

# Comparison of Healthy and Osteoarthritic Human Meniscus-derived Matrix Scaffolds for Meniscus Repair

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**INTRODUCTION:** The meniscus is frequently injured due to both sports injuries and age-related degeneration (1,2). Meniscus tissue engineering is a promising approach to replace lost and/or damaged meniscus tissue. Meniscus-derived matrix (MDM) scaffolds contain biological cues that can enhance cell infiltration, remodeling, and regeneration of injured meniscus tissue (3,4). MDM can be isolated from allogeneic or xenogeneic meniscus tissues; however, similarity of the composition of xenogenic scaffolds and availability of healthy human meniscus tissue remains challenging. On the other hand, there is a large abundance of human osteoarthritic menisci (5). Therefore, the objectives of this study were to generate and compare healthy (H) and osteoarthritic (OA) MDM scaffolds to assess *in vitro* meniscus cellular responses and integrative meniscus repair using an *ex vivo* meniscus repair model system.

**METHODS:** **Scaffold fabrication:** Healthy medial meniscus allografts (N=10) from 12-30 year old donors were provided by JRF Ortho and surgical waste OA meniscus tissue from 54-81 year old joint replacement patients (N=30) were collected using an IRB approved waste protocol and frozen at -80°C. Fig. 1 shows the methods used for fabricating healthy (H) and OA scaffolds (3,7). ***In vitro* cellular response:** Human meniscus fibrochondrocytes (hMFCs) were enzymatically isolated from fresh surgical waste OA menisci (N=3 pooled) and vacuum seeded onto healthy and OA MDM scaffolds at  $1.32 \times 10^5$  cells/scaffold (3,4,8). Cell viability was measured on days 3 and 7 by Live/Dead staining (n=3). Proliferation was quantified on days 4 and 14 by Edu staining (n=3). At days 1, 4, 7, and 14, MDM scaffolds were digested in papain for biochemical assessments (n=3) (3). ***Ex vivo* meniscus repair model:** Tissue repair model explants (8mm diameter) were harvested along the centerline of fresh OA human menisci and then cut to a uniform thickness of 2 mm (Fig. 3A). A 3 mm diameter inner core was removed from the explant to simulate a full-thickness defect. For control samples (Meniscus), the inner core was immediately returned to the defect (Fig. 3B). For the experimental groups (scaffold + meniscus), the defect was filled with H (Fig. 3C) or OA (Fig. 3D) MDM scaffolds. After 28 days, push-out testing was used to determine the integrative shear strength of repair (n=18). The MDM scaffolds were digested in papain and biochemical assessments (n=18) were performed. **Statistical analyses:** All data were normally distributed. For *in vitro* experiments, a two-way ANOVA followed by Šidák's multiple comparisons post-hoc test and for *ex vivo* experiments t-tests were performed. p<0.05 was considered statistically significant.

**RESULTS:** Human MFCs showed more than 90% viability within both the H and OA MDM scaffolds at days 3 and 7 with no detectable differences between the scaffolds. Higher proliferation of hMFCs was observed at day 4 than day 14 (p<0.05) for both the H and OA MDM scaffolds but no difference was observed between the H and OA scaffolds (Fig. 2A). DNA content significantly increased over 14 days (Fig. 2B, p<0.05), sGAG content increased from days 7 to 14 (p<0.05), and collagen content remained stable in both H and OA scaffolds. Interestingly, both DNA (Fig. 2B) and collagen content were higher in the OA than the H scaffolds (p<0.05). After 28 days in the *ex vivo* meniscus repair model, H and OA MDM scaffolds contained similar amounts of DNA, sGAG, and collagen and were ~3-fold lower than meniscus tissue controls (Fig. 4 A-C). However, both the H and OA MDM scaffolds showed similar repair strength to meniscus tissue controls (Fig. 4D). Histological analysis revealed that the H and OA scaffolds had integrated with the surrounding meniscus tissue (Fig. 4E).

