Meniscus Cell Migration Through Dense Fibrous Networks Is Regulated By Nuclear Mechanics

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Introduction

Cell migration to a wound site is required for tissue repair[1]. However, the small pores of dense connective tissue extracellular matrix (ECM) present an obstacle to migration.This is primarily the result of the cell nucleus, the largest and stiffest organelle in the cell[2]. Modulating nuclear stiffness is, therefore, one potential strategy for enhancing cell mobility that could be leveraged to improve repair of dense connective tissues. Previously, we showed that Trichostatin A (TSA, a histone deacetylase inhibitor) induced chromatin relaxation and decreased nuclear stiffness in adult meniscus cells (aMCs), enhancing their migration through micron- sized pores using a transwell assay[3]. Here, we extend this work to a physiologic context, and determine whether such chemically induced nuclear softening modulates migration through fiber networks of varying porosity and through native tissue.

Methods

To assess migration through nanofiber networks, a custom-PDMS chamber was implemented[3]. The system consisted of a top reservoir containing basal growth media (BM) and a bottom reservoir containing BM supplemented with 200 ng/mL PDGF (as a chemoattractant, Fig. 1A). Fluorescently labeled (Cell Tracker) poly(ε-caprolactone)/poly(ethylene oxide) (PCL/PEO) composite aligned fibrous scaffolds (composed of 0%, 25% or 50% sacrificial PEO fibers, Fig. 1B) were interposed between the two reservoirs (Fig. 1A).Adult meniscus cells (aMCs), passage were seeded on the top of each scaffold and cultured in BM with/without TSA (200 ng/ml) for an additional 2 days (Fig. 1A). After a total of 3 days, 3D reconstructions of cell and scaffolds were obtained from confocal z-stacks to quantify infiltration3. Additionally, a cell/ECM model was developed in which the nucleus was taken to be a compressible neo-Hookean solid and the critical force required to pull the nucleus through these fiber networks was predicted (COMSOL Inc., Stockholm). To assess the impact of nuclear softening in the longer term, aMCs were seeded onto PCL/PEO 25% aligned scaffolds and cultured in TGF-β3 containing chondrogenic media for 4 weeks. TSA was applied once a week for 1 day (Fig. 2A). At 4 weeks, cryosections were obtained and stained with Picrosirius Red and DAPI. To quantify cell infiltration into the constructs, cell nuclei through the scaffold depth were counted using Image J. Finally, to investigate the role of nuclear softening on migration in native tissue, meniscus tissue explants (6 mm diameter, 6 mm height, Fig. 3A) were devitalized at their periphery and re-colonization was evaluated over time. For this, cells along the periphery of the explants were selectively lysed via a 2-cycle freeze-thaw process (-20°C for 30 min followed by thawing at room temperature for 30 min, repeated twice on Day -2, Fig. 3A). Freeze-thawed explants were treated with TSA for 1 day (Day -1, Fig. 3A) and the explants were then cultured in fresh BM for an additional 3 days.At day 3, LIVE/DEAD staining was used to assess the number of live cells within 1 mm of the periphery in 8

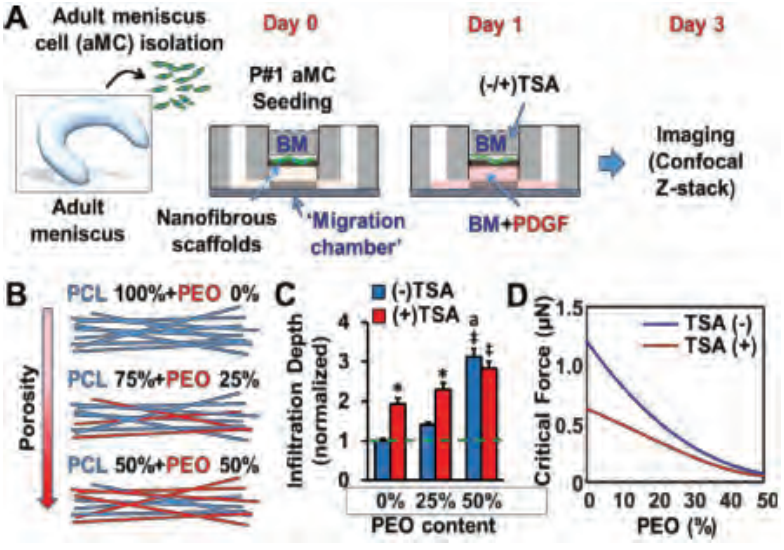

Figure 1. Schematics of **(A)** study design and **(B)** removal of sacrificial PEO fibers to increase porosity. **(C)** Quantification of cell infiltration [n = ~30, *p<0.05 vs (−) TSA, ‡p<0.05 vs 0% PEO, [a]p<0.05 vs 25% PEO, mean ± SEM, normalized to the control 0% PEO group]. **(D)** Predicted force required for nuclear entry into the scaffold as a function of PEO content.

regions of the cutting plane using Image J. Statistical analysis was carried out in Graphpad Prism; sample number for each assay is as indicated in the figure legends.

