Bioactive CPC-Loaded Scaffold Enhances Meniscal Repair in a Yucatan Minipig Model

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Disclosures: Braden C. Fleming (3C - MIACH Orthopedics LLC, 7B & 8 - American Journal of Sports Medicine, 9 - Orthopedic Research Society), Brett D. Owens (8 - American Journal of Sports Medicine), All other authors (N)

INTRODUCTION: Meniscal injuries are a common source of knee pain and dysfunction and often progress to osteoarthritis (OA) due to altered joint mechanics and limited healing capacity [(1)]. While suture repair is the standard treatment, it may be insufficient to fully restore the meniscal function or prevent OA. Previous work in our lab demonstrated that intra-articular injection of cartilage progenitor cells (CPCs) can enhance meniscus repair and prevent post-traumatic OA in a rat model [(2,3)]. This study aims to evaluate the therapeutic potential of allogeneic CPCs delivered with a fibrin-thrombin scaffold enriched with Stromal Cell-Derived Factor-1 (SDF-1) to support suture repair in a Yucatan minipig model, which closely mimics human knee anatomy and biomechanics. We hypothesize that this combination of biologic and surgical approaches will accelerate healing and improve meniscal repair outcomes in a large animal model.

METHODS: ***Isolating and culturing Porcine CPCs:*** Allogeneic porcine CPCs (P-CPCs) were isolated from articular knee and hip cartilage of Yucatan minipigs, with IACUC approval. Pronase and Collagenase Type IA was used to extract cells. CPCs were enriched by fibronectin adhesion and cultured in DMEM growth media. ***Meniscus Injury Model and Treatment groups:*** A longitudinal tear was created in the red-white zone of the medial meniscus of young adult male Yucatan minipigs (15–18 months old) using a 6-mm linear osteotome. Synovial fluid was also collected from the knee joint during surgery. Menisci were treated with either (I) P-CPCs (4.0×10^6) in fibrin scaffold (50 µL) containing SDF-1 (50 ng/mL); (II) fibrin scaffold with no P-CPCs containing only SDF-1 (scaffold control); or (III) left untreated (negative control). Following treatment, all menisci were sutured using the standard clinical inside-out method, and the animals were allowed to recover for 3 months. Male minipigs were used in this initial study to minimize variability associated with hormonal cycles. Inclusion of females is planned in future studies to evaluate potential sex-related differences. ***Tissue Processing and Staining:*** After euthanasia, synovial fluid from the knee joints were collected and menisci and tibial plateau were harvested for histopathological analysis involving decalcifying and paraffin embedding. Sections of both menisci and tibial plateau were cut at thickness of 4 µm and stained with Safranin O/Fast Green. ***Quantification of Meniscus Defect Filling:*** Average depth of the tear was quantified using ImageJ as an assessment of the extent of healing observed between groups. ***Cartilage OARSI Scoring:*** Scoring was conducted on the medial tibial plateau of the injured knee by three blinded assessors, independently. The results from these individual scorers were then averaged to provide a final score. ***ELISA:*** Synovial fluid protein concentration of MMP-13, IL-6 and IL-17 were determined by Sandwich ELISA according to manufacturer's protocol. ***Statistical Analysis:*** Statistical analysis was performed using a Brown-Forsythe and Welch ANOVA test to examine the effects of treatment and Pearson correlation test was performed to assess the relationship between meniscal tear severity and cartilage degradation severity, n≥5 per experimental group.

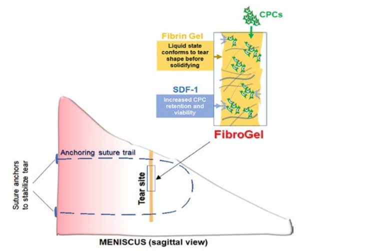

Fig.1. Bioactive cell carrier designed to conform to – and interface with meniscal tear for stimulating healing. The hydrogel contains P-CPCs encapsulated in a fibrin sealant mimicking a clot, infused with SDF-1 to enhance P-CPC viability and retention at the treatment site. Upon administration into the meniscal tear, stabilizing sutures are used to secure the tear and prevent reopening during load bearing.

