Anatomically Precise Meniscus Derived Scaffolds with Zonal and Regional Fidelity for Functional Repair

Fatima Elzahra Essassi and Solaiman Tarafder
Department of Mechanical Engineering, South Dakota State University, Brookings, South Dakota
Email: FatimaElzahra.Essassi@sdstate.edu / Solaiman.Tarafder@sdstate.edu

DISCLOSURES: Fatima Elzahra Essassi (N), Solaiman Tarafder (N)

INTRODUCTION: The knee meniscus is a structurally specialized tissue essential for load transmission, shock absorption, and joint stability. Its outer vascularized zone, composed primarily of type I collagen and fibroblast-like cells, provides tensile strength, while the inner avascular zone, rich in type II collagen and chondrocyte-like cells, contributes to compressive resistance and viscoelasticity. The interplay of these distinct regions is critical for long-term joint function. Meniscal injuries remain a major clinical challenge and are strongly associated with osteoarthritis if untreated or inadequately repaired. Current repair strategies, including synthetic implants and decellularized scaffolds, have shown limited success, largely due to their inability to reproduce the meniscus's inherent zonal and regional heterogeneity. Meniscus-derived matrix (MDM) scaffolds preserve native extracellular matrix components and show promise in promoting cell recruitment and integration, yet most studies have treated the meniscus as a bulk tissue, without considering zonal or medial–lateral differences. Here, we develop and characterize ECM scaffolds from four anatomical regions - inner and outer zones of the medial and lateral menisci. We hypothesize that region- and zone-specific scaffolds exhibit distinct biochemical and biomechanical profiles reflective of their native functions, providing a foundation for biomimetic, anatomically precise meniscal repair strategies.

METHODS: Extracellular matrix (ECM) scaffolds were prepared from anatomically defined regions of fresh bovine menisci: inner zone medial (IZM), outer zone medial (OZM), inner zone lateral (IZL), and outer zone lateral (OZL). Following harvest and cleaning, tissues were decellularized using freeze–thaw cycles and sequential treatments with NH_4Cl, NaOH, HCl, EDTA, and guanidine hydrochloride at 4 °C, then washed, freeze-dried, and milled into powder. Scaffolds were fabricated by casting meniscus-derived ECM solution into 3D printed customized molds (2 × 2 in., 3.5 mm thick) followed by freeze-drying. Scaffolds were assigned to non-crosslinked or crosslinked groups, the latter treated with EDC/NHS for 5 h at room temperature, then washed with phosphate buffer and DI water. Characterization included porosity testing, SEM for microstructure, and mechanical testing (compression on 4 mm punches, tensile on 3 × 12 mm strips) after PBS equilibration, using a Univert mechanical tester. Crosslinking was verified by FTIR (amide I/II shifts, hydroxyl reduction). Viscoelastic properties were measured via oscillatory rheology, and swelling/degradation assessed in PBS. Meniscal tissue regeneration was evaluated by culturing hBMSCs on the scaffolds for 4 weeks followed by histological evaluations.

RESULTS: SEM imaging confirmed compact and organized pore networks in both crosslinked (CL) and non-crosslinked (N-CL) scaffolds (as shown in **Fig. 1A** for CL scaffold). Non-crosslinked scaffolds exhibited slightly higher porosity across all regions, with IZL reaching up to 86%. Crosslinked scaffolds showed slightly reduced porosity, indicating matrix densification following EDC/NHS treatment (**Fig. 1B**). FTIR spectroscopy confirmed successful crosslinking, evidenced by characteristic shifts in amide I and II bands and a reduction in free hydroxyl and amine groups (Data not shown here). Rheological analysis showed that ECM derived from outer zone had higher storage and loss moduli (**Fig. 1C**) than the inner zone (**Fig. 1D**), indicating better viscoelastic performance under dynamic loading. Degradation profile (**Fig. 1E**) conducted in PBS demonstrated greater structural stability in crosslinked scaffolds, whereas non-crosslinked scaffolds showed faster degradation, consistent with their more open microarchitecture (data not shown here). Mechanical testing was performed on PBS-equilibrated scaffolds under hydrated conditions. Inner zone scaffolds (IZM, IZL) demonstrated higher compressive modulus (**Fig. 1F**) compared to outer zones (OZM, OZL), consistent with their denser structure. Interestingly, scaffolds derived from inner zone ECM also exhibited higher tensile moduli (**Fig. 1G**) and tensile strength (**Fig. 1H**) compared to those derived from outer zone ECM. Preliminary histological analysis is ongoing to assess cell-scaffold interactions and regional differences in functional meniscus tissue regeneration.

