Development of a Delivery Method for Human Subacromial Bursa Tissue Using Alginate-Based Hydrogels

Peyton A. Hadfield[1,3], Stefan L. Minyayluk[2], Mary Beth McCarthy[2], Augustus D. Mazzoca[2], Joseph P. DeAngelis[1], Benjamin R. Freedman[1]
[1]Department of Orthopaedic Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA, USA
[2]Department of Orthopaedic Surgery, Mass General Brigham, Harvard Medical School, Boston, MA, USA
[3]Harvard College, Cambridge, MA, USA
phadfiel@bidmc.harvard.edu

INTRODUCTION: Rotator cuff tears are a prevalent shoulder disorder with retear rates between 12-94% [1]. While removal of the subacromial bursa often accompanies arthroscopic rotator cuff repair, recent studies have found a high prevalence of multipotent mesenchymal stem cells in this tissue, prompting research into rotator cuff healing implications [2]. Alginate hydrogels present a potential delivery strategy due to their excellent biocompatibility, biodegradability and ability to control expression of tenogenic differentiation markers [3,4]. These markers may be further amplified by tuning hydrogel stiffness using ionic crosslinking to induce mechanical signaling and biochemical responses [5]. This study aimed to engineer an injectable alginate hydrogel that allows for the administration and long-term localization of autologous minced bursa tissue to the rotator cuff repair site. We hypothesized that the storage modulus of high and low molecular weight alginate hydrogels could be engineered using varied concentrations of ionic crosslinkers and that these hydrogels could maintain viability of encapsulated subacromial bursa-derived mesenchymal stem cells and tissue.

METHODS: Hydrogel Formation: 6% w/v solutions of PRONOVA UP MVG and VLVG alginate and 200, 400, and 600mM solutions of calcium sulfate dihydrate were prepared with cell suspension, minced tissue, or water. Rheology: After crosslinking, gels were swollen for 24 hours in DMEM at 37°C. Rheology was performed to compare the G', G", and tan(δ) using 8mm parallel sandblasted plates with a 1% strain, 1Hz oscillation, and a 0.1N axial preload at 37°. Cell Extraction: Human subacromial bursa tissue (IRB Exemption Protocol # 2025D000656 at BIDMC) was minced, plated, and incubated in complete media for 7 days before tissue was removed and cells were passaged, grown near confluency, and then passaged in a 2M cells/ml suspension. Live/Dead Assay: Hydrogels were punched and placed in a 24-well plate in complete media. Following 7 and 14 days of incubation, hydrogels were stained using calcein/ethidium and imaged using confocal microscopy. Image Analysis: FIJI was utilized to quantify cell viability. Statistical Analysis: Unpaired t-tests were used to compare groups (Prism 10, GraphPad).

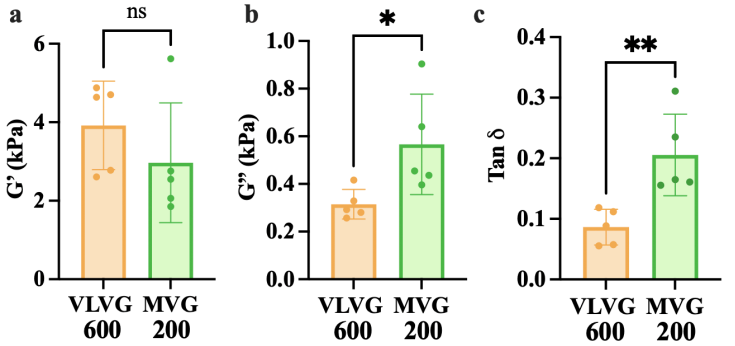

Figure 1: *Stiffness and Viscoelastic Properties of Alginate Hydrogels can be Decoupled.* Rheological properties (**a**) G', (**b**) G" and (**c**) Tan(δ) for VLVG w/ 600mM and MVG w/ 200mM calcium sulfate dihydrate. Data shown as mean ± S.D.

