Supplementary Material (ESI) for Lab on a Chip

Supplementary Information for

Simple Benchtop Patterning of Hydrogel Grids for Living Cell Microarrays

Scott A. Zawko[1], *Christine E. Schmidt*[1,2*]

[1]Department of Chemical Engineering, The University of Texas at Austin, Austin, Texas

[2]Department of Biomedical Engineering, The University of Texas at Austin, Austin, Texas

* To whom correspondence should be addressed (schmidt@che.utexas.edu)

This supplement contains:
Materials and Methods
Table I
Figs S1 to S4

Materials and Methods

Nylon mesh measurements For each mesh size ten random fields of view were imaged by Brightfield microscopy with either a 4x or 10x objective. These images were analyzed with ImageJ software by thresholding and inverting to obtain images with white backgrounds and black squares in the foreground. The area of each black square (i.e., mesh opening) was then quantified by the ImageJ Analyze Particles feature. The width of each mesh opening was calculated by taking the square root of each area measurement. Then the distribution of mesh openings was plotted in Excel.

Atomic force microscopy Images were acquired with lateral resolution 175 nm using an Asylum Research Molecular Force Probe 3D operating in contact mode in air. Specimen was an alginate hydrogel grid patterned with Nylon mesh possessing 48 μm mesh openings.

Confocal laser scanning microscopy Images were acquired with a Leica SP2 AOBS mounted on an inverted microscope and equipped with a 63x oil objective. Fluorescent hydrogel grids were prepared by spiking a 1% w/v sodium alginate solution with 0.02% w/v fluorescent TRITC-conjugated dextran (MW=70 kDa, Sigma). The fluorescent dextran was entangled inside the alginate hydrogel by the high alginate crosslink density thus permitting confocal fluorescent imaging. The grid was then covered with 30 μL of water, coverslipped, and sealed with nail polish. The specimen was imaged with a 63x oil objective. To create three-dimensional renderings of the hydrogel grids a series of 146 z-slices were captured with a z-step of 0.2 nm. After image acquisition Imaris 5.0 software was used to render three-dimensional images of the serial slices.

Table I. Nominal openings and thread diameters of Nylon mesh

Mesh Opening (μm)	Thread Diameter (μm)	Mesh Count per cm^2
90	39	6,009
64	33	10,628
48	38	13,521
35	33	21,626

