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Material-Mediated Degradation of the Meniscus Wound Interface Enhances Integration

[1,2]Feini Qu, BS
[1,2]Michael P. Pintauro
[1,2]Elizabeth A. Henning, BS
[1,2]John L. Esterhai, MD
[1,2]Matthew B. Fisher, PhD
[1,2]Robert L. Mauck, PhD

[1]McKay Orthopaedic Research Laboratory, University of Pennsylvania, Philadelphia, PA, USA

[2]Translational Musculoskeletal Research Center, Philadelphia VA Medical Center, Philadelphia, PA, USA

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feini@seas.upenn.edu

Introduction

Meniscus tears are one of the most common indications for arthroscopic surgery, but the limited healing of adult meniscus directs clinical practice towards resection (partial meniscectomy) rather than repair, increasing the risk for early-onset osteoarthritis.[1] As such, methods to augment endogenous repair could promote long-term joint health after injury. Previously, we showed that the meniscus extracellular matrix (ECM) increases in density with age, and hypothesized that this constitutes a physical barrier to repair.[2] To test this hypothesis, we treated the adult bovine meniscus wound edge with a matrix-degrading enzyme (collagenase) and showed improved cellularity and tissue integration *in vitro*.[3] To localize enzymatic treatment, we developed a material-mediated delivery system in which bioactive collagenase was released from polymer nanofibers. In this study, we tested the hypothesis that these novel scaffolds would enhance cellularity and matrix formation in bovine meniscus 'repair' constructs *in vivo* (placed subcutaneously in athymic rats).

Methods

Scaffold Fabrication

Nanofibrous composites were formed via electrospinning using a custom tri-jet device[4] to contain a poly(ε-caprolactone) (PCL) structural fiber fraction along with two water-soluble poly(ethylene oxide) (PEO) fiber fractions (~50-60% PEO by mass). PEO fibers contained either no collagenase (PCL/PEO) or 15.6% type IV collagenase by mass (PCL/PEO-C).[3] Scaffolds were cut to 5x5 mm with a 2 mm diameter fenestration to permit tissue-to-tissue contact inside the construct.

Repair Construct Preparation

Cylindrical adult bovine meniscal explants (8 mm diameter) were harvested, trimmed to a height of 4 mm, and incised to create a horizontal defect. Four treatment groups were prepared: empty defect incubated in basal media (control, BM), empty defect incubated for 6 hours in BM with 0.4 mg/mL collagenase (C), defect with a control scaffold (PCL/PEO), and defect with a collagenase-releasing scaffold (PCL/PEO-C).

Implantation Surgery

All procedures were approved by the Institutional Animal Care and Use Committee of the Philadelphia VA Medical Center. Four dorsal pockets were prepared in each of five male athymic nude rats (Crl:NIH-*Foxn1^{rnu}*, 300-350 g, Charles River). One repair construct per group was placed in each pocket. At 1 week (n=1) and 4 weeks (n=4) rats were euthanized by CO_2 asphyxiation, and samples were harvested for analysis.

