Platelet-Derived Growth Factor Promotes Interstitial Cell Migration in the Knee Meniscus

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Introduction:

Few regenerative approaches exist for injuries to adult dense connective tissues. Compared to fetal tissues, adult tissues are hypocellular and lack a sufficient population of endogenous cells at the wound site to affect repair.[1] We hypothesized that this deficiency is exacerbated by the dense and stiff adult extracellular matrix (ECM), a biophysical barrier that restricts cell mobility.[2] We also hypothesized that chemotactic cues[3] might help overcome this barrier and promote cell migration through small pores. Using the knee meniscus as a test platform, we investigated the age-dependent response of cells to physical migratory barriers and developed a novel 'tissue Boyden chamber' system to determine whether interstitial cell migration through native tissue is enhanced by a local platelet derived growth factor AB (PDGF-AB) gradient.

Methods:

Transwell Migration Assay: Menisci were isolated from fetal and adult cows and minced to isolate cells. To assess migration in the presence of physical barriers, 96-well transwell migration assays with pore diameters of 3, 5, or 8 μm were used (Millipore). Cells (Passage 1) in DMEM + 1% FBS were seeded at 50k cells per top chamber and incubated for 16 hours before being fluorometrically quantified. Three media conditions in the bottom chamber were tested (n = 3 wells/group): DMEM + 1% FBS (Control) and DMEM + 1% FBS with either 50 or 100 ng/mL human recombinant PDGF-AB. Fluorescence signal intensity was normalized to the 3 μm pore group for each condition. To visualize cell migration, fetal and adult tissue explants (8 mm ø) were incubated in CellTracker™ Green for 1 hour and then placed atop microporous membranes. After 48 hours in DMEM + 1% FBS, the egressed cell nuclei were stained with 4', 6-diamidino-2-phenylindole (DAPI). Membranes were mounted onto glass slides, and confocal z-stacks obtained at 20X in the FITC, DAPI, and TRITC channels to visualize cells, corresponding nuclei, and membranes (autofluorescent in the TRITC channel). Migration Through Native Tissue: To generate physiologic microenvironments, fetal meniscal bodies were cryotomed into transverse sections (~35 μm thick) and mounted onto glass slides to cover four holes (1 mm ø). To fabricate a 'tissue Boyden chamber' that allows interstitial migration of cells towards a chemotactic gradient, the tissue-mounted slide was set atop a concave slide containing 140 μL of either DMEM + 1% FBS (Control) or 200 ng/mL PDGF-AB. The top and bottom slides were sealed, and fetal or adult tissue explants incubated in CellTracker™ Green were placed atop the tissue sections (n = 3/group). After 48 hours in DMEM + 1% FBS, slides were stained as above. Confocal z-stacks were obtained at 10× in the FITC and DAPI channels to visualize cells and nuclei engaging with the devitalized tissue spanning the slide holes (autofluorescent in the DAPI channel). To assess the quantity of egressed cells around and within the holes, cell signal area was determined using maximum z-stack projections in Fiji (n = 9 holes/group). To quantify interstitial migration, cells from five tissue cross-sections were counted for each hole (n = 3 holes/group) and categorized as either Surface or Migrated, where a Migrated cell was entirely embedded within the tissue or had emerged onto the other side. Statistics: Significance was assessed by one- or two-way ANOVA with Tukey's HSD post hoc tests to determine the impact of pore size, media condition, and/or age ($p \leq 0.05$). Data are presented as the mean ± SD unless otherwise noted.

Results:

Transwell migration of fetal and adult meniscal cells was dependent on pore size and PDGF dose. Confocal images revealed that for both age groups, nuclei were able to deform and pass through the 5 and 8 μm pores, but could not pass through 3 μm pores (Figures 1A and 1B). In the absence of a PDGF gradient (Control), there was no difference between pore sizes for either age group (Figures 1C and 1D). With the addition of PDGF to the bottom chamber, pore size had a significant effect, with cell migration increasing as a function of pore size for both ages ($p \leq 0.05$). Addition of 100 ng/mL PDGF-AB (High PDGF) to the bottom chamber significantly increased migration through both the 5 and 8 μm pores compared to the 3 μm pores. A similar trend was seen for the 50 ng/mL PDGF-AB (Low PDGF) group, though migration was increased for only the 8 μm pores. While

