Electronic Supplementary Material

A microscale anisotropic biaxial cell stretching device for applications in mechanobiology

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Device fabrication and assembly

To fabricate the 3-layer PDMS device, two master molds were prepared by photolithography on polished silicon wafers with SU-8 2050 negative photoresist (Microchem, Newton, MA, USA). Masters were further exposed to Trichloro(1H,1H,2H,2H-perfluorooctyl)silane vapor (Sigma-Aldrich, USA) to ease PDMS removal after curing. The elastic properties of the PDMS were controlled by varying the ratio of curing agent relative to the base monomer. The bottom and top sections of the device used a ratio of 1:15 and was cured for 2 h at 80 °C, whereas the membrane had a ratio of 1:20 to increase the elasticity and was cured for 1 h at 80 °C. Fluorescent beads (FluoSpheres, 200 nm, Invitrogen, CA, USA) were embedded into the membrane to act as stationary markers in order to calibrate the membrane deformation during stretching. Initially suspended in an aqueous solution, the fluorescent beads were resuspended in isopropanol at a ratio of 1:10 to enhance mixing with the PDMS. PDMS (1:20) with embedded beads was spin-coated on a clean silicon wafer coated with the releasing agent at a speed of 3000 rpm for 5 min. The bottom section and the membrane were air-plasma treated for 30 s at 30 W, bonded together and further cured for 30 min at 80 °C. The bottom section, which is now bonded to the membrane, was peeled off from the underlying wafer. The membrane was then punctured manually with a needle in line with the low pressure and the fluidic channel ends. Holes were punched in the top section using a biopsy punch, in order to accommodate external tubing. The bottom (with membrane) and top sections were again air-plasma treated. The top section was carefully aligned with the bottom part using a mask aligner (OAI, CA, USA). The whole assembly was further cured for 30 min at 80 °C.

Incubation chamber

Since PDMS is porous to gas, the device is kept at 37 °C in a humid atmosphere of 5% CO_2 and 95% air to maintain culture medium pH at a physiological value. As depicted in Supplementary Fig. 1 in Online Resource 4, the device in placed in a petri dish, which is put into a petri dish heater, and kept at a temperature on 37 °C. A lid with holes allows the insertion of external tubing and gas input of 5% CO_2 and 95% air at a flow rate of 100 ml/min. To maintain humid conditions, sponges filled with water are placed inside the petri dish. The whole set up is covered with a plexi-glass heating box to prevent condensation from forming on the lid covering the petri dish heater, and to keep the lid temperature at 38 °C.