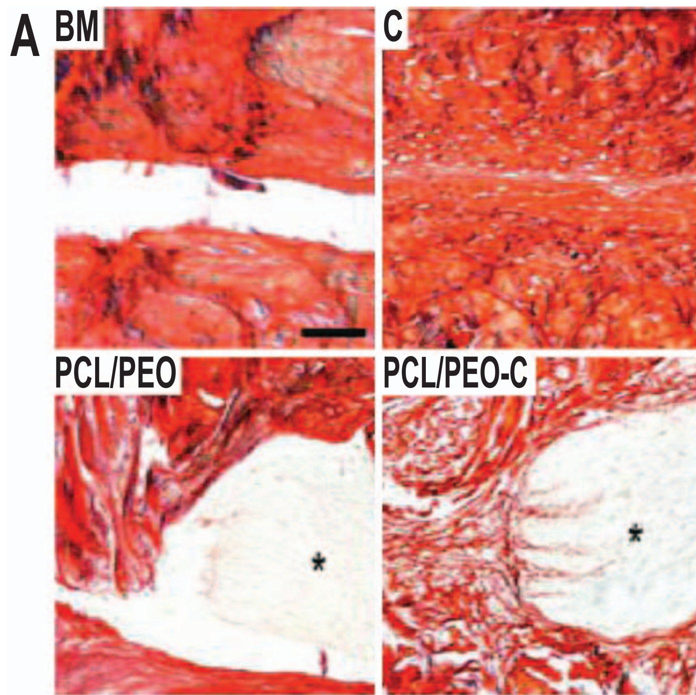
Sample Processing and Analysis

Samples were fixed with paraformaldehyde, paraffin embedded, sectioned, and stained with picrosirius red (PSR) for collagen or 4', 6-diamidino-2-phenylindole (DAPI) for cell nuclei. Immunohistochemistry for the cell surface marker CD45 was performed to identify cells of hematopoietic origin. Percent integration of the interface was calculated by dividing the cumulative length of cohesive tissue-tissue and tissue-scaffold segments by the defect length in PSR sections (n=3-4/group at 4 weeks). Cell signal intensity at the interface (n=4/group at 4 weeks) was determined by converting the cell nuclei stained with DAPI to white (intensity=255) and the background to black (intensity=0) using ImageJ (NIH). The pixel intensity along the defect was averaged starting from the interface up to 700 μm perpendicular to the interface and binned into intervals of 100 μm. Significance was assessed by one- and two-way ANOVA with Tukey's HSD post hoc tests to compare integration and signal intensity between groups, respectively ($p \leq 0.05$).

Results

After 1 week *in vivo*, untreated controls (BM) and samples containing control scaffolds (PCL/PEO) had a dense collagenous ECM and lacked matrix deposition at the center of the defect. In contrast, samples treated with aqueous collagenase (C) and collagenase-releasing scaffolds (PCL/PEO-C) showed a qualitative decrease in matrix staining and increased

cellularity at the interface, with defect closure across the construct. After 4 weeks, staining for collagen showed improved defect fill for all groups compared to 1 week. Tissue-tissue and tissue-scaffold integration was most abundant for PCL/PEO-C samples, with regions of the scaffold staining positive for collagen (Figure 1A). There was a trend towards improved integration for PCL/PEO-C samples (87 ± 9%) compared to BM controls (59 ± 16%) (Figure 1B, p=0.07). Staining of nuclei showed a significant increase in cell signal intensity at the interface for PCL/PEO-C samples vs. all other groups (Figure 2B, p≤0.05). This increase in cell number was significant for a distance of 300 μm from the interface



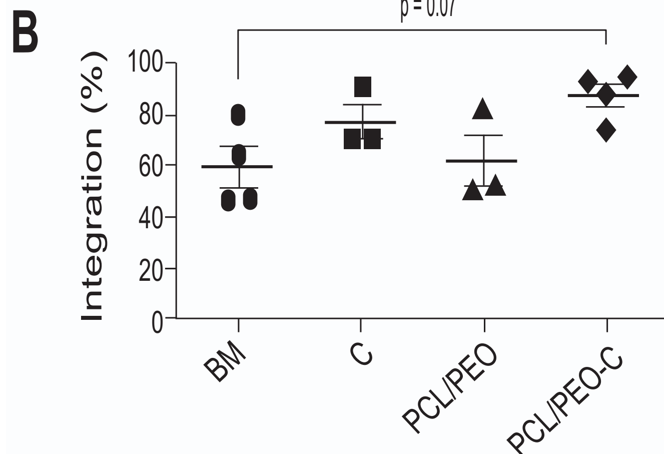


Figure 1. Improved integration of meniscus repair constructs with collagenase treatment (C and PCL/PEO-C) compared to controls (BM and PCL/PEO) at 4 weeks. (A) PSR staining of collagen at the defect. Asterisk indicates scaffold. Scale = 100 μm. (B) Integration normalized to defect length (n=3-4/group).