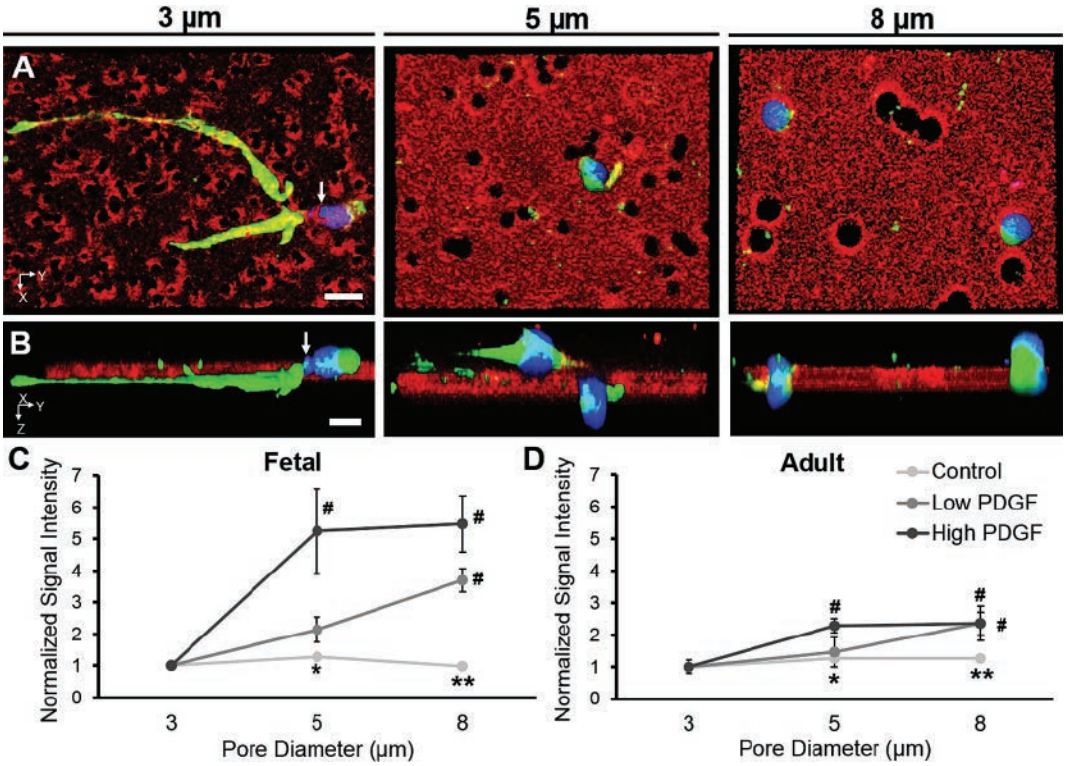

Figure 1. PDGF-AB enhances cell migration through intermediate size pores. (A) Confocal reconstructions of adult cells (green) passing through microporous membranes (red) of 3, 5, and 8 μm diameter. Arrow points to constricted nucleus (blue). Scale = 10 μm. (B) Cross-section of the cells in (A). Scale = 10 μm. (C) Migrated fetal and (D) Adult cell signal intensity normalized to 3 μm pore for each media condition. = $p \leq 0.05$ vs. High PDGF. **= $p \leq 0.05$ vs. all other media conditions. #= $p \leq 0.05$ vs. 3 μm pore.

types,[2] meniscus cell migration declines with decreasing pore size, and eventually cells are rendered immobile. Notably, the nucleus is the rate-limiting organelle in migration due to its large size and stiffness.[2] Since 3D migration depends on deformability through interstitial space, age-related changes in cell and/or nuclear mechanics may play a role in the differential mobility seen between fetal and adult cells. While low interstitial cell mobility may contribute to the lack of cell-mediated repair in the adult meniscus, this limitation may be partly overcome via the provision of a soluble chemotactic gradient that promotes migration to the injury site. Here, we show that a PDGF-AB gradient enhances cell infiltration through membranes of intermediate pore sizes, as well as through migration increased with PDGF dose for both age groups, fetal cells were more mobile than adult cells. To study interstitial migration towards a PDGF gradient, a 'tissue Boyden chamber' was fabricated (Figure 2). When fetal and adult tissue explants were placed atop the devitalized meniscal tissue, egressing cells adhered, spread, and began migrating into the substrate within 48 hours. In all conditions, the cell signal area (% of total area) was greater for fetal cells than adult cells (Fig. 2D, $p \leq 0.05$). Without PDGF in the bottom chamber (Control), few cells from either age migrated through the tissue (Figure 2E). Addition of 200 ng/mL PDGF-AB to the bottom chamber increased the adult cell signal area as well as the number of Migrated cells for both age groups compared to Controls ($p \leq 0.05$).

