Meniscus Cellular Responses are Similar on Different Aged Meniscus-derived Matrix Scaffolds

Saman Firoozi[1], Rohan Shirwaiker[2], Matthew B. Fisher[2], Amy L. McNulty[1,3]
[1]Duke University School of Medicine, Durham, NC; [2]North Carolina State University, Raleigh, NC; [3]Duke University, Durham, NC
saman.firoozi@duke.edu

Disclosures: Saman Firoozi (N), Rohan Shirwaiker (N), Matthew Fisher (N), Amy McNulty (8-Connective Tissue Research)

INTRODUCTION: Injured meniscus tissue has limited repair potential and current therapeutic strategies do not restore functional meniscus tissue or prevent osteoarthritis (OA) development (1). Tissue engineering aims to promote the regeneration of tissues using bioactive scaffolds to replace damaged meniscus tissue (2). Meniscus-derived matrix (MDM) scaffolds contain biological cues that can improve cell infiltration, remodeling, and regeneration of injured meniscus tissue (3,4). Age-dependent changes in the vasculature and composition of the meniscus tissue (5) may influence cellular responses to MDM scaffolds generated from different aged tissues. Therefore, the objective of this study was to characterize vascularity and extracellular matrix (ECM) composition from different aged porcine menisci and evaluate *in vitro* meniscus cellular responses to MDM scaffolds derived from these tissues. We hypothesized that MDM scaffolds would contain age-dependent biological cues that would lead to different cellular responses.

METHODS: Tissue harvest: Menisci were harvested from the knees of 6 week, 7 month, and 3 year old pigs either sacrificed for other studies or from a local abattoir. The medial menisci from 6 week old pigs were from both female (N=5) and male (N=3) animals. Both medial and lateral menisci were harvested from 7 month old female pigs (N=4). Both the inner and outer zones of medial menisci were collected from 3 year old female pigs (N=7). All tissues were frozen at −80°C. Histological analysis: A cross-section of each tissue was taken for safranin O, fast green, and hematoxylin staining. Scaffold fabrication: MDM tissue was pulverized and either papain digested for biochemical assays or 8% MDM scaffolds were fabricated (6). Fabricated scaffolds were decellularized in 0.6mg/ml $MgCl_2$, 0.18mg/ml $CaCl_2$, 2.5% (v/v) Tris-HCl, and 50U/mL DNase at 37°C for 24h and then lyophilized (3,7). *In vitro* cellular response: Primary meniscus cells were enzymatically isolated from 3 year old female porcine medial meniscus tissue (N=3 pooled) and vacuum seeded in MDM scaffolds at $1.32x10^5$ cells/scaffold (3,4,8) (n=3/group/outcome). At days 1, 4, 7, and 14, the Cell counting kit-8 (CCK-8) was used to evaluate cell growth on the scaffolds. Scaffolds were papain digested and sGAG and collagen content were measured by DMMB and hydroxyproline assays respectively (3,4). Cell viability was measured on days 3 and 7 by Live/Dead staining. Proliferative cells were quantified on days 4 and 14 by Edu staining. Statistical analyses: All data were normally distributed. One-way ANOVA followed by Tukey post-hoc testing was performed for tissue characterization. Two-way ANOVA followed by Tukey's post-hoc test was performed for cell seeded experiments. Subgroup analyses (sex, location, and zone) were performed by t-tests with multiple comparison correction. $p<0.05$ was considered statistically significant.

RESULTS: Vascularity and tissue composition varied with tissue age (Fig. 1A). Inner zone vessel number (Fig. 1B, $p<0.05$) and DNA content (Fig. 2A, $p<0.05$) was highest in 6 week menisci and lowest in 3 year menisci. sGAG content was higher in 3 year old tissues than other ages (Fig. 2B, $p<0.05$), while collagen content was similar in all aged tissues (Fig. 2C). For the cell seeded scaffolds, meniscus cells numbers increased in all scaffolds over time and there were no detectable differences by age (Fig. 3A). Meniscal cells were greater than 80% viable on all scaffolds (Fig. 3B). There were no detectable differences among age or timepoint in either viability or proliferation (Fig. 4). sGAG content of 6 week old scaffolds was significantly lower than MDM scaffolds from 7 month and 3 year old tissues (Fig. 5A, $p<0.05$). While sGAG content in all scaffolds increased in early culture and then decreased from days 7 to 14 (Fig. 5A), collagen content was retained throughout culture in all scaffolds (Fig. 5B). Interestingly, in both tissue (Fig. 2C) and scaffolds (Fig. 5B) from 3 year old menisci, the collagen content of the outer zone was significantly higher than the inner zone. Further analyses by sex, location, and region are ongoing.

