Investigating the Effect of Cellular Augmentation on the Metabolic and Mechanical Properties of Frozen Meniscus Allografts

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INTRODUCTION: Meniscus allograft transplantation (MAT) has become a common procedure for symptomatic meniscus deficiency. The goal of this procedure is to reduce further joint degeneration and avoid knee replacement in young patients. Fresh-frozen meniscus allografts are the current standard for MAT procedures, due to long shelf-life, ease of preservation, high availability, and allows for size matching to occur. However, the rapid freezing process kills any viable cells and alters fiber orientation and mechanical properties. Moreover, fresh-frozen meniscus allografts often lead to graft extrusion, shrinkage, tears, and minimal integration with remaining meniscus rim. A promising alternative are fresh meniscus allografts, but they have significantly shorter shelf-life and demanding preservation steps to maintain graft viability. Therefore, there is a clear need for an improved meniscus allograft, which maintains the long shelf-life, while improving clinical outcomes. Our group is investigating cellular augmentation as a method of improving MAT procedures. We hypothesize that the addition of viable cells into frozen allografts will increase matrix metabolism, helping to maintain graft size, and improve fiber orientation and mechanical properties. The goal of this study is to understand if cellular augmentation, with adipose derived MSCs (AMSCs) and meniscons (meniscus cell with its pericellular matrix), improves the metabolic and mechanical properties of frozen allografts.

METHODS: Frozen meniscus allografts were injected with a combination of 80% AMSCs and 20% meniscons [1]. Meniscons were digested from meniscus tissue collected from total knee arthroplasty procedures conducted at Mayo Clinic (N = 23 – 12F, 11M). For mechanical testing, frozen allografts were injected at 5 evenly spaced locations (N = 7 – 6F, 1M). For metabolic experiments 4mm biopsies of frozen allografts were injected at a single location (N = 10 – 3F, 7M). All injections consisted of 600,000 cells suspended in 50uL of lactated ringer solution. Injected allografts were then cultured for 28 days in DMEM with 1% pen/strep, 1% ascorbic acid, 1% ITS-X and 50ug/mL L-proline. Live/Dead and histological staining was performed after culture to evaluate cell viability and migration. Microindentation and macro-mechanical testing were performed to evaluate local properties and load distribution differences.

RESULTS: Live/Dead images of injected biopsies showed cellular migration away from injection site and maintained viability at 28 days (Figure 1). Histological images revealed tissue production at the site of injection working to close the defect created by the needle (Figure 2). Microindentation testing revealed significant differences between fresh and frozen allografts (Fresh N = 9 – 1F, 8M, Frozen N = 28 – 9F, 19M), with frozen injected allografts shifting away from that of frozen and towards the mechanical properties of fresh allografts (Figure 3). Macro-mechanical testing revealed significant differences in load distribution between fresh and frozen medial and lateral menisci (Fresh N = 2 – 2M, Frozen N = 2 – 2M)(Figure 3).

DISCUSSION: The results of this study confirm that the addition of cells to fresh-frozen meniscus allografts improve mechanical properties following 28 days of culture. Histological findings reveal that cells may be producing matrix. We are currently applying metabolic labeling techniques to quantify newly formed matrix within injected biopsies. Picrosirius Red staining and polarized light microscopy are currently being used to evaluate fiber orientation changes in frozen injected meniscus allografts.

SIGNIFICANCE/CLINICAL RELEVANCE: MAT procedures are commonly conducted following meniscectomies and the development of a symptomatic meniscus deficiency. However, MAT is associated with suboptimal clinical outcomes and high reoperation rates. Fresh-frozen meniscus allografts have altered tissue properties that could negatively impact the clinical outcomes. With the addition of an active cell population that improves mechanical properties and matrix metabolism, a revitalized fresh-frozen meniscus allograft could be the solution to improving MAT procedures.

REFERENCES: **1.** Struijk C, Lydon KL, Husen M, Verdonk P, Michielsen J, van Wijnen AJ, Krych AJ, Saris DBF. Cellular Enhancement of Frozen Meniscus Allograft Combining Native Meniscus and Mesenchymal Stromal Cell Injections. Cartilage. 2024 Feb 7:19476035231224802. doi: 10.1177/19476035231224802. Epub ahead of print. PMID: 38321966; PMCID: PMC11569627.

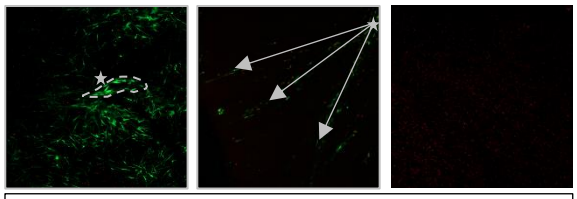

Figure 1: Live/Dead images of injection hole at top of biopsy (left), cell migration away from injection in center of biopsy (middle) and control frozen biopsy without injection (right).

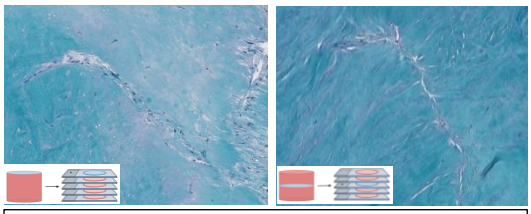

Figure 2: Safranin-O, fast green and hematoxylin staining of injection hole at top of biopsy (left) and center of biopsy (right). High cell density within injection hole, and matrix production is demonstrated by shrinkage of injection hole at center of biopsy.

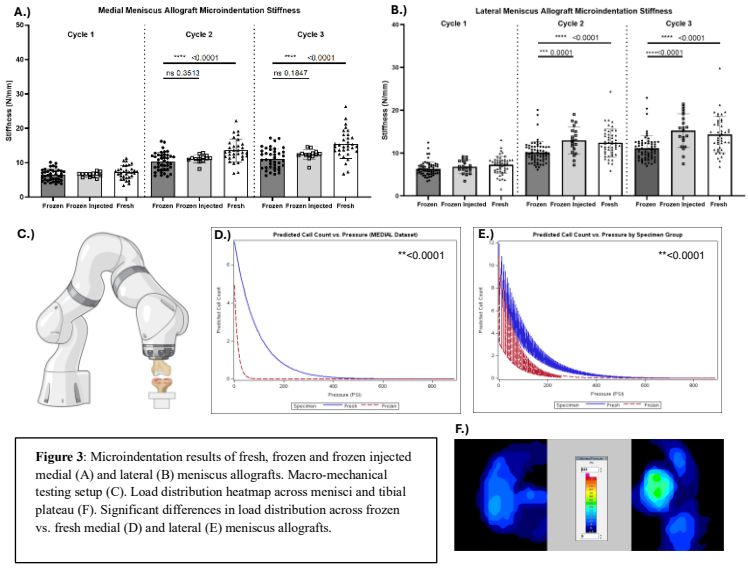

Figure 3: Microindentation results of fresh, frozen and frozen injected medial (A) and lateral (B) meniscus allografts. Macro-mechanical testing setup (C). Load distribution heatmap across menisci and tibial plateau (F). Significant differences in load distribution across frozen vs. fresh medial (D) and lateral (E) meniscus allografts.