Electronic Supplementary Material (ESI) for Lab on a Chip

Electronic Supplementary Information

Three-Dimensional Microfiber Devices that Mimic Physiological Environments to Probe Cell Mechanics and Signaling

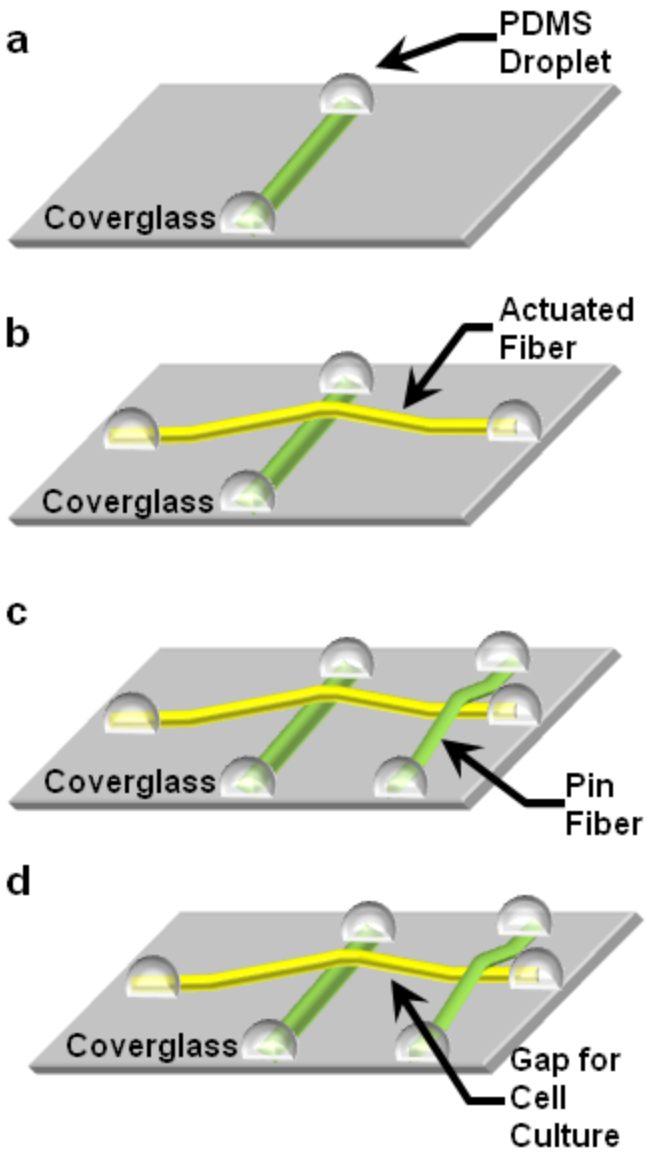
Warren C. Ruder[1], *Erica D. Pratt*[1,2], *Sasha Bakhru*[1], *Metin Sitti*[1,2,3], *Stefan Zappe*[1], *Chao-Min* Cheng[1,2,4], James *F. Antaki*[1,5*], and *Philip R. LeDuc*[1,2,6,7]*

