Matrix Degradation Enhances Cell Mobility in Dense Connective Tissues

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Introduction

Cell migration plays a pivotal role during tissue repair, where cells must first proliferate and colonize the wound site. Both high extracellular matrix (ECM) stiffness and density have been implicated as barriers to 3D migration,[1,2] especially when cell-mediated proteolysis via matrix metalloproteinases (MMPs) is inhibited. Although the highly organized ECM of mature dense connective tissues enables mechanical function,[3] these densely packed collagen fibers may inhibit cell migration after injury, resulting in poor healing in adults. We hypothesized that the adult ECM is a biophysical impediment to cell mobility during repair, and that reduction of this steric constraint would expedite cell migration to the wound site. Using the adult knee meniscus as a platform, we show that modulating the ECM microstructure via an exogenous matrix-degrading enzyme enhances interstitial cell mobility, and that this acts synergistically with endogenous MMPs to promote cell migration through the dense ECM.

Methods

Tissue Microstructure Analysis: Tissue explants (8 mm ø) were excised from adult bovine meniscal bodies and cryosectioned onto glass slides (~35 µm thick). Devitalized sections were UV sterilized and hydrated. Three substrates were tested: untreated adult ECM (Control), and adult ECM pre-treated with 0.05 or 0.1 mg/mL collagenase in basal media (BM) for 1 hour (LowC or HighC). To visualize fibrillar collagen, second harmonic generation (SHG) imaging was performed.[4] Maximum z-stack projections were used to identify discrete areas of positive and negative signal, representing aligned fibers parallel to the substrate and inter-fibrillar material that constitutes the remaining ECM (n = 10 stacks/group). The average diameter of discrete inter-fibrillar regions was quantified using Fiji's Local Thickness plugin (n = 10 stacks/group). *Interstitial Migration Analysis:* To visualize cell invasion, adult meniscal explants (4 mm ø) were incubated in CellTracker™ Green for 1 hour and then placed atop sections to allow for cell egress onto the section. Two media conditions were tested for each substrate group (n = 3 samples/group): BM with or without 1 µg/mL of the broad spectrum MMP inhibitor GM6001 (MMPi). After 48 hours, explants were removed and the nuclei of egressed cells were stained with 4', 6-diamidino-2-phenylindole (DAPI). Confocal z-stacks were obtained in the FITC and DAPI channels to visualize cells, nuclei, and devitalized matrix (autofluorescent in the DAPI channel). Cell area and aspect ratio (elongation) were determined from maximum z-stacks projections (n=100 cells/group). Cell infiltration depth was measured as the distance between the apical tissue surface and the basal cell surface (n = 100 cells/group). *Statistics:* Significance was assessed by one- or two-way ANOVA with Tukey's HSD post hoc tests to compare substrate and media conditions between groups ($p \leq 0.05$). A cumulative distribution plot, coupled with the Kolmogorov-Smirnov test, was used to determine whether the distribution of