**DISCUSSION:** Our study compared scaffolds derived from both healthy and OA menisci, while prior studies have used meniscus from OA patients (9), cadavers (10), or both (11) as a tissue source. We hypothesized that healthy MDM scaffolds, which were also from younger donors, would contain biological cues that would lead to improved cellular responses and integrative repair. However, we found that OA scaffolds showed more favorable cellular growth and collagen content over 14 days *in vitro* and healthy and OA scaffolds performed similarly for repair. Interestingly, while *in vitro* cell viability and proliferation at the measured time points were not detectably different between the healthy and OA scaffolds, the overall DNA content and collagen content were both significantly increased in the OA scaffolds, suggesting that more hMFCs were able to attach to the OA scaffolds at the time of seeding and produce more collagen throughout culture. However, in the *ex vivo* model, where the hMFCs grew into the scaffolds from the surrounding meniscus tissue, there were no detectable differences in the scaffolds after 28 days. Overall, we found that both healthy and OA MDM scaffolds had similar *ex vivo* repair with OA human meniscus tissue. While the scaffolds contained lower biochemical content in the repair model than the meniscus tissue control, this may be due to the use of meniscus tissue from end stage OA. It would be interesting to see if the repair responses were improved in non-OA, acutely-injured meniscus tissue, which would likely be more amenable for meniscus repair.

**SIGNIFICANCE:** OA meniscus tissue is a readily available and useful source for generating MDM scaffolds that support cellular growth, ECM production, and potentially integrative repair.

**References:** [1] Barrett+ JA&RS 1998, [2] Baker+ AJSM 1985, [3] Ruprecht+ SR 2019, [4] Lyons+ IJMS 2019, [5] Yuan+ JB 2016, [6] Kara+ JBA 2021, [7] Rowland+ Biomat 2016, [8] Andress+ JOR 2020, [9] Liang+ BMES 2019, [10] Spierings+ JEO 2022, [11] Liang+ Ac.Bio 2018.

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Fig 1. Fabrication procedure of H and OA MDM scaffolds.

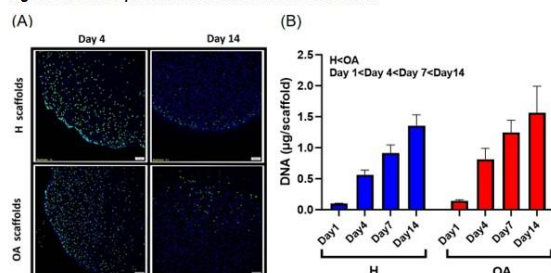


Fig 2. Cellular response of hMFCs to the H and OA MDM scaffolds. (A) EDU (green) staining on days 4 and 14 (B) DNA content of scaffolds over the 14 days of culture.

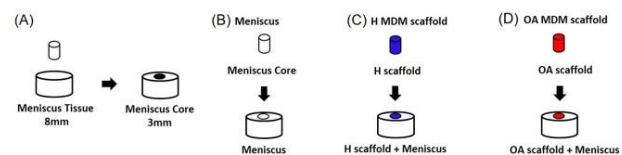


Fig 3. Schematic of meniscal defect model (A). Implantation of meniscus (B), H MDM scaffold (C) and OA MDM scaffold (D).

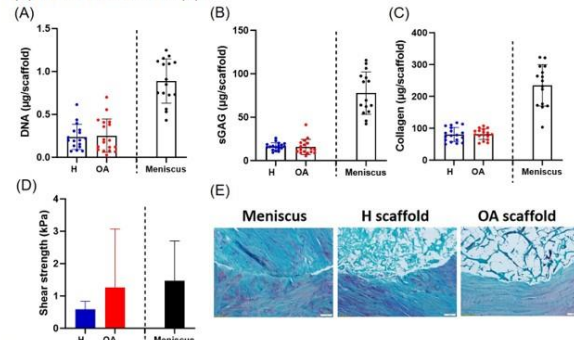


Fig 4. DNA content (A), sGAG (B), and collagen content (C) of the inner core meniscus and MDM scaffolds. Shear strength of repair (D). Histological assessments (E).