*Corresponding authors e-mail addresses: antaki@cmu.edu, prleduc@cmu.edu

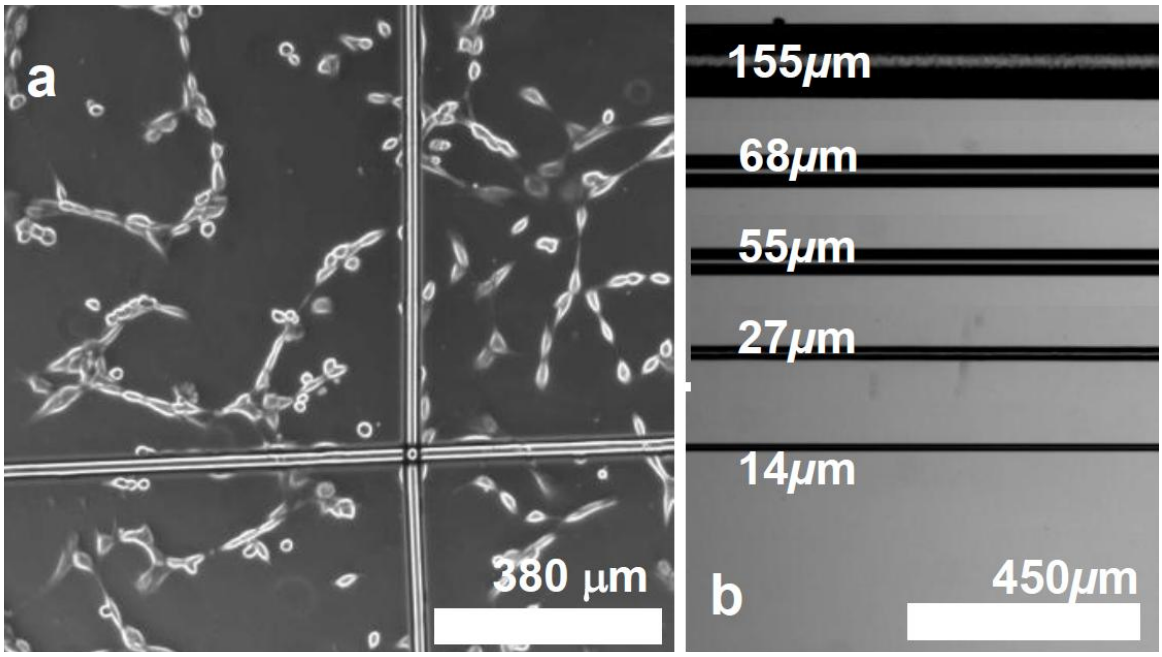
[1]Department of Biomedical Engineering
[2]Department of Mechanical Engineering
[3]Robotics Institute
Carnegie Mellon University
5000 Forbes Avenue
Pittsburgh, PA 15213

[4]Institute of Nanoengineering and Microsystems
National Tsing Hua University
Hsinchu 300 Taiwan

[5]Department of Computer Science
[6]Department of Computational Biology
[7]Department of Biological Sciences
Carnegie Mellon University
5000 Forbes Avenue
Pittsburgh, PA 15213



Supplementary Figure S1. Methodology of MP3D device construction. (a) First, a single 40 mm X 22 mm #1 borosilicate coverslip is placed on a hot plate (turned off) and a polypropylene microfiber is placed on top. This microfiber is created by melting polypropylene stock with a laboratory burner until viscous. Forceps are then placed in the melted stock and pulled away to form a microfiber. After the microfiber is placed in position, the hot plate is turned on and two droplets of uncured PDMS are placed at the ends to secure it to the glass. The hot plate is then turned off until cool. (b) After the support fiber is secured, the actuated fiber is assembled using the same process. (c) The pin fiber is then created with the same process as both the support and actuated fibers. (d) Finally, the completed MP3D structure is sterilized in 70% ethanol, followed by incubation with a fibronectin solution to promote cell adhesion. Cells are then cultured on the device using standard cell culture procedures discussed in this supplement.



Supplementary Figure S2. Cell interactions with the MP3D device. (A) Cells divide normally and spread across the glass substratum while interacting with the MP3D device. The horizontal fiber is the actuated fiber. (B) A composite image of multiple fiber diameters. The plastic drawing process allows the development of fibers ranging from approximately 15 to 150 µm in diameter, which can be used to create a range of boundary conditions for the cells.

SUPPLEMENTARY MATERIALS AND METHODS

Cell Culture on Devices

NIH 3T3 fibroblasts were cultured in high glucose Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% bovine calf serum and 1% GPS solution (29.2 mg mL^{-1} L-glutamine, 10 U mL^{-1} penicillin, and 10 mg mL^{-1} streptomycin). Cells were maintained in a humidified incubator at 37° C with 5% CO_2. Fibroblasts were plated onto sterilized MP3D devices and grown overnight.

