Nuclear Softening Enhances Meniscus Cell Migration into Dense Fiber Networks and Native Tissue

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Introduction

Cell migration is essential for healing of dense connective tissues[1]. However, the cell nucleus, which is the stiffest organelle in mammalian cells, is an obstacle to efficient migration due to its inability to squeeze through the small pores that typify the dense extracellular matrices (ECM) of these tissues[2]. Modulation of nuclear stiffness is therefore a potential target for enhancing cell mobility. Nuclear mechanics are established in part by the proteins that make up the nuclear lamina, and in part by the packed DNA (chromatin) within[3]. Trichostatin A (TSA) is a histone deacetylase (HDAC) inhibitor that induces hyperacetylation and chromatin relaxation, decreasing nuclear stiffness[3]. We hypothesized that treatment of meniscus cells with TSA would result in more deformable nuclei and thus increase their mobility through both dense fiber networks and the dense ECM of the native tissue. To test these hypotheses, we first developed a novel PDMS/nanofiber membrane cell migration chamber and evaluated whether nuclear softening by TSA pre-treatment improved meniscus cell migration. Next, using sections of adult meniscus, we evaluated whether this same approach could improve meniscus cell migration into native tissue.

Methods

Adult bovine meniscal fibrochondrocytes (MFCs) were seeded on aligned (AL) or non-aligned (NAL) nanofibrous scaffolds (2×105 cells, Passage 1) in basal media (BM: DMEM + 10 % FBS)[3]. A subset of scaffolds was treated with TSA (400 nM) for 3 hours. An image-based edge detection algorithm was used to determine the degree of chromatin condensation (the CCP) in individual DAPI stained nuclei[3] with and without TSA treatment. Additionally, constructs were stretched from 0 to 15% grip-to-grip strain on a custom tensile device and the change in nuclear aspect ratio (NAR) was measured with and without TSA pre-treatment[3]. As an initial assessment of MFC migration, a 96-well transwell migration assay was employed, with pore diameters of 3 or 5 μm (Millipore)[4]. To assess cell contractility with and without TSA pre-treatment, MFCs were seeded onto 10 kPa polyacrylamide gels and traction force was measured[5]. To assess cell migration through dense nanofiber networks, a custom-PDMS 'migration assay chamber' was implemented. The device consisted of a top reservoir containing BM and a bottom reservoir containing BM + 200 ng/mL PDGF as a chemoattractant (Figure 2A). Fluorescently labeled (Cell Tracker Red) aligned (AL) or non-aligned (NAL) nanofibrous PCL scaffolds (thickness: ~ 150 μm) were interposed between the reservoirs, and MFCs (1000 cells, passage 1) were seeded onto the top of each scaffold and cultured in BM with/without TSA for 3 days. At the end of three days, cells were fixed and visualized by actin/DAPI staining. Confocal z-stacks were obtained at 40× magnification and maximum z-stack projections were used to assess cellular morphology. The % of infiltrated cells was quantified, with cells located beneath fibers categorized as 'infiltrated'. To evaluate meniscus cell migration in native tissue, adult meniscus tissue was cryosectioned onto glass slides (~35 μm thick)[4]. To visualize cell invasion, additional living adult meniscal explants (5 mm Φ) were incubated in Cell Tracker™ Green for 1 hour and then placed atop the tissue sections to allow for cell egress onto and invasion into the section[4]. These samples were cultured in BM with/without TSA for 48 hours, at which point maximum z-stack projections were acquired and cell infiltration depth was measured as the distance between the apical tissue surface and the basal cell surface[4]. Statistical analysis was performed using ANOVA (with Tukey's post hoc) or with a Student's t-test ($p < 0.05$).

Results

TSA treatment decreased in the number of visible edges in MFC nuclei (decreased the CCP), indicating efficient relaxation of the chromatin (Figure 1A, B). When TSA-treated cells were deformed, their nuclei increased in NAR to a greater extent than control cells, indicating a softer nucleus (Figure 1C). Treatment with TSA also significantly increased MFC migration through both 3 and 5 μm pores in the transwell assay (Figure 1D). TSA treatment did not, however, alter traction force generation in these cells (data not shown). When MFCs were placed atop scaffolds, the % of cells that had infiltrated was higher in the NAL group than the AL group, and infiltration increased in both groups with TSA treatment (Figure 2B, C). Of note, compared to control