DISCUSSION: In contrast to our hypothesis, primary meniscus cells showed similar responses to all age-related MDM scaffolds over 14 days of culture despite the differences in vascularity and ECM composition in the different aged meniscus tissues. We found that the composition of the aged scaffolds was reflective of the ECM components in the different aged meniscus tissue. Vasculature and matrix composition of the meniscus are modulated throughout development (9) and many biological factors in the meniscus tissue change during aging (10). While these compositional changes did not appreciably affect differentiated meniscus cellular responses on the MDM scaffolds, it is possible that these differences may have a larger effect on stem and progenitor cells that may grow into scaffolds implanted *in vivo*. All aged MDM scaffolds showed high biocompatibility and supported cellular growth and expansion throughout culture. The reduction in sGAG content at day 14 was independent of age and may be due to leaching into the media. Our prior work has shown that scaffold crosslinking can help to retain sGAG content (6). Collagen content, one of the main ECM components, remained stable throughout culture in all scaffolds.

SIGNIFICANCE: All aged MDM scaffolds showed high biocompatibility and supported cellular growth and expansion throughout culture. Primary meniscal cells showed similar cellular responses to the different aged MDM scaffolds and thus different aged scaffolds may be useful for meniscus tissue engineering.

References: [1] Lamplot+ Bone Joint J. 2016, [2] Lin+ Biomat 2020, [3] Ruprecht+ SR 2019, [4] Lyons+ IJMS 2019, [5] Aidos+ Bioeng 2022, [6] Ruprecht+ SR 2019 [7] Rowland+ Biomat 2016, [8] Andress+ JOR 2020, [9] Smith+ Histochem.Cell. Bio. 2010, [10] Alessia+ JBRHA 2016 [10] Alessia+ JCCM 2017.

Acknowledgments: Research funding was provided in part by NIH grants AR078245 and AR073221.

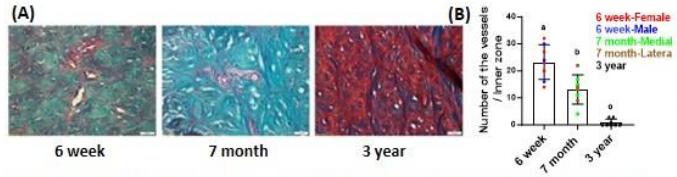

Fig.1 Safranin O/fast green staining of inner zone meniscus tissue (A). Quantification of the number of vessels (B). All groups not sharing the same letter have p-values < 0.05.

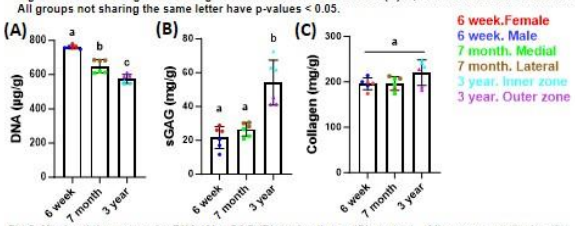

Fig 2. Meniscal tissue powder DNA (A), sGAG (B), and collagen (C) content. . All groups not sharing the same letter have p-values < 0.05.

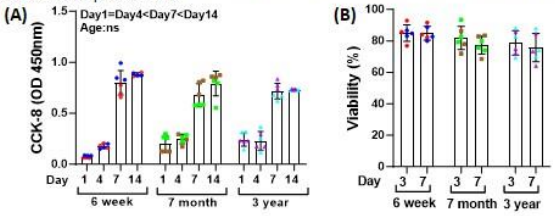

Fig 3. Cell seeded scaffold CCK8 assay (A) and quantification of the % viability (B).

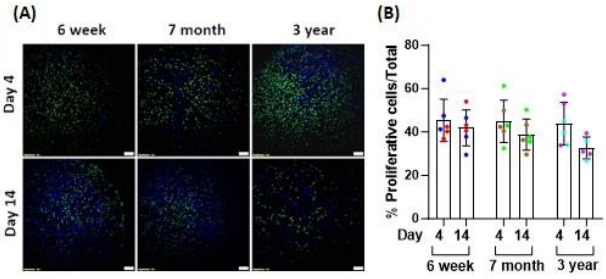

Fig 4. 4 Representative images of Edu+ (greens) cells and total cells (blue) (A) and percent proliferative cells per total cells on aged MDM scaffolds.

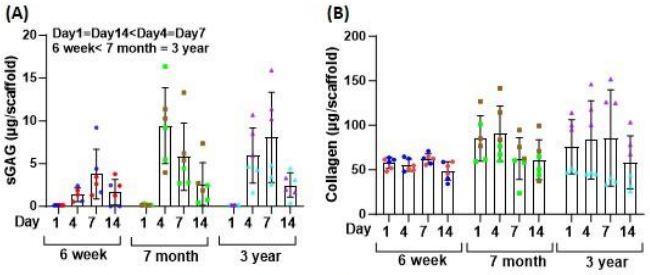

Fig 5. Cell seeded scaffold sGAG (A) and collagen (B) content.