix. *Indicates ($p < 0.05$).

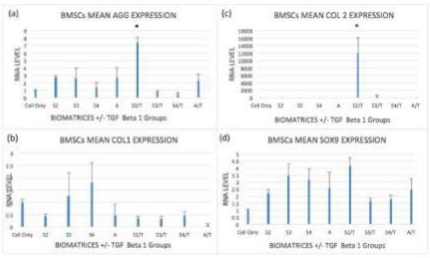

Fig 3(a-d). BMSCs mRNA expression after normalization with glyceraldehyde 3-phosphate (GAPDH). Data were shown as mean ± standard deviation. Key: S=SurgiMend, A=Allopatch, T=TGF-β1 added. Roman numeral 2, 3, 4 means thickness in millimeter of SurgiMend biomatrix. *Indicates ($p < 0.05$).