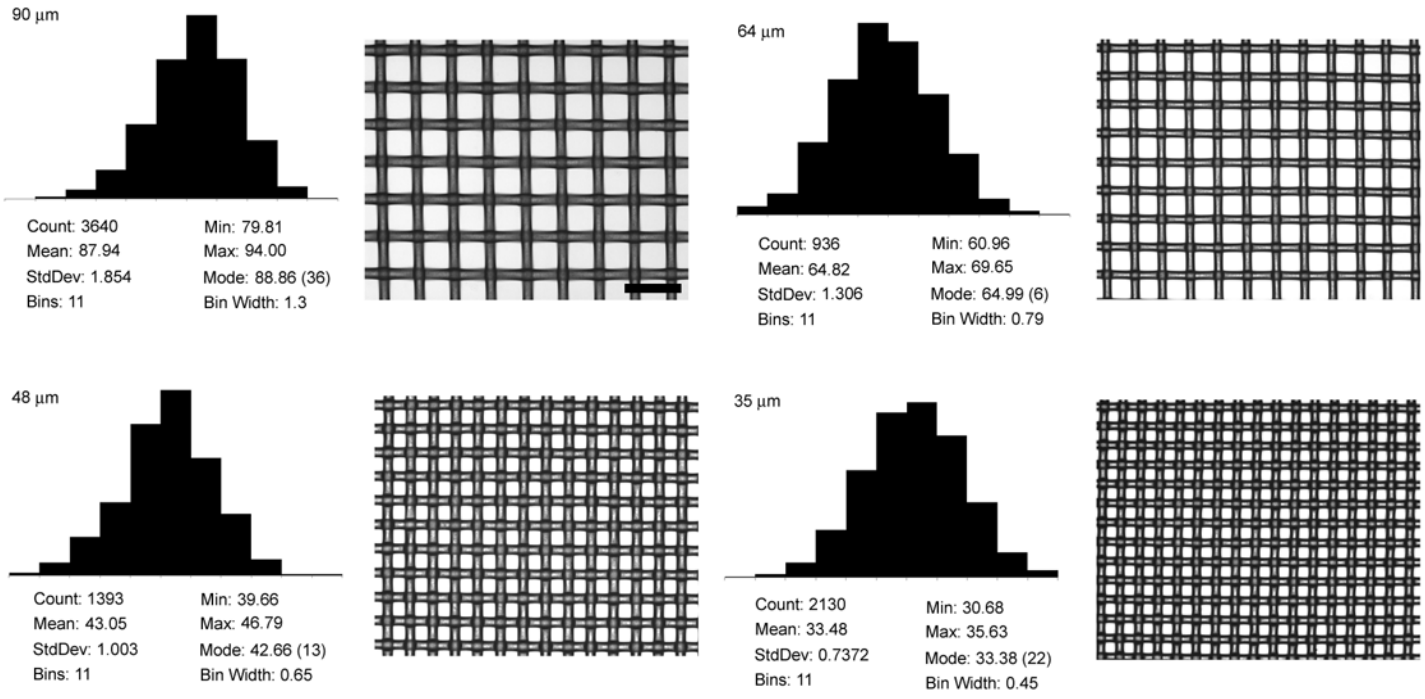

Figure S1. Measurements of Nylon mesh openings and representative Brightfield images. Scale bar is 200 µm.

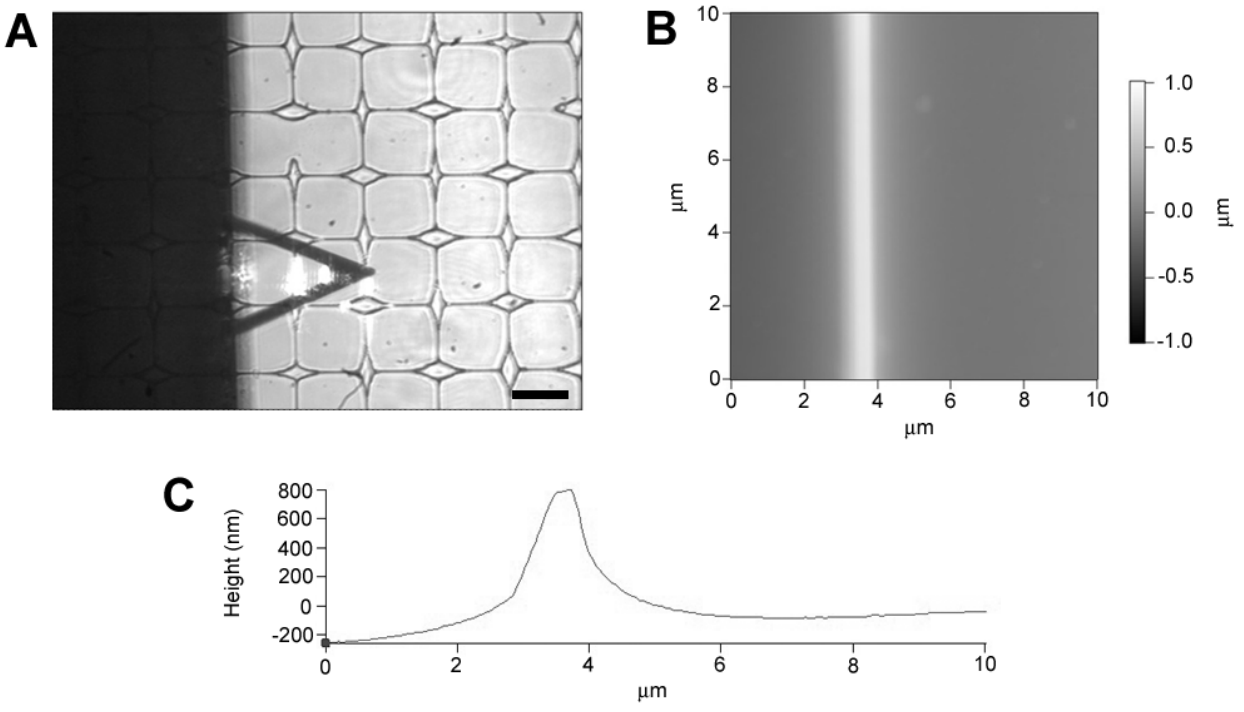

Figure S2. Atomic force microscopy imaging of alginate hydrogel grid. (**A**) Optical light image of the hydrogel grid and cantilever. Scale bar is 100 µm. (**B**) Height map of the hydrogel barrier. (**C**) Height profile across a hydrogel barrier. This is the least high portion of the microwell. The wall is 1000 nm high and 2 µm wide.

