Improved Meniscus Integration via Controlled Degradation of the Wound Interface

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Introduction

Meniscal tears are prevalent and poor intrinsic healing (especially in avascular zones) directs clinical treatment towards partial removal rather than repair. However, removal alters joint biomechanics and can lead to early osteoarthritis.[1] As there are limited restorative strategies (e.g. replacement using allografts), methods to foster repair by promoting cell growth, extracellular matrix (ECM) production, and integration would represent a marked clinical advance. Previously, we showed that fetal and juvenile menisci have greater intrinsic healing capacity compared to adult meniscus,[2] and hypothesized that the high ECM density and low local cell density in adult meniscus may present physical and biologic barriers to endogenous healing. Such considerations have also arisen in cartilage-to-cartilage integration, where decreasing the local ECM density improves tissue repair.[3] To test this hypothesis in the context of the meniscus, we explored how controlled degradation of the local ECM at the wound interface might expedite healing by facilitating cell migration and division at the wound site and subsequent tissue remodeling. Furthermore, we used enzyme-releasing scaffolds to demonstrate how this technology might be applied clinically to promote meniscal repair.

Methods

Modulation of adult meniscus ECM density: Tissue cylinders were excised with a biopsy punch from fetal and adult bovine menisci (8 mm diameter) and cored with a 4 mm punch. Samples were incubated in basal media (BM) containing 0.05 mg/ml collagenase (type IV) for 6 hours, after which the cores were replaced within the annuli (Fig. 1). Controls were incubated in BM only. Repair constructs were cultured in a chemically-defined medium[2] with transforming growth factor β3 for 4 weeks. Paraffin sections were stained with hematoxylin and eosin (H&E) to visualize cell nuclei and ECM density (n=1-2). To investigate the long-term effect of collagenase treatment on mature menisci, adult samples were incubated with 0 (BM), 0.01 (low collagenase, LC), or 0.05 mg/ml (high collagenase, HC) collagenase, reassembled into repair constructs, and cultured for 1, 4, or 8 weeks. Samples (n=4) were saturated in Lugol's solution and imaged via microcomputed tomography (μCT, ScanCo, VivaCT 70). Afterwards, sections were stained with either H&E, picrosirius red (PSR) for collagen, or 4', 6-diamidino-2-phenylindole (DAPI) for cell nuclei. Integration was defined as the cumulative distance of annulus-to-core contact normalized by the core perimeter (n=3-4 samples/group). Cell density at the interface was determined by counting the number of nuclei present within 100 μm of the interface (n=4 samples/group). Significance was assessed by two-way ANOVA with Tukey's HSD post hoc tests to compare groups ($p \leq 0.05$).

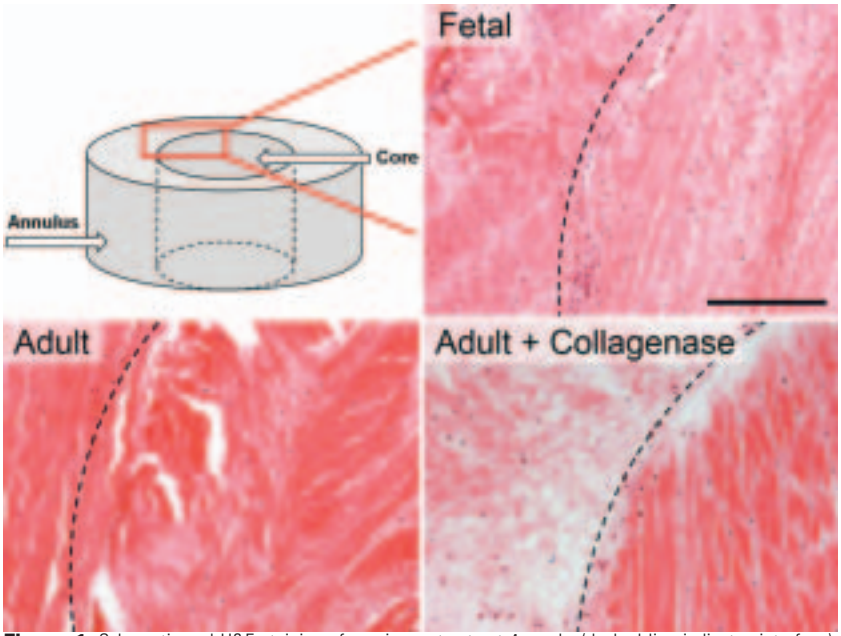

Figure 1. Schematic and H&E staining of repair constructs at 4 weeks (dashed line indicates interface). Scale = 0.25 mm.

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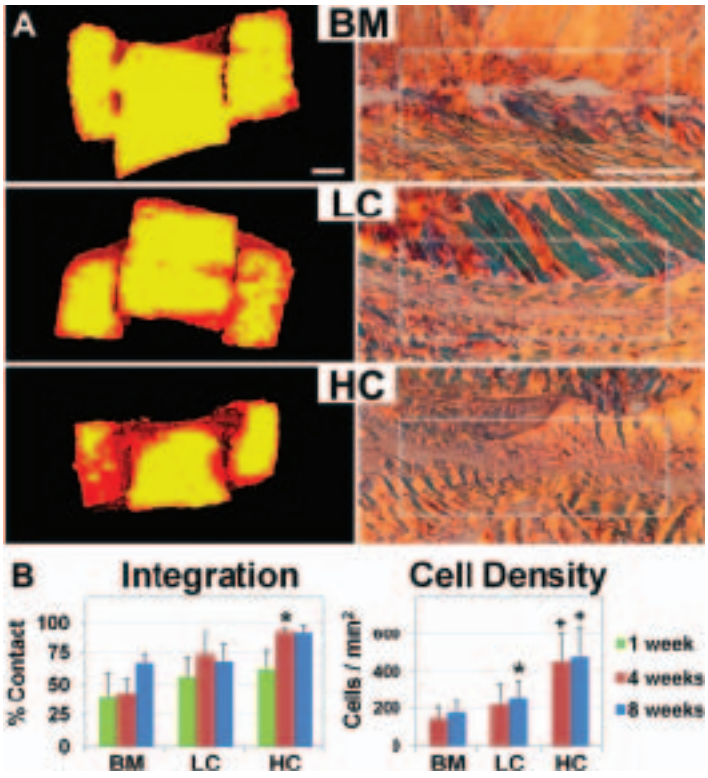