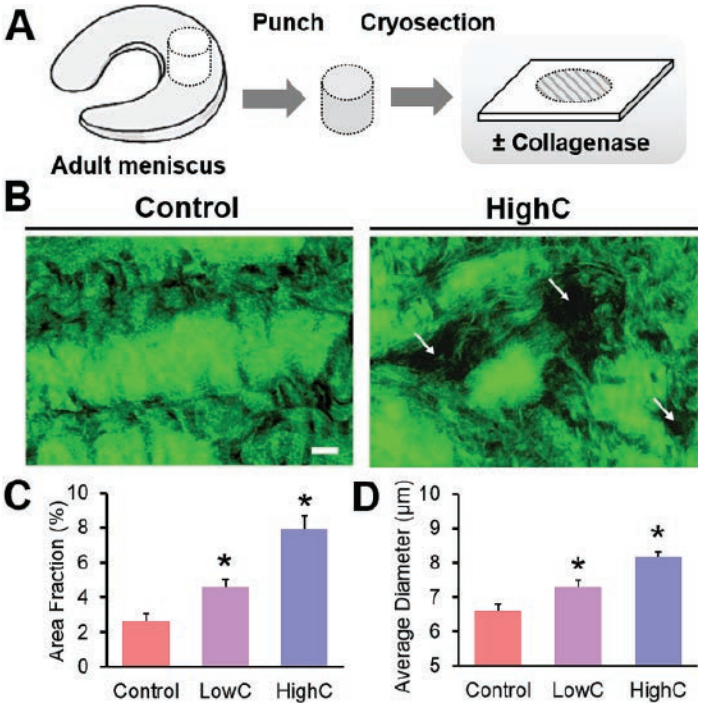

Figure 1. Matrix degradation decreases ECM density. (A) Experimental schematic. (B) SHG signal (green) of substrates. Arrows indicate inter-fibrillar regions. Scale = 20 µm. (C) Inter-fibrillar area fraction and (D) Diameter. * = $p \leq 0.05$ vs. all other groups.

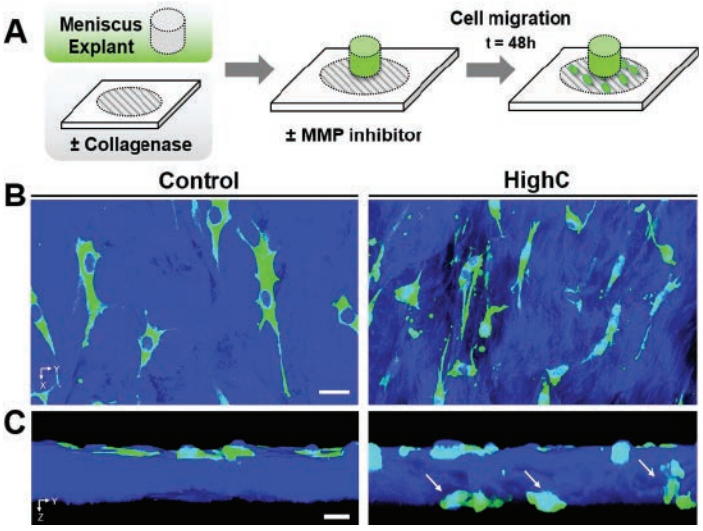

Figure 2. Matrix degradation expedites interstitial cell migration. (A) Experimental schematic. (B) Confocal reconstructions of adult meniscal cells (green) on tissue substrates (blue). Scale = 20 μm. (C) Cross-sectional view of (B). Arrows point to infiltrating cells. Scale = 10 μm.

infiltration was different between groups ($p \leq 0.05$). Data are presented as mean ± SEM.

Results

SHG imaging of adult meniscal sections pre-treated with various levels of collagenase revealed distinct microenvironments (Figure 1B). Qualitatively, the untreated Control substrate had thicker and more organized collagen bundles than the low-dose (LowC) and high-dose (HighC) collagenase groups. The area fraction of inter-fibrillar ECM, indicated by the absence of SHG signal, increased with collagenase dose (Figure 1C, $p \leq 0.05$). The average diameter of discrete inter-fibrillar regions also increased with collagenase dose, suggesting local disruption of the native collagen network (Figure 1D, $p \leq 0.05$). Adult meniscal cells from explants adhered to and infiltrated the devitalized tissue substrates within 48 hours (Figure 2B). Cells in the untreated Control group remained spread on the tissue surface, whereas cells in the collagenase groups were found within or below the tissue surface (Figure 2C). Cell infiltration depth was significantly greater for the HighC group (10.9 ± 0.6 μm) compared to the LowC (5.1 ± 0.2 μm) and Control (3.6 ± 0.1 μm) groups (Figures 3A and 3B, $p \leq 0.05$). Inhibition of cell-produced MMPs (MMPi) decreased infiltration depth for the HighC group only (7.5 ± 0.6 μm). While cells on all substrates aligned in the fiber direction of the underlying tissue, cell morphology was dependent on the substrate and media condition. Cell area decreased with increased substrate degradation, but was not affected by the addition of MMPi (not shown, $p \leq 0.05$). On the other hand, cell aspect ratio (elongation) remained constant with substrate degradation, but increased with MMPi for LowC and HighC substrates (Figure 3C, $p \leq 0.05$).

