A NOVEL APPROACH TO HARNESS MESENCHYMAL STROMAL CELLS TO AUGMENT ARTICULAR CARTILAGE REGENERATION: AN IN VITRO AND IN VIVO ASSESSMENT

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INTRODUCTION

Clinical need for cartilage repair methods

- Globally, 595 million patients suffer from Osteoarthritis (OA), a leading cause of disability worldwide[1]
- Focal chondral defects (FCD) and cartilage injuries are known to contribute to progressive joint degradation and are implicated in the onset and progression of OA[2, 3]

Current Treatment Options

- Osteoarthritis (OA) is not well managed globally
- NSAIDs are the current standard of care for non-surgical OA management
- Microfracture, the current leading surgical treatment for articular cartilage damage, has established long-term failure rates as high as 66%, with a mean time of 4.0 years to failure[4]

Articular Cartilage Paste Graft Procedure

- The articular cartilage paste graft (paste graft) is a cartilage repair technique that utilizes an autologous graft of healthy bone and cartilage harvested from the intercondylar notch of the knee
- Morselization of the damaged cartilage allows for infiltration of bone marrow MSCs, resulting in *de novo* cartilage formation
- Clinically, Paste Graft results in a mean benefit time of 16.6 ± 0.9 years in validated metrics of pain, function, and activity levels[5]
- Histology outcomes demonstrated 63.3% of patients had robust articular surface replacement, with approximately 1/3 patients showing re-development of normal, hyaline cartilage[6]

Hypothesis:

- Addition of allogeneic human MSCs (hMSCs) will amplify the effects of bone marrow MSCs, and the addition of hydrogel to the paste graft will improve handling characteristics and integration of the graft with surrounding native tissue

METHODS

In Vitro

- Alamar Blue assay to assess cellular metabolic activity and viability to determine strongest paste preparation method (cut vs. smashed)
- Long-term (28 day) culture of optimized 3D tissue engineered cartilage constructs with the addition of MSCs
- Cell viability and metabolic activity data analyzed via one-way ANOVA and subsequent Post-Hoc Tukey test
- Long-term (28 day) culture of Optimized Tissue-Hydrogel (n=9) and Tissue-Hydrogel-MSCs
- Microtiter plate quantitative assays: S-GAG as a measure of proteoglycan; OH-Proline as a measure of collagen

In Vivo

- One articular cartilage defect (3mm diameter, 5 mm deep) was made on the medial femoral condyle of the right knee in n=26 rabbits
- Gross imaging, histological staining via Safranin O/Fast Green staining, and confirmatory microCT with Hexabrix contrast to assess repair
- Histology analyzed for percent defect fill and attachment to surrounding native articular cartilage

RESULTS: *IN VITRO*

Figure 1: Cell Viability and Metabolic Activity from Differential Preparations

Tissue Preparation	Cell Viability/Metabolic Activity (Mean ± SD)
Cartilage and Bone Smashed Paste (n=7)	33.4 ± 16.7%
Cartilage and Bone Cut Paste 1 (n=8)	99.3 ± 32.6%
Cartilage and Bone Cut Paste 2 (n=3)	107.8 ± 56.7%

Figure 2: Long-Term (28-day) Culture of Cartilage Constructs

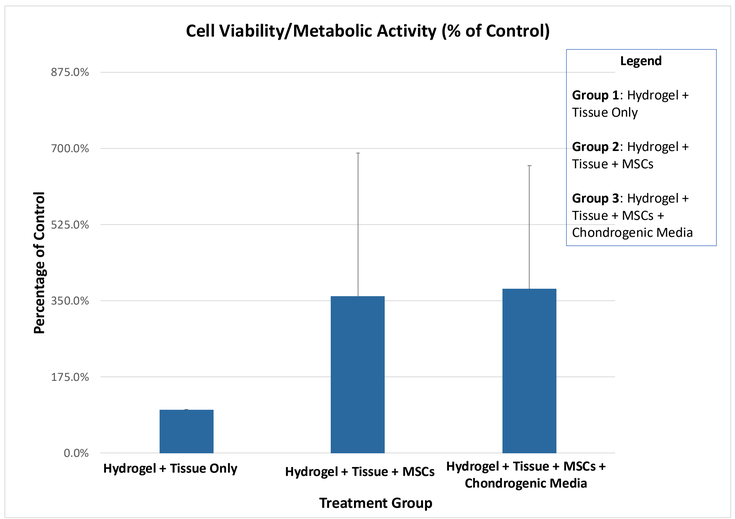

Fig. 2: Long-term (28 days) of culture in n=10 samples of human cartilage and bone tissue with PEGDA-hydrogel revealed cell viability/metabolic activity was enhanced by 370% in Group 3. ANOVA and subsequent Post-Hoc Tukey test showed a statistically significant difference between Groups 1 and 3 (**p = 0.03**)