Results

Cell infiltration depth increased as a function of PEO content in the absence of TSA [(−)TSA, Fig. 1C] and TSA treatment [(+) TSA] enhanced this infiltration into scaffolds of lower porosity (lower % PEO groups, <25%) (Fig. 1C). The cell model predicted a decrease in critical force for nuclear entry into the scaffold as the PEO content (and so porosity[4]) increased (Fig. 1D). Consistent with the experimental data, the model predicted no effect of nuclear softening at higher PEO percentages (~50%). Taken together, these findings suggest that decreasing nuclear mechanics and/or increasing scaffold porosity enhance interstitial cell infiltration into dense fiber networks. In longer term cultures, control groups [(-)TSA], showed collagen deposition only at the border of the constructs (Fig. 2B). With TSA treatment [(+)TSA, 1x per week], the deposition and distribution of collagen was increased (Fig. 2B). Similarly, DAPI-stained cross-sections revealed a greater depth of aMC infiltration into scaffolds treated with TSA [Fig. 2C, D]. In native tissue, the 2-cycle freeze-thaw process effectively eliminated live cells at the explant periphery (Day -2, Fig. 3B, D), while preserving viability in the center (Fig. 3B). A greater number of these viable cells migrated into the previously devitalized border region over three days of TSA treatment, compared to untreated control groups (Fig. 3C and D).

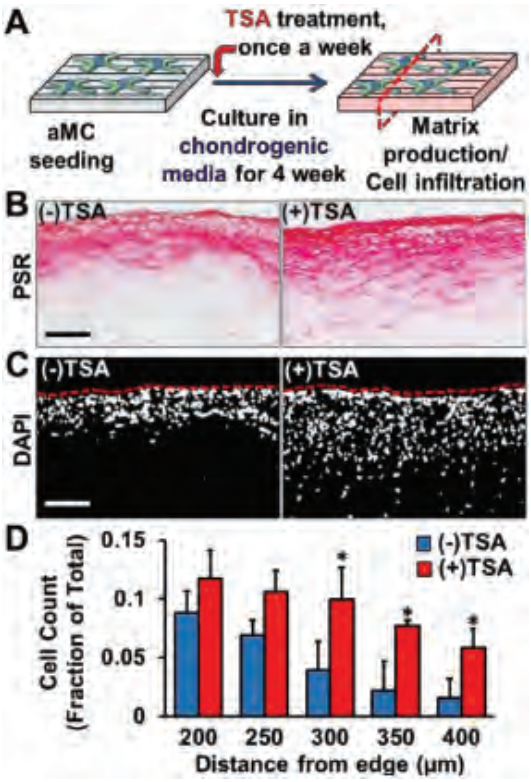

Figure 2. (A) Schematic of study design. Representative cross-sections of aMC-laden nanofibrous constructs at week 4 stained for collagen (PSR: picrosirus red staining, **(B)**) and cell nuclei (DAPI, **(C)**), bar = 100 μm. **(D)** quantification of MFC infiltration with/ without TSA treatment [n = 3 images].

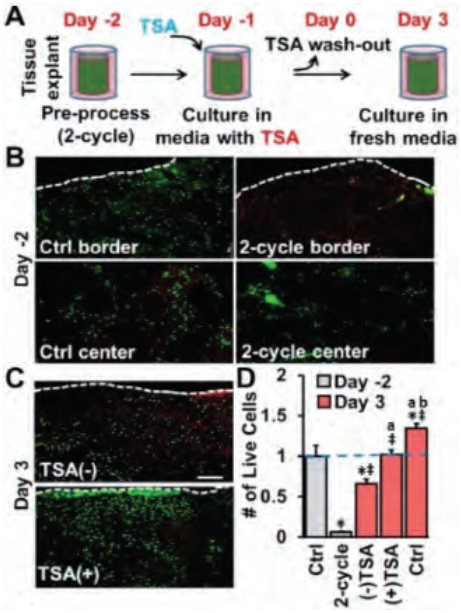

Figure 3. (A) Study design. **(B)** Representative Live (green)/Dead (red) images at the periphery or center of control explants (Ctrl) and freeze- thawed explants (2-cycle) at day -2. **(C)** Live/Dead staining 3 days post TSA treatment. **(D)** Quantification of live cells at the periphery [n = 24~32 images from 3~4 explants, normalized to cell number in ctrl groups at day -2 (dashed line), mean ± SEM, *p<0.05 vs. Ctrl, ‡p<0.05 vs. 2- cycle, [a]p<0.05 vs.(−)TSA, [b]p<0.05 vs. (+)TSA.

Discussion

This work shows that both decreasing meniscus cell nuclear mechanics (via chromatin decondensation) and increasing scaffold porosity (via removal of sacrificial fibers) enhances adult meniscus cell interstitial migration in dense fibrous networks. The finding of increased collagen deposition in scaffolds subjected to repeated TSA treatment also suggests that de-condensation does not permanently interrupt cellular phenotype or matrix forming capacity. Notably, nuclear softening enhanced migration even in the context of dense, adult, meniscus ECM, suggesting that mobility of cells can be increased while preserving the loadbearing structure of the native tissue. Ongoing studies are reducing this finding to practice via the programmed release of de-condensing agents from implanted nanofibrous scaffolds and testing this as a therapeutic in a large animal (ovine) model of endogenous meniscus repair.

Significance

Our findings support the concept that decreasing physical impediments to migration through nuclear softening can improve dense connective tissue repair by enabling more cells to migrate to and colonize the wound site after injury. This will have widespread application in the promotion of endogenous repair in all poorly healing dense connective tissues.

References

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