Discussion

Restricted interstitial cell migration may prevent proper healing of the adult meniscus and other dense connective tissues. An innovative strategy to promote repair may be to first free native cells from the matrix so as to facilitate migration to the wound site. Our findings suggest that interstitial cell mobility increases with matrix degradation. Adult meniscal cells on devitalized adult tissue sections pre-treated with collagenase were smaller and more invasive than the same cells on untreated tissue. In the untreated condition, migrating cells must navigate through narrow spaces between rigid, aligned collagen fibers, analogous to the constraints imposed by a decreasing pore size in transwell assays.[1] Partial enzymatic digestion improved cell mobility by increasing the area fraction and size of inter-fibrillar regions and also by decreasing the local ECM stiffness.[4] Blocking cellular MMPs increased cell elongation and decreased infiltration depth, suggesting that exogenous and endogenous MMPs act synergistically to remodel the ECM during migration. However, this effect was only observed in the HighC group, indicating that when the biophysical barrier is too great, endogenous MMPs play

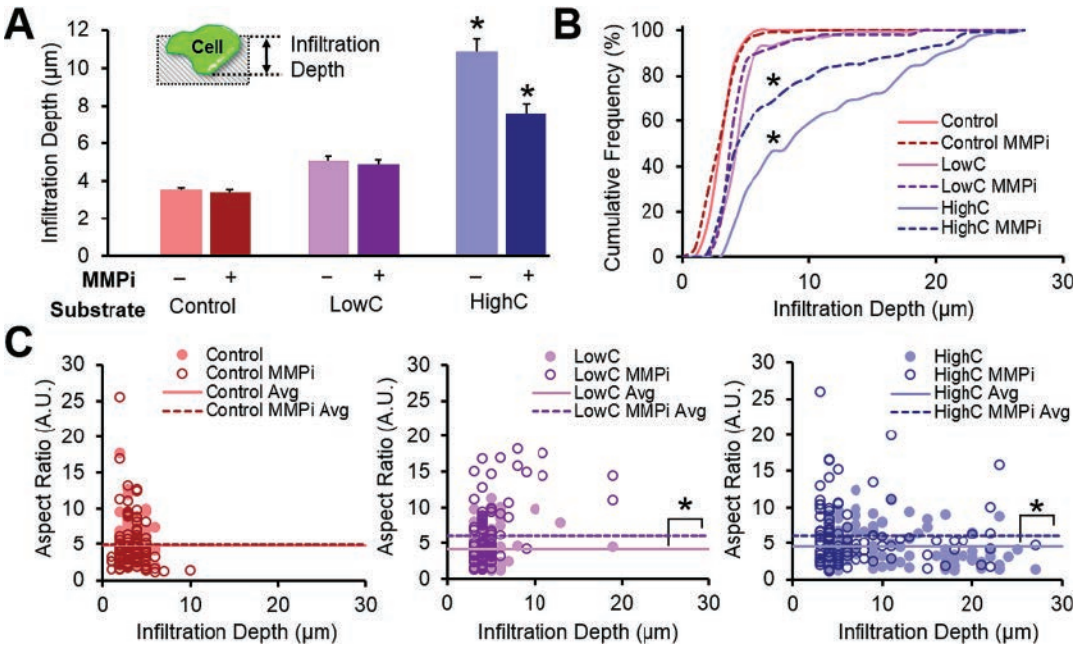

Figure 3. Exogenous and endogenous MMPs work synergistically to enhance migration. (A) Average and (B) Cumulative frequency distribution of cell infiltration depth. *= $p \leq 0.05$ vs. all other groups. (C) Aspect ratio of individual cells (circles) as a function of infiltration depth for different substrate and media conditions. Lines indicate averages. *= $p \leq 0.05$ between groups.

a limited role and migration relies more on cell deformation through the inter-fibrillar regions. Taken together, our data indicate that providing the proper microenvironment for interstitial migration ultimately results in enhanced cellularity and integration at the wound interface.[4] By addressing the inherent limitations to repair imposed by the mature ECM, these studies may define a new clinical paradigm for repairing damaged dense connective tissues

Significance

Partial enzymatic digestion of the ECM expedites interstitial cell migration, which may promote dense connective tissue repair.

Acknowledgments

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