Figure 3: Long Term (28-day) Culture of Tissue-Hydrogel Constructs

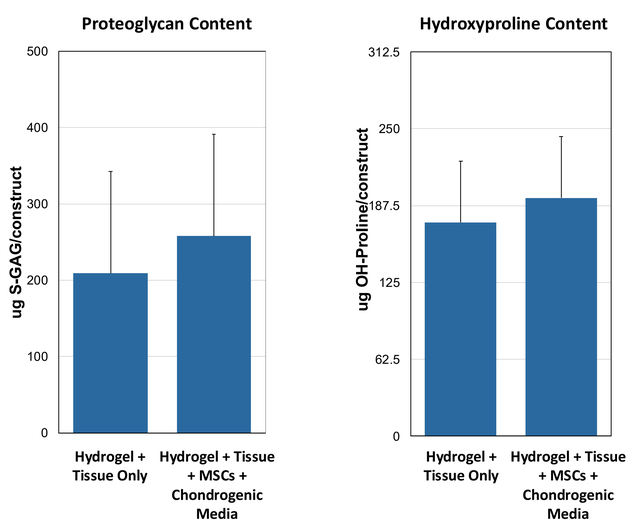

Fig. 3: S-GAG and OH-Proline were quantified as measurements of proteoglycan and collagen, respectively during long-term (28 days) culture. S-GAG content of constructs containing hMSCs with chondrogenic media was elevated by 23% (**p = 0.02**) compared to Hydrogel + Tissue alone. No significance reported for OH-Proline.

RESULTS: *IN VIVO*

Figure 4: Representative *In Vivo* Results (Gross Imaging, Histology, and Micro-CT)

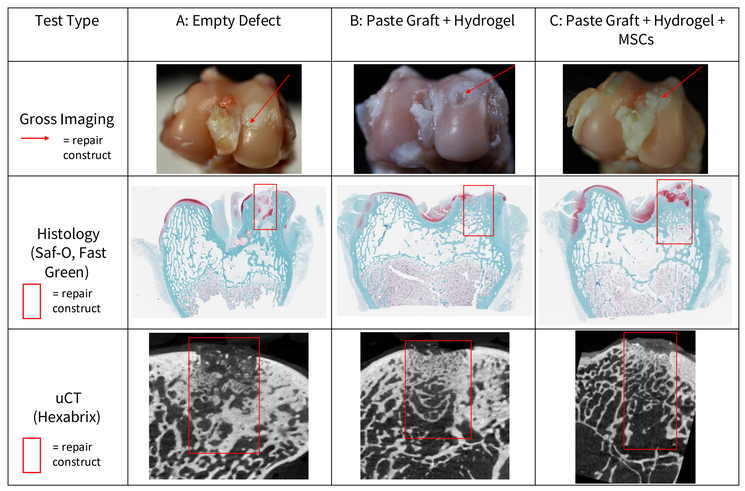

Figure 5: Histology Fill and Attachment

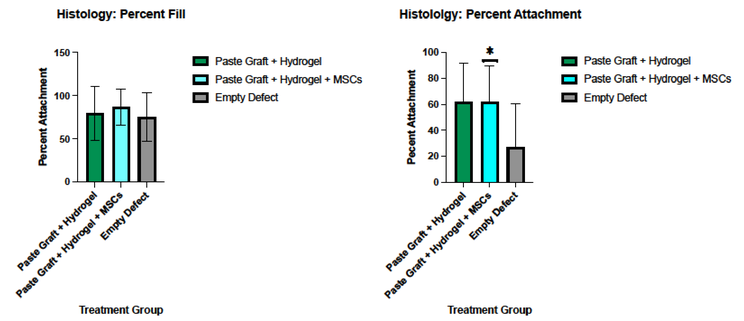

Fig. 5: Percent Fill and Percent Attachment to surrounding articular cartilage were calculated for n = 21 defects (defects positioned too close to the notch and thereby not surrounded by articular cartilage were excluded). There was no statistically significant difference in repair tissue fill, but the Paste Graft + Hydrogel + MSC group had significantly improved (**p = 0.03**) attachment to surrounding tissue.

DISCUSSION & CONCLUSION

Paste Graft augmented with hydrogel and MSCs results in increased proteoglycan content and significantly improved attachment to surrounding articular cartilage.

Findings suggest that both hydrogel and MSCs aid in the generation of a robust cartilage repair construct.

Next Steps: We are currently investigating the augmented Paste Graft in an equine model under FDA & CVM supervision.

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