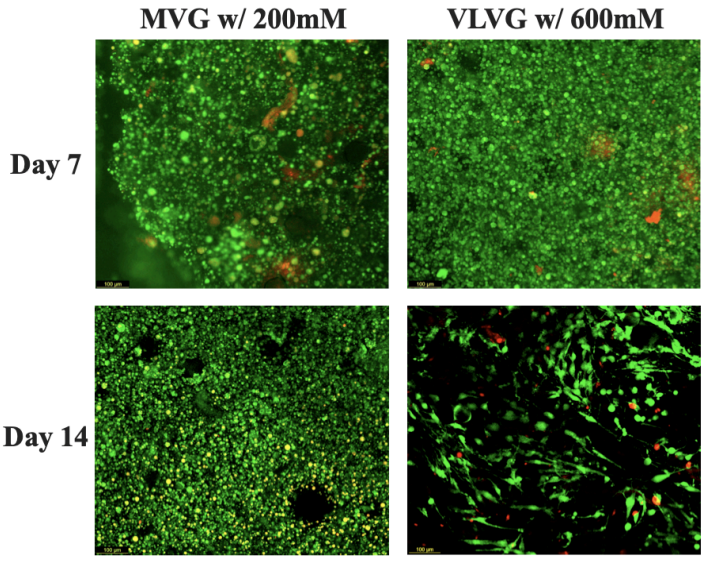

Figure 2: *Bursa Tissue Encapsulated in Alginate Hydrogel Systems is Viable at 7 and 14 days Post-Encapsulation.* Confocal images of calcein/ethidium-stained minced bursa tissue encapsulated in MVG aliginate with 200mM calcium sulfate, and VLVG alginate with 600mM calcium sulfate following 7 and 14 days of incubation.

RESULTS: Stiffness and viscoelasticity of alginate hydrogel systems were decoupled through varying alginate molecular weight and ionic crosslinking (Fig. 1). The G' of 6% w/v VLVG alginate with 600mM calcium sulfate and 6% w/v MVG alginate with 200mM calcium sulfate were found to not be statistically different while viscoelastic properties G" and tan(δ) varied significantly as molecular weight was increased (Fig.1). Human bursa cells encapsulated in MVG and VLVG alginates were found to be viable for at least 7 days (data not shown). Confocal images of hydrogel-encapsulated bursa tissue stained with calcein/ethidium stain indicated that the tissue remained viable after 7 and 14 days (Fig. 2).

DISCUSSION: This study successfully developed an alginate-based delivery system for encapsulated subacromial bursa tissue. As in agreement with previous studies, stiffness and viscoelastic properties of alginate hydrogels were decoupled by varying calcium sulfate dihydrate crosslinker concentrations [6]. This decoupling allows for the effects of alginate type and mechanical cell signaling on viability to be examined independently. Quantification of calcein/ethidium-stained cell-encapsulated gels under confocal imaging confirmed cell viability. Confocal images of bursa-tissue encapsulated gels confirmed viability in VLVG and MVG alginates after 7 and 14 days. This indicates that both cell and tissue viability can be maintained without the use of RDG-modified alginate, unlike seen in previous studies [7]. Future work will study additional hydrogel systems, assess the gene expression (COL1/COL3) of the cells and tissue encapsulated within, and confirm correct localization using large animal models.

SIGNIFICANCE/CLINICAL RELEVANCE: This study aimed to assess an alginate-based hydrogel delivery system of human subacromial bursal cells for arthroscopic rotator cuff repair.

REFERENCES: [1] Trasolini NA. Arthroscopy. 2022;38:2413–2416; [2] Song N. Tissue Eng Part A. 2014;20:239–249; [3] Chen R. Genes Dis. 2023;11:1010191; [4] Marturano JE. J Biomech. 2016;49:3281–3288; [5] Freedman BR. Acta Biomater. 2022;143:63–71; [6] Charbonier F. Curr Protoc. 2021;1:124; [7] Dumbleton J. Cell Mol Bioeng. 2016;9:277–288.