Supporting Information

Influence of fiber stiffness on meniscal cell migration into dense fibrous networks

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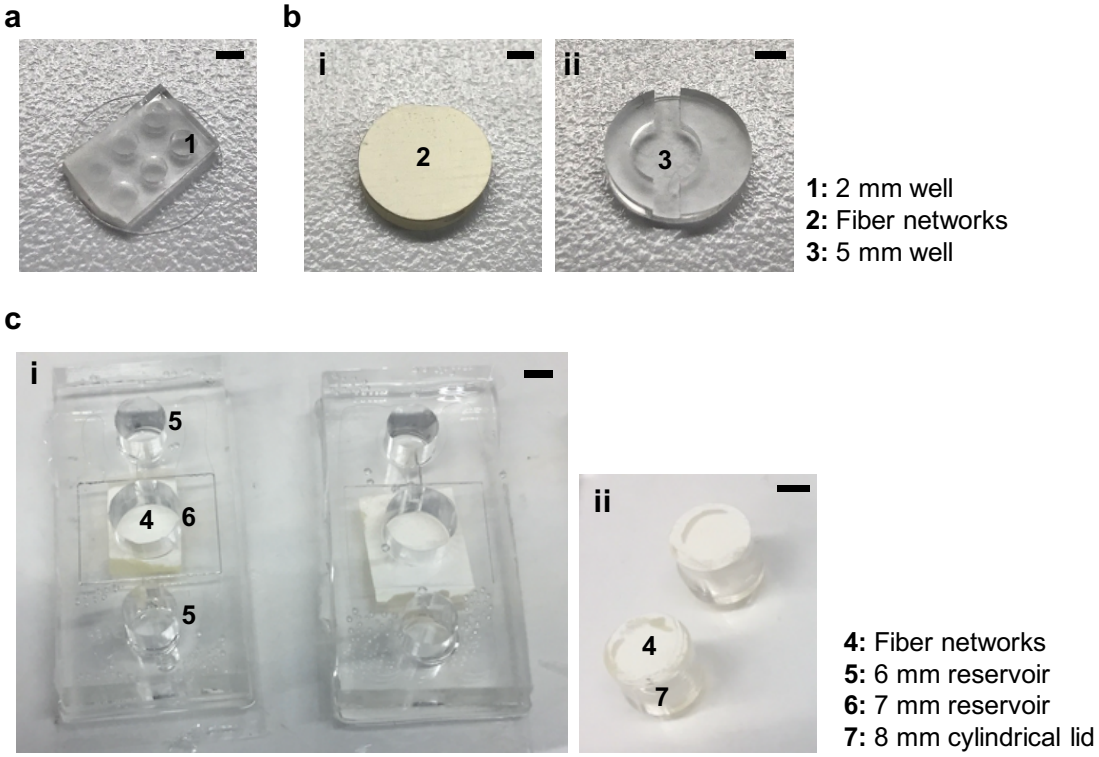
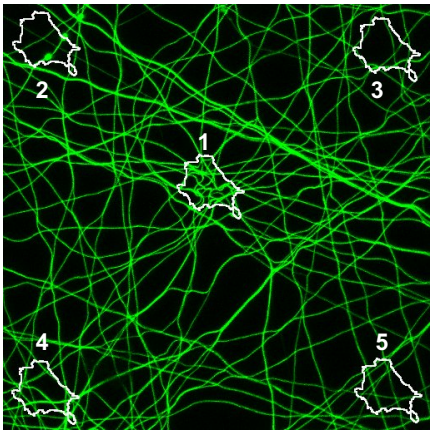


Figure S1. Images of polydimethylsiloxane (PDMS) wells and cell migration chambers used throughout the experiments. (a) 2 mm PDMS wells with thin layer of fibrous networks. Scale bar is 2 mm. (b) A 5 mm PDMS well (i) with or (ii) without a thick fibrous network layer. The PDMS well contains a ditch to avoid entrapping air. Scale bars are 2 mm. (c) Cell migration chambers consisting of (i) bottom and (ii) top parts with a thick fibrous network layer. Enclosing sliced tissues with fibrous networks (#4) at the 7 mm reservoir (#6) and 8 mm lid (#7) allowed cell migration through fibers. Two 6 mm reservoirs (#5) at the bottom are connected via a 2 mm width channel to provide media to cells. Scale bars are 4 mm.



Normalized fiber intensity:

$$\frac{\text{Fluorescence signal of cellular region (1)}}{\text{Average of fluorescence signal of acellular regions (2, 3, 4, 5)}}$$

Figure S2. Analysis of fiber density in the cellular region. Fluorescence signal of cellular region (#1) was normalized by the average of the fluorescence signals of 4 different acellular regions (#2, #3, #4, #5). The area of the cellular region (outline of a cell, white) was used to set the sizes of acellular regions at the corners of each image.

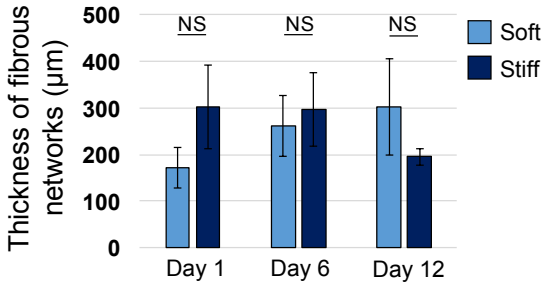
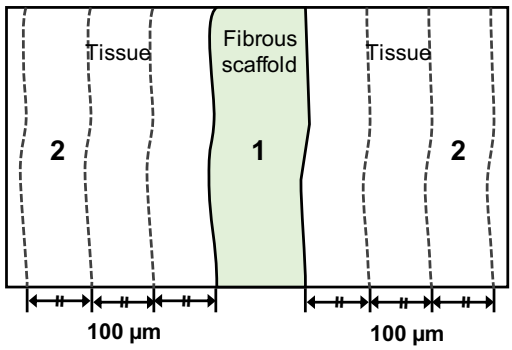


Figure S3. Quantification of the thickness of fibrous networks used for MFC migration assay within cell migration chambers. Fluorescent images of cryo-sectioned fibrous networks were analyzed to obtain thicknesses of fibrous networks. Two-way ANOVA with Tukey's post hoc testing. NS: not significant. $n = 5$ tissue constructs.



Normalized cell density:

$$\frac{\text{\# of cells at region 1}}{\text{\# of cells at region 2}}$$

Normalized collagen intensity:

$$\frac{\text{Intensity at region 1}}{\text{Intensity at region 2}}$$

Figure S4. Analysis of cell density and collagen type I and II deposition. To analyze cell density and collagen deposition, the number of cells and collagen intensity at the fibrous scaffold (region #1) were normalized by the number of cells and collagen intensity at region #2 (200 μm away from the fibrous scaffold).

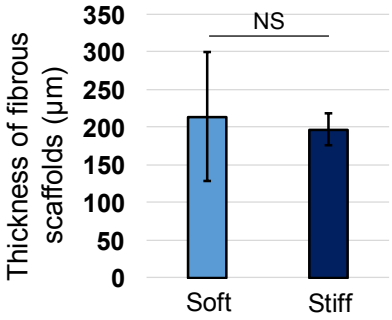


Figure S5. Quantification of the thickness of fibrous scaffolds after 4 weeks of implantation. Fluorescent images of sectioned fibrous scaffolds were analyzed to obtain the thicknesses of fibrous scaffolds. Unpaired t-tests. NS: not significant. $n = 6$ tissue constructs.