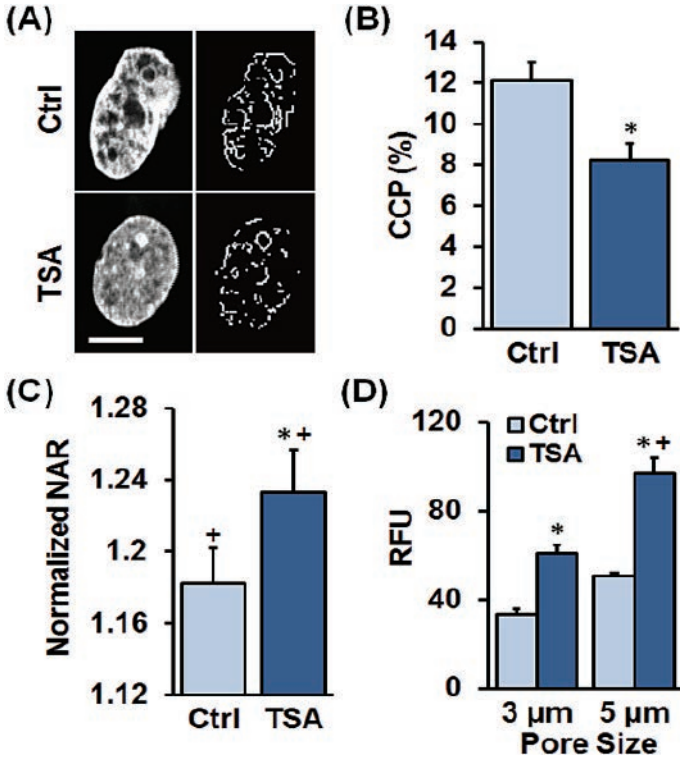

Figure 1. (A) DAPI stained nuclei (left) and corresponding edge detection (right) with/without TSA treatment (Ctrl/TSA, bar = 3 µm), and quantification of chromatin condensation parameter (CCP, right). (B) Quantification of nuclear deformation (NAR) with 15% scaffold stretch (n = ~48, *p < 0.05 vs. Ctrl, +p < 0.05 vs. 0%, normalized 0%). (D) Fluorescence intensity of migrated MFCs in a traditional transwell assay with TSA treatment (n = 5, *p < 0.05 vs. Ctrl, +p < 0.05 vs. 3 µm pore).

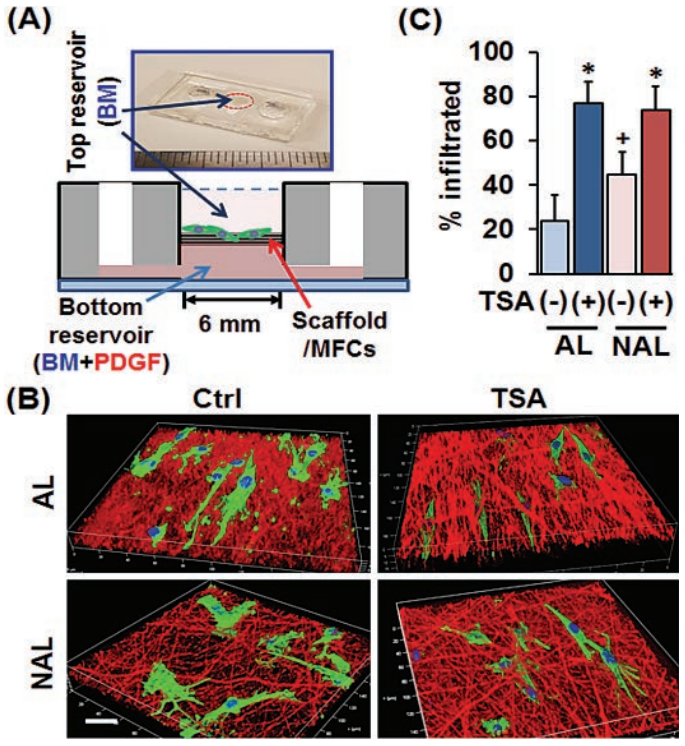

Figure 2. (A) Schematic of PDMS/nanofiber migration chamber. **(B)** Fluorescent images of cells (green), nuclei (blue), and nanofiber scaffolds (red) with/without TSA treatment (Ctrl/TSA, bar = 20 µm). **(C)** Quantification of % infiltrated cells [*p < 0.05 vs. no TSA (−), + p < 0.05 vs. aligned scaffold (AL), n = ~35].

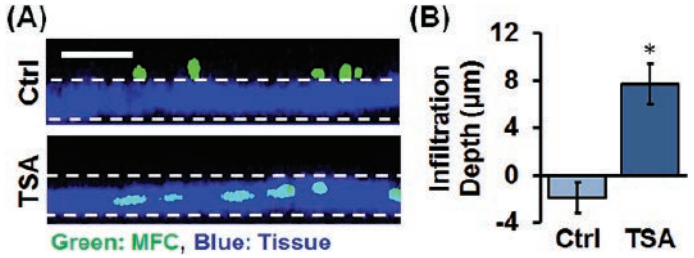

Figure 3. (A) Cross-section view of confocal reconstruction of MFCs (green) migrating through tissue substrates (blue); bar = 50 µm, dashed lines: tissue borders. **(B)** Quantification of cell infiltration depth (n = 45~70 cells, *p < 0.05 vs. Ctrl).

MFCs, TSA treated-cells had more elongated nuclei with higher nuclear aspect ratios (NAR, data not shown). When placed on tissue sections, untreated MFCs (Ctrl) remained primarily on the tissue surface; whereas TSA treated MFCs were found below the tissue surface (Figure 3A). Quantification showed that MFC infiltration depth was significantly greater with TSA treatment compared to controls (Figure 3B).

Discussion

Endogenous cell recruitment is required for healing of injured dense connective tissues. In this study, we demonstrated that nuclear softening via pharmacological decondensation of chromatin in MFCs enhanced their migration through dense fibrous networks and through native tissue. In a previous study, we had shown that partial enzymatic digestion of the ECM also expedites interstitial cell migration[4]. Together, these findings suggest that decreasing the physical impediments to migration (i.e., the properties of the cells themselves and/or the matrix through which they are traveling) can enhance interstitial cell mobility and foster repair.

Significance

Nuclear softening of meniscus cells increased their interstitial migration in dense fiber networks and in native tissue. This approach may improve dense connective tissue repair by enabling more cells to migrate to and colonize the wound site after injury.

Acknowledgements

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