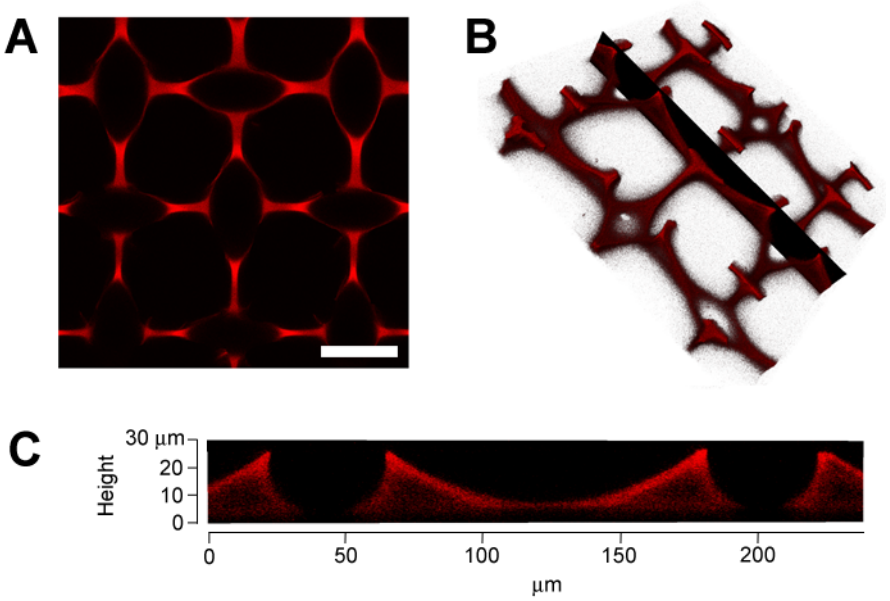

Figure S3. Confocal laser scanning microscopy of a fluorescent alginate hydrogel grid. (**A**) Single scan of the alginate grid in the xy-plane. Scale bar is 50 µm. (**B**) Three-dimensional volume rendering of a stack of xy-confocal scans. The black xz-plane that bisects the image is depicted in C. (**C**) An xz-slice of B showing the height profile of the hydrogel grid.

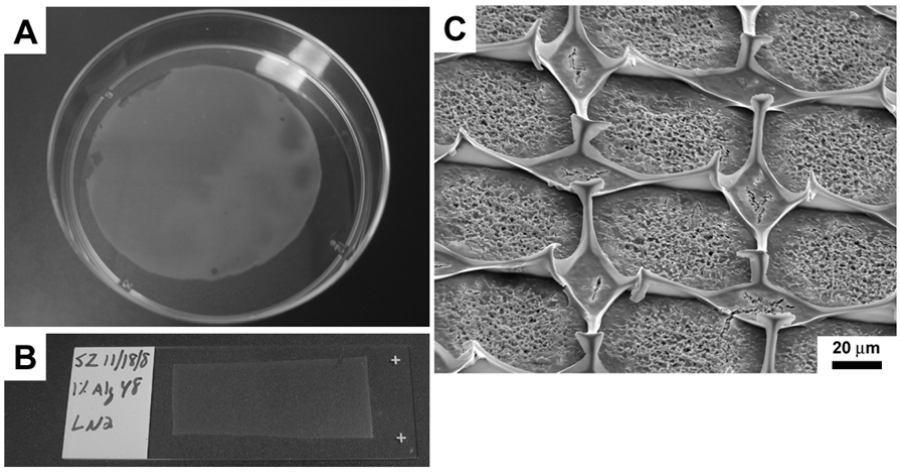

Figure S4. Three substrates coated with alginate hydrogel grids. (**A**) 150 mm TC dish patterned with 1.8 million compartments. (**B**) Glass microscope slide patterned with a hydrogel grid with a compartment density of 13,500 per square centimeter. (**C**) Hydrogel grids can conform to the rough surface of porous PVDF membrane.