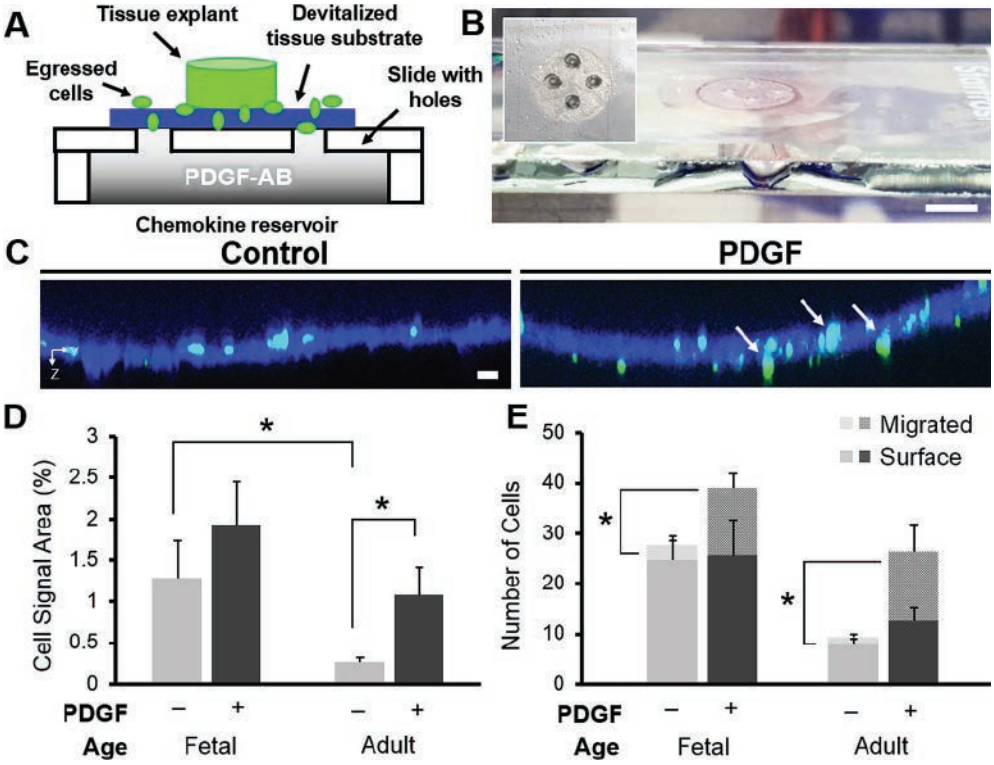

Figure 2. PDGF-AB enhances cell migration through native tissue. (A) Schematic of a 'tissue Boyden chamber.' (B) Tissue slide atop concave slide containing media. Inset shows tissue cryosection. Scale = 5 mm. (C) Confocal cross-section of cells migrating through the tissue section. Arrows point to Migrated cells. Scale = 20 μm. (D) Fetal and adult cell signal area for each media condition (mean ± SEM). *= $p \leq 0.05$ between groups. (E) Fetal and adult cells within the slide hole. *= $p \leq 0.05$ comparing Migrated cells between groups.

Discussion:

The ECM of dense connective tissues is a physically restrictive microenvironment. As with other cell

a physiologic microenvironment using a novel 'tissue Boyden chamber.' Given the potent chemotactic effect of PDGF-AB, we are currently investigating whether this factor can expedite cell migration to the wound site after localized matrix degradation, which decreases ECM density at the wound interface.[4] By combining these diverse but complementary approaches to augment interstitial migration, we aim to recapitulate the robust healing response of fetal tissues.

Significance

Platelet-derived growth factor-AB stimulates interstitial migration, which may facilitate cell-mediated repair of dense connective tissues.

Acknowledgments

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