Imaging of Perfused Cells

MP3D devices were mounted in an open-bath perfusion chamber (Series 20, Warner Instruments) and bathed in Ringer's solution containing: 121 mM NaCl, 2.4 mM K_2HPO_4, 0.4 mM KH_2PO_4, 1.2 mM $CaCl_2$, 1.2 mM $MgCl_2$, 5.5 mM glucose, and 10 mM Hepes/NaOH, pH 7.43. The chamber was mounted on the stage of an inverted epifluorescent conventional microscope (Zeiss Axiovert 200) equipped with both halogen and 100W mercury arc light sources. A precision (0.8 μm $degree^{-1}$) micromanipulator (Newport Instruments) was used to position a needle (tip radius < 40 μm) probe to displace individual polymers. Images were captured by a CCD camera (Insight QE), and post-processing was performed using NIH Image/ImageJ software.

Live-Cell Fluorescent Imaging

For calcium imaging experiments, sub-confluent MP3D devices were incubated with 2 μM Fluo-4-AM for 30 minutes prior to mounting in perfusion chamber and rinsing with Ringer's solution bath. Dye-loaded cells were exposed for 150 ms to mercury arc light every 5 seconds and fluorescent emission was captured by CCD camera (Insight QE). During chemical stimulation, 100 μM ATP was continually superfused until the chamber volume was exchanged. Mechanical stimulation was applied as described above. Cell image stacks were analyzed with NIH Image/ImageJ software and average pixel intensity in user-defined ROIs was plotted using MS Excel.

Scanning Electron Microscopy

MP3D devices cultured with cells were exposed to fixation buffer (4% paraformaldehyde in PBS) for 20 minutes. Devices were then rinsed with ddH_20 and sputtered with 2 nm of platinum. Samples were rotated 26° horizontally so that imaging was performed at a 64° angle to the substratum surface. SEM images were collected as previously described.[1]

Fluorescent Labeling

Fluorescent labeling of NIH 3T3 actin cytoskeletons was accomplished by rinsing cells in phosphate buffered saline (PBS), and fixing with 4% paraformadehyde (in PBS) for 15 minutes at 37 °C. Following an additional three washes with PBS, cells were permeabilized in 0.2% Triton-X/PBS for 5 minutes. Permeabilized cells were rinsed again three times with PBS, and incubated with Alexa-Fluor-596-conjugated-phalloidin (5 units/mL) for actin-labeling, for 2 hours at 37 °C. After another three rinses with PBS and once with distilled water, coverslips were mounted to an additional coverslip using Fluoromount G (Invitrogen).

---

[1] C. M. Cheng and P. R. LeDuc, *Mol Biosyst*, 2006, **2**, 299-303.

Confocal Microscopy

Confocal images were acquired with a Leica SP5 confocal system. MP3D samples were imaged using a Leica Plan-Apochromat 63X 1.2 NA immersion objective and excited with the 488 nm laser line. Spectral windowing of a photomultiplier tube (PMT) allowed capture of distinct emission spectra for the dye and transmitted light was captured by an additional PMT. Stacks of images were acquired with approximately 135 nm separation and analyzed using NIH ImageJ software.

VIDEO DESCRIPTIONS

Supplementary Video 1. Confocal characterization of actin distribution in MP3D system. A full animation of the image stack slices from the first row of Fig. 3 spanning from z = 0 μm to z = 12.5 μm is provided here, showing actin distribution from the glass surface up to and following the curvature of the dorsal (upper) fiber. With the exception of contrast stretching and background subtraction, these data are unmodified with no other image processing applied.

Supplementary Video 2. Confocal characterization of actin distribution on glass alone. A full animation of the image stack slices from the second row of Fig. 3 spanning from z = 0 μm to z = 4.8 μm is provided here, showing actin distribution from the glass surface upwards. With the exception of contrast stretching and background subtraction, these data are unmodified with no other image processing applied.

Supplementary Video 3. MP3D devices allow for displacement of the dorsal cell membrane with simultaneous monitoring of the SAC calcium response. This video provides the full sequence of calcium activation resulting from stimulation with MP3D fiber displacement and 100 μm ATP. First, the MP3D fiber is briefly displaced and calcium increases inside the cell. After recover to basal calcium levels, ATP stimulus is applied, and calcium again increases inside the cell. This is the full time series highlighted in Fig. 4.