**Effect of Nuclear Softening and Fiber Stiffness on Cell Migration into Dense Fibrous Networks**

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**Statement of Purpose:** Cell migration through dense fibrous structures is essential for tissue repair, including with biomaterial scaffolds [1]. However, the nuclear stiffness hinders migration since the nucleus is too stiff to squeeze through small pores of dense fibers [2]. Also, the mechanics of fibers may influence the ability of cells to displace them for invasion [3]. To address these issues, we investigated the influence of nuclear softening and fiber stiffness on cell migration through dense fibrous networks, with a focus on meniscus cells. We developed PDMS devices containing multi-layered chambers compartmentalized with dense fiber networks and with chemotactic signals (e.g., PDGF) and analyzed invasion with changes in: (i) nuclear softening with polycaprolactone (PCL) fibers and (ii) fiber stiffness through alterations in methacrylated hyaluronic acid (MeHA) modification [4]. Outcomes can help to design tissue repair strategies to promote cell migration.

**Methods:** To fabricate devices, PDMS was used to sandwich electrospun fiber networks (e.g., PCL, MeHA) onto a glass support (Fig. 1A). Adult bovine meniscal fibrochondrocytes (MFCs) were cultured in DMEM media containing 10 % FBS and treated with trichostatin A (TSA 400 nM, histone deacetylase inhibitor) for 3 hours to soften nuclei [5]. Using a custom tensile device, grip-to-grip strain was applied to stretch cells seeded onto PCL fibers and the changes in nucleus aspect ratio (NAR) were analyzed (Fig. 2A). Top and bottom chambers were filled with culture media and media containing 200 ng/ml PDGF, respectively, and MFCs (1000 cells, passage 1) were seeded onto top chambers. After culturing 3 days with/without TSA, cells were fixed and actin/nucleus were visualized with fluorescence (Fig. 2B). The % of infiltrated cells were analyzed by counting cells beneath fibers (Fig. 2C). HA fibers were electrospun with 4 wt% MeHA with coupled RGD and rhodamine, 3.5 wt% PEO and 0.05 wt% I2959 photoinitiator in water and exposed to UV 15 mW/cm$^2$ for 20 min (Fig 3A). Using confocal microscopy, fluorescence images of cells were acquired (Fig. 3B, C, E). The mechanics of fibrous networks were calculated by forces and indentations recorded until the yielding point (Fig. 3D) [3].

**Results:** Multi-layered PDMS chambers were well-compartmentalized with dense fiber networks generating growth factor (PDGF) gradients from the bottom to top chamber to encourage invasion (Fig. 1). Nuclei of TSA-treated MFCs were more elongated (higher NAR) than those of control cells in response to tensile forces, implying that TSA treatment successfully softened nuclei of MFCs (Fig. 2A). As a result, the % of MFC infiltration into PCL fiber networks was increased with TSA treatment (Fig. 2B,C). MeHA fibers were generated with RGD and fluorescence and cells attached and spread on the MeHA fibers (Fig. 3). The stiffness of the fibers is readily altered through changes in fiber mechanics (Fig. 3D) [4] and the influence of mechanics is being explored.

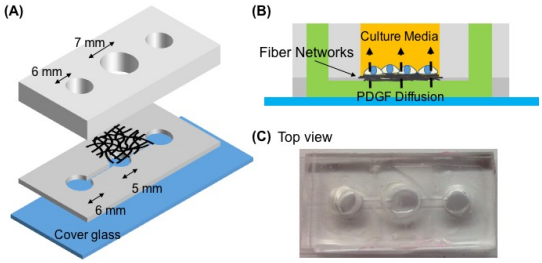


Fig 1. (A, B) Fabrication (A) and cross-section view (B) schematic of PDMS devices for the study of cell migration into fibrous networks. (C) A top view picture of PDMS device.

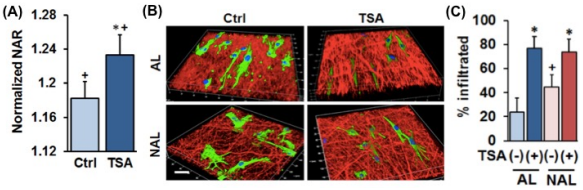


Fig. 2. (A) Analysis of nucleus deformation (NAR) on 15%-stretched fiber networks (Normalized to 0%-stretched, $p<0.05$ vs. Ctrl, $+p<0.05$ vs. 0%-stretched). (B) Fluorescence images of cell cytoplasm (green), nucleus (blue), and fiber networks (red) with/without TSA treatment (Scale bar = 20 μm). (C) % of cells infiltrated to aligned (AL)/non-aligned (NAL) fiber networks ($p<0.05$ vs. TSA (-), $+p<0.05$ vs. AL).

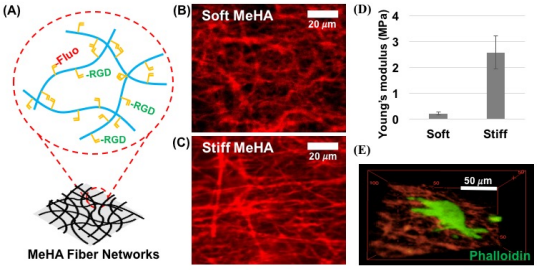


Fig. 3. (A) Schematic of MeHA fibrous networks. (B, C) Fluorescence images of hydrated soft (B) and stiff (C) MeHA fiber networks. (D) Young's modulus of fibrous networks. (E) 3D fluorescence image of a cell interacting with fibrous networks. Phalloidin (green), Fibers (red).

**Conclusions:** We developed PDMS devices with compartmentalized fibrous networks and a chemotactic gradient from the bottom to the top. Using this system, the migration of MFCs into dense fibrous networks in response to the gradient was observed and analyzed. TSA treatment successfully softened the nucleus of MFCs and resulted in increased cell infiltration into PCL fiber networks. To study effect of fiber stiffness on cell migration, fibrous networks with varied crosslink density were generated that supported MFC interactions.

**References:** [1] Elena Scarpa. JCB. 2016;212:143-155. [2] Patricia M. Davison. Cell Mol Bioeng. 2014;7:293-306. [3] Brendon M. Baker. Nat Mater. 2015;14. [4] Iris L. Kim. Biomat. 2013;34:5571-5580. [5] Su-Jin Heo. 2015;5:16895.