Figure 2A-B. Changes with exposure to no (BM), low (HC), and high-dose (HC) collagenase. (A) Left: 1 week μCT scans with low (red) and high (yellow) signal intensity. Scale = 1 mm. Right: 8 week PSR sections of the interface under polarized light. Scale = 0.25 mm. (B) Left: integration normalized to core perimeter. Right: cell density at the interface. *=p≤0.05 compared to BM. +=p≤0.05 compared to BM and LC.

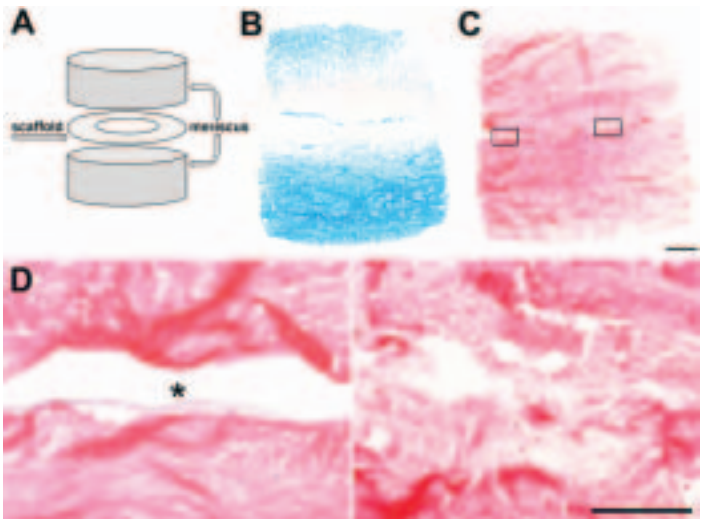

Figure 3A-D. Adult meniscus interface treated with collagenase-releasing scaffold at 7 days. (A) Schematic. (B) AB and (C) H&E staining. Scale = 1 mm. (D) Magnified areas from C. Left: lack of integration at the edge by the scaffold (asterisk). Right: bridging tissue in the interior. Scale = 0.25 mm.

Scaffold-mediated degradation of the wound interface: Electrospun nanofibrous scaffolds containing collagenase were placed inside a horizontal defect in cylindrical adult bovine meniscal explants (Fig. 3A). The scaffolds were comprised of poly(ε-caprolactone) (PCL) structural fibers and water-soluble poly(ethylene oxide) (PEO) fibers that released collagenase upon hydration.[4] Scaffolds were constructed in annular form (8 mm diameter with 5 mm core) to permit tissue-to-tissue contact within the construct. The defect was pinned closed and the repair construct cultured for 7 days. Paraffin sections were stained with either H&E or alcian blue (AB) for proteoglycans (PG).

Results

Collagenase digestion of adult meniscus resulted in a porous microenvironment that closely resembled fetal tissue (Fig. 1). Matrix degradation increased with enzyme dosage, reflected by the lower μCT signal at the construct edges (Fig. 2A). Long-term culture of adult constructs showed improved cellularity and integration with increasing collagenase digestion. By 4 weeks, cells and new collagen fibrils closed approximately 92% of the wound gap in adult HC samples, which exhibited superior integration compared to LC samples and BM controls (Fig. 2A and 2B, p≤0.05). Cell density at the annulus-core boundary was significantly higher for HC samples compared to all other groups, with a 213% and 170% increase over BM controls at 4 and 8 weeks, respectively (Fig. 2B, p≤0.05). Integration was also observed in adult explants exposed to enzyme-releasing scaffolds after 7 days, although the scaffold physically inhibited repair at the construct edges (Fig. 3D). AB staining confirmed localized digestion and loss of PG at the wound site (Fig. 3B).

Discussion

Our *in vitro* results suggest that partial digestion of the wound interface may benefit meniscal repair. As hypothesized, a dense ECM inhibited cell migration, proliferation, and matrix remodeling, whereas an ECM made penetrable and fetal-like was more conducive to these cell activities. High-dose collagenase treatment significantly improved cellularity at the wound margins while fostering the production of new contiguous tissue spanning the wound site. To ensure targeted degradation of the defect, we developed a delivery system in which collagenase was stored inside electrospun PEO nanofibers and released upon hydration.[4] Preliminary data demonstrated that collagenase-releasing scaffolds acted locally and resulted in a cellular response similar to that of global treatment with soluble collagenase. In the future, these scaffolds can be made more porous to facilitate cellular infiltration and further functionalized by incorporating growth factors that promote cell migration and matrix deposition, released either directly from nanofibers[5] or from drug-delivering microspheres.[6] Given the long time course and high failure rate of fibrous tissue healing, methods to enhance integration and instruct tissue formation will improve treatment of meniscal injuries.

Significance

Endogenous meniscal repair was improved via the targeted delivery of a matrix-degrading enzyme to the wound interface. This innovative approach may aid the many patients that exhibit meniscus tears, thereby circumventing the pathologic consequences of partial meniscus removal.

Acknowledgements

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