HARNESSING ENDOGENOUS BONE MARROW STEM CELLS TO AUGMENT RABBIT AND EQUINE ARTICULAR CARTILAGE RENEGERATION: PRE-CLINICAL 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 (ArtCart) 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 stem cells, 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 mesenchymal stromal cells (MSCs) will amplify the effects of bone marrow stem cells, and the addition of hydrogel to the paste graft will improve handling characteristics and integration of the graft with surrounding native tissue

METHODS

In Vivo Rabbit

- One articular cartilage defect (3-mm diameter, 5-mm deep) was made on the medial femoral condyle of the right knee in n=26 rabbits
- Gross imaging, histology via Safranin O/Fast Green staining, and confirmatory microCT with Hexabrix contrast to assess repair
- Histology was analyzed for percent defect fill and attachment to surrounding native articular cartilage and graded based on the ICRS II scoring system[7]
- Statistical analysis performed using one-way ANOVA and subsequent Bonferroni Post-Hoc Test ($p \leq 0.05$)

In Vivo Equine

- Bilateral knee articular cartilage defects (15-mm diameter, 2.5-mm deep) were made in the medial trochlear ridge of n=10 horses
- Gross imaging, immunohistochemistry (IHC), and histology via Safranin O/Fast Green staining graded based on ICRS II[7]
- Statistical analysis via SAS Version 9.4

RESULTS: *IN VIVO* RABBIT

Figure 1: Representative *In Vivo* Rabbit Results (Gross Imaging, Histology, and Micro-CT)

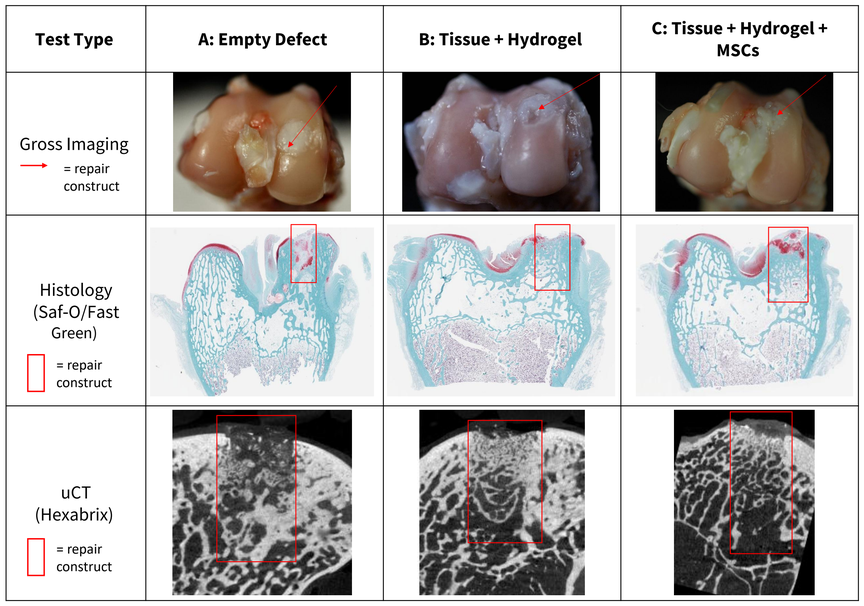

Figure 2: Histology Fill and Attachment

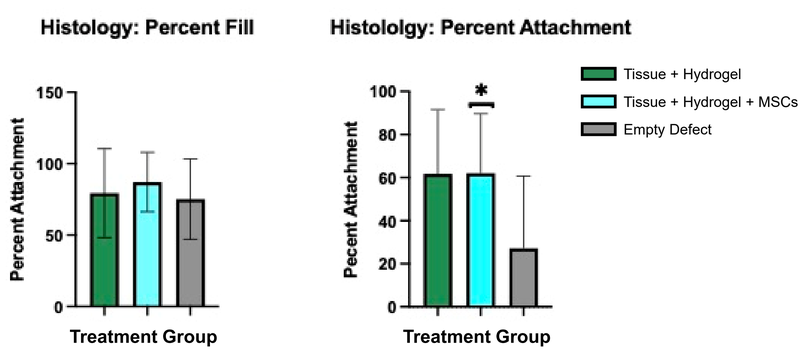

Fig. 2: Percent Fill and Percent Attachment to surrounding articular cartilage were calculated for n = 21 defects (defects positioned too close to the notch and thereby not surrounded by articular cartilage were excluded). There was no statistically significant difference in repair tissue fill, but the Tissue + Hydrogel + MSC group had significantly improved (**p = 0.03**) attachment to surrounding tissue.