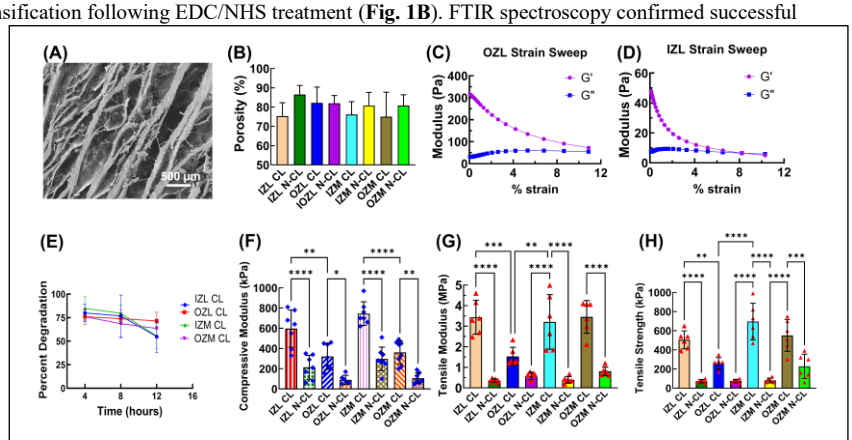

Fig. 1. Physico-mechanical characterization of anatomically precise meniscus-derived ECM scaffolds: (A) Representative SEM image of a crosslinked (CL) porous scaffold prepared from the inner-zone meniscus ECM matrix, (B) Porosity of ECM-derived scaffolds from different anatomical locations and zones, (C–D) Rheological strain sweep analysis showing storage modulus (G') and loss modulus (G'') as a function of strain for scaffolds derived from (C) outer-zone lateral (OZL) and (D) inner-zone lateral (IZL) regions, (E) Degradation profiles of native bovine meniscus tissue, non-crosslinked (N-CL) scaffolds, and crosslinked (CL) scaffolds, (F) Compressive modulus, (G) tensile modulus, and (H) tensile strength of scaffolds before and after crosslinking. (Data represent n = 4–8 per group; *p < 0.05).

DISCUSSION: Separating meniscal tissue into four anatomically defined regions enabled systematic evaluation of how zonal and regional origin influences scaffold properties. Inner zone scaffolds (from both medial and lateral) exhibited higher compressive modulus and superior viscoelasticity, consistent with their denser microarchitecture and type II collagen content, which supports water retention and resistance to axial loading. Unexpectedly, inner zone scaffolds also demonstrated higher tensile strength and modulus than outer zone scaffolds, suggesting that decellularization and processing preserved functionally relevant collagen interactions that enhanced tensile behavior beyond native expectations. These findings underscore the importance of direct experimental evaluation rather than relying solely on assumptions from native tissue composition. Crosslinking further improved mechanical integrity across all scaffold types, enhancing cohesion and resistance to degradation while maintaining porosity for cell infiltration. Rheological testing confirmed increased dynamic mechanical properties, particularly in inner zone scaffolds. Beyond zonal specialization, medial–lateral comparisons revealed additional distinctions. Medial scaffolds, especially IZM, retained porosity and compressive properties more effectively, suggesting regional differences in collagen organization and matrix composition that may influence repair dynamics. Together, these findings highlight the need for scaffold designs that account for both zonal and regional identity. Anatomically precise scaffolds may better recapitulate native function, improve integration, and advance next-generation strategies for meniscal repair.

SIGNIFICANCE/CLINICAL RELEVANCE: This study establishes the first anatomically precise meniscus-derived scaffolds that preserve both zonal and regional heterogeneity, providing critical insight into structure–function relationships overlooked in prior work. By aligning scaffold properties with native meniscal specialization, these findings lay the groundwork for developing more effective, biomimetic implants that may improve integration, durability, and clinical outcomes in meniscus repair and osteoarthritis prevention.

ACKNOWLEDGEMENTS: The authors acknowledge the support from the National Science Foundation (NSF) under the award # 2347673 to Dr. Solaiman Tarafder.