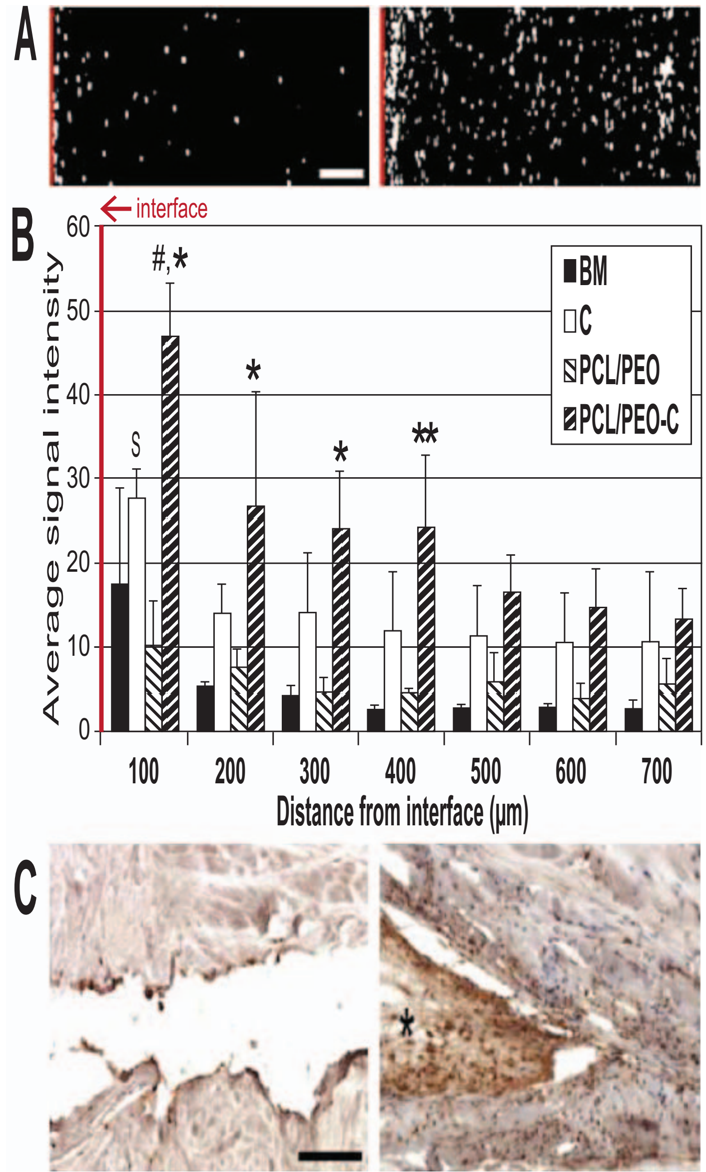


Figure 2. Collagenase treatment increased cellularity at the wound interface. (A) DAPI staining of cell nuclei for BM (left) and PCL/PEO-C (right) at 4 weeks. Interface is to the left (red line). Scale = 100 μm. (B) Average cell signal intensity vs. distance from the interface. $=p≤0.05 vs. C distances ≥400 μm, #=p≤0.05 vs. all other PCL/PEO-C distances, *=p≤0.05 vs. all groups, **=p≤0.05 vs. BM and PCL/PEO. (C) CD45 staining with hematoxylin counterstain for BM (left) and PCL/PEO-C (right) at 4 weeks, with more CD45+ cells (dark brown) evident in enzyme-treated samples. Asterisk indicates scaffold. Scale = 100 μm.

compared to collagenase alone, and 400 μm compared to BM and PCL/PEO groups. Immunohistochemistry revealed CD45+ cells at the constructs' periphery, with some penetrating the interior (Figure 2C).

Discussion

Our *in vivo* results suggest that material-mediated and localized delivery of a matrix-degrading enzyme to the meniscus wound interface enhances cellular infiltration and tissue integration. Without matrix digestion, few cells populated

the wound site or the PCL/PEO scaffold alone, leading to little or no integration at the defect center. However, collagenase-treated groups showed significant increases in cellularity and deposition of new matrix across the length of the defect, including within the PCL/PEO-C scaffold. The fenestration in the scaffold promoted integration, as cells colonized the empty space and synthesized collagen that bridged the wound gap. While some CD45+ cells, possibly macrophages, entered the repair, the majority of reparative cells did not stain positive and are likely meniscal cells or host-derived fibroblasts. Scaffolds may be modified to deliver anti-inflammatories[5] and growth factors[6] to limit inflammation and further promote matrix synthesis. Future studies will evaluate the efficacy of these scaffolds to enhance meniscus repair in an orthotopic large animal model. By combining multiple bioactive components that promote cellularity, colonization, and matrix deposition at the wound interface, these novel scaffolds offer a promising approach towards improving meniscus repair.

Significance

Meniscus integration was enhanced by localized degradation of the extracellular matrix via collagenase-releasing scaffolds in a rat subcutaneous implantation model. This technology may be combined with meniscus repair to expedite healing, thus avoiding the need for partial meniscus removal.

Acknowledgments

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