Figure 3: Histology ICRS II Analysis

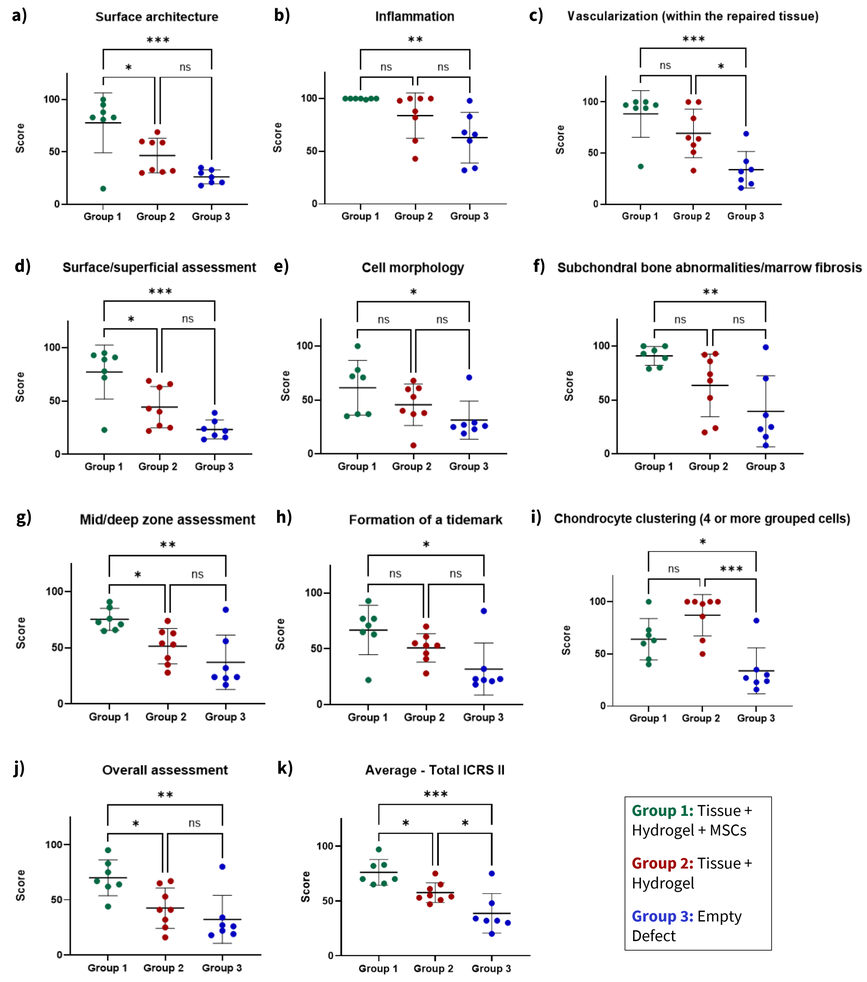

Fig. 3: Graphical representation of a-i) ICRS II scoring components and k) Total ICRS II. ***p = 0.05, **p = 0.01, ***p = 0.001**. Histological evaluation using the ICRS II scoring system showed that Group 1 (Tissue + Hydrogel + MSCs) consistently achieved the highest scores across multiple key parameters associated with cartilage regeneration and tissue quality. Statistically significant improvements **(p = 0.001)** in Group 1 compared with Group 3 (Empty Defect) were observed in a) surface architecture, b) inflammation, c) vascularization, d) surface/superficial assessment, e) cell morphology, f) subchondral bone abnormalities/marrow fibrosis, g) mid/deep zone assessment, h) formation of a tidemark, i) chondrocyte clustering, j) overall assessment, and k) total ICRS II score.