RESULTS: Both treatment groups (CPCs + scaffold, and scaffold alone) showed significantly better healing compared to untreated controls ($p \leq 0.05$) **(Fig. 2)**. The CPC group demonstrated improved cartilage histopathology with lower OARSI scores, though statistical significance approached but did not reach the threshold ($p = 0.052$). A strong positive correlation was found between meniscal tear depth and cartilage degeneration ($r = 0.738$, $p < 0.001$), underscoring the clinical relevance of effective tear repair **(Fig. 3)**. ELISA data showed that MMP-13 increased in the two experimental groups compared to the control group, while IL-6 was unchanged, and IL-17 was reduced in both the experimental groups. The standard deviation between groups was moderate to high. N≥4 **(Fig. 4)**.

DISCUSSION: Incorporating CPCs within a bioactive hydrogel scaffold alongside suture repair significantly enhances meniscal healing in a large animal model. Notably, cartilage protection was also significantly improved, as evidenced by a lower OARSI score. The strong positive correlation between meniscal tear depth and cartilage degeneration highlights the need for effective treatments for deeper tears. These results suggest that biologic and cell-based therapies are promising for enhancing meniscal repair and preventing cartilage degeneration in a large animal model.

CLINICAL RELEVANCE: This study supports the therapeutic potential of combining CPCs with a bioactive scaffold for meniscus repair. Such strategies may improve healing outcomes and reduce the risk of OA development, paving the way for translational studies in human musculoskeletal soft tissue repair.

REFERENCES: *(1) Horie, M. et al. Stem Cells. 2009 (2) Desai et al. Front. Bioeng. Biotechnol., 2022 (3) Jayasuriya et al. Stem Cells. 2019*

ACKNOWLEDGEMENTS: This research is supported by DOD Grant W81XWH-20-1-0773

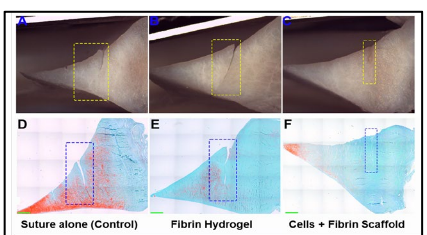

Fig.2. Macroscopic and microscopic images of medial meniscus tears treated with three different conditions at 3-months post-injury. Sagittal plane images of medial meniscus tears and corresponding histology sections stained with Saf-O/Fast Green. (A, D) Tear repaired with suture alone; (B, E) Tear repaired with fibrin hydrogel without CPCs followed by suture; (C, F) Tear repaired with fibrin hydrogel containing CPCs followed by suture; N≥5. *, p≤0.05.

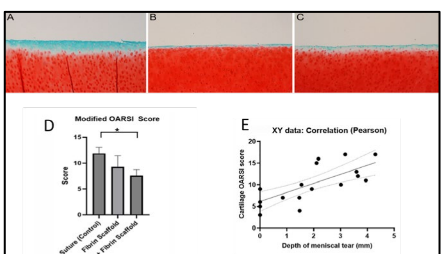

Fig.3. Histological assessment of Cartilage. Articular cartilage of the tibial plateau in Yucatan minipigs that were treated for a meniscal tear by **(A)** Standard suture repair control; **(B)** fibrin scaffold + suture repair; and **(C)** fibrin scaffold + CPCs + suture repair. All tissues were collected at 3-months post injury and stained with Saf-O/Fast Green. Red staining signifies rich proteoglycan content whereas blue indicates the loss of proteoglycans. **(D)** OARSI scoring results for each respective group. N≥5. **(E)** Pearson Correlation between cartilage OARSI and meniscal tear depth shows strong positive correlation ($r = 0.7380$, $p = 0.0003$).

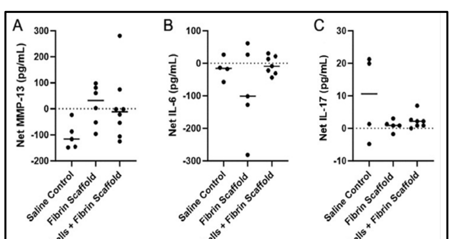

Fig.4. Catabolic marker changes in animals following treatment. (A) Net Matrix metalloproteinase (MMP)-13, **(B)** Interleukin (IL)-6, and **(C)** IL-17 levels at three months post-treatment in each experimental group. Data suggests that MMP-13 is increased in the two experimental groups compared to the control group, while IL-6 is generally unchanged, and IL-17 is reduced in both the experimental groups. The standard deviation between groups is moderate to high. N≥4.