RESULTS: *IN VIVO* EQUINE

Figure 4: Representative *In Vivo* Equine Results (Gross Imaging and Histology)

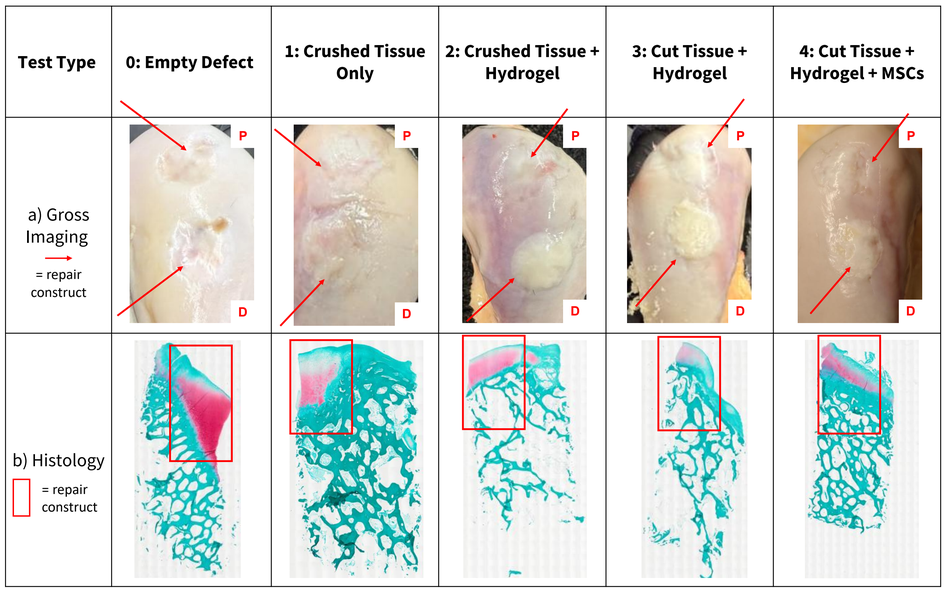

Fig. 4: a) Endpoint gross imaging (12-month) of bilateral defects, proximal (P) and distal (D); and b) histology biopsy (6-month) with Saf-O/Fast Green staining.

Figure 5: Histology (ICRS II) and IHC Analysis

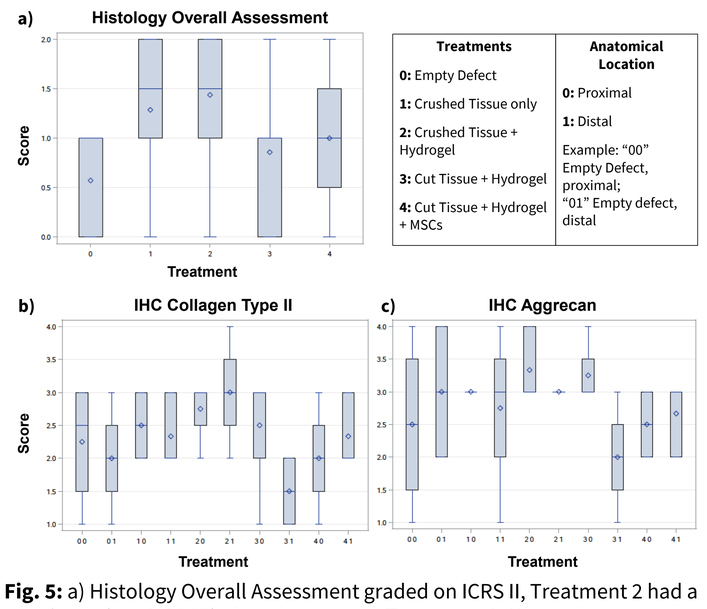

Fig. 5: a) Histology Overall Assessment graded on ICRS II, Treatment 2 had a significant **(p = 0.0045)** higher score than Treatment 0. No statistically significant difference was observed in b) collagen type II and c) aggrecan IHC. However, the proximal defect in Treatment group 3 yielded a higher aggrecan score. Treatment group 2 showed greater collagen type II score compared to Treatment 0 and 3.

DISCUSSION & CONCLUSION

Overall, our findings support the therapeutic potential of the Articular Cartilage Paste Graft with hydrogel and MSCs for the repair of focal cartilage defects *in vivo*.

The addition of hydrogel and MSCs to ArtCart enhances structural repair quality and promotes more hyaline-like regeneration of cartilage.

Next Steps: We will further evaluate the Tissue + Hydrogel + MSCs construct as the lead therapeutic candidate in an *in vivo* equine study under FDA & CVM supervision.

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