Electronic Supplementary Material (ESI) for Lab on a Chip

Multi-gradient hydrogels produced layer by layer with capillary flow and crosslinking in open microchannels

SUPPLEMENTARY INFORMATION

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Supplementary figures

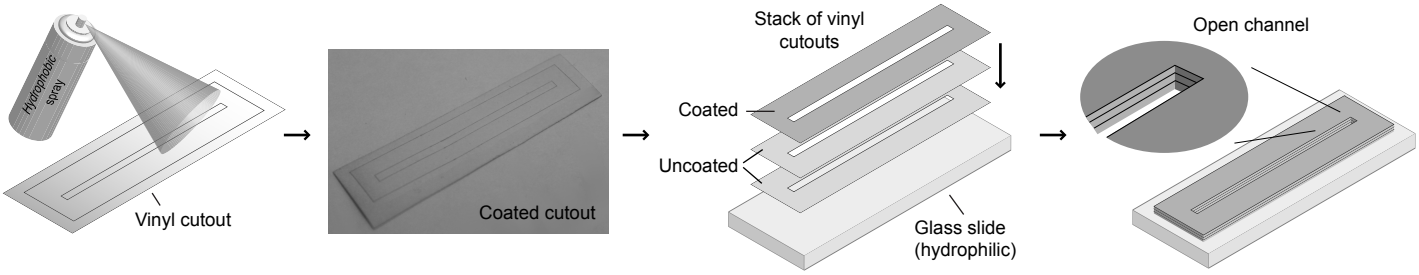

Figure S1. Fabrication protocol for open channel device. A MACtac vinyl sheet with channel cut with plotter is coated with hydrophobic spray. Coated and uncoated vinyl channel cutouts are stacked on a glass slide to form an open channel.

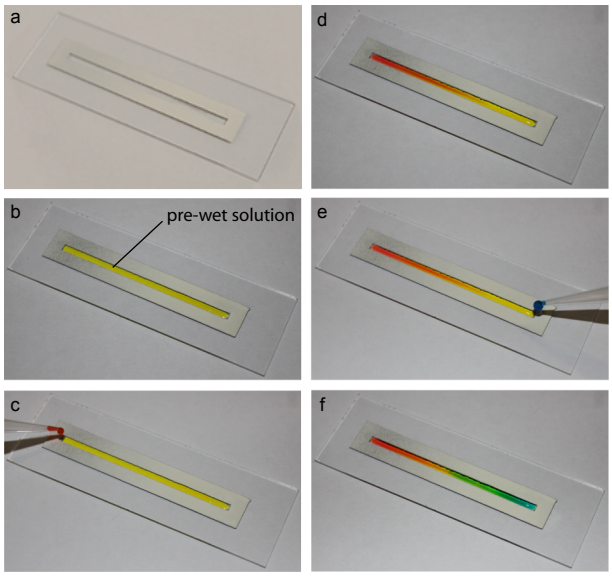

Figure S2. Protocol for creating multi-layered hydrogels with gradient layers. (a) Open channel device. (b) Open channel pre-wet with precursor solution. (c) Droplet added to one end of pre-wet open channel. (d) The solution is left at rest to achieve desired vertical and lateral uniformity. The gradient precursor solution is then crosslinked, for example by ultraviolet (UV) light. (e-f) The process a-c is repeated for subsequent layers.

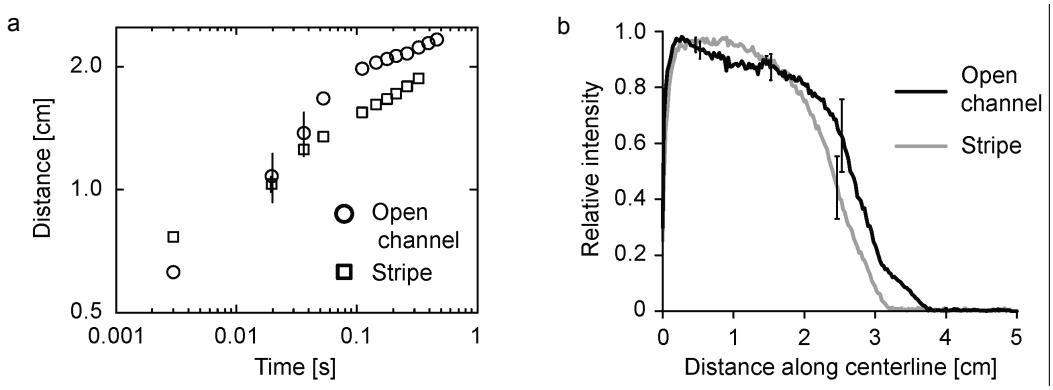

Figure S3. Similar capillary flow speeds and gradient profiles are observed for the current single layer open channel device and our previous coated slide device.[1] (a) Position vs. time of the tip of a 10 µL droplet of dye plus 20% PEGDM 1000 added to a 20 µL pre-wet solution of 20% PEGDM 1000 in the open channel device and coated slide devices. Speed in open channel is somewhat faster than in stripe. Experiments were repeated at least two times and the standard deviation was less than the symbol size except where noted by error bars. (b) Centreline averaged relative intensity profiles for fluorescent gradients produced by adding a 10 µL droplet of phosphate buffered saline with 0.1% fluorescein to a 20 µL pre-wet volume of PBS in the open channel device and coated slide devices. Error bars indicate the average standard deviation in the intervals [0,1], [1,2] and [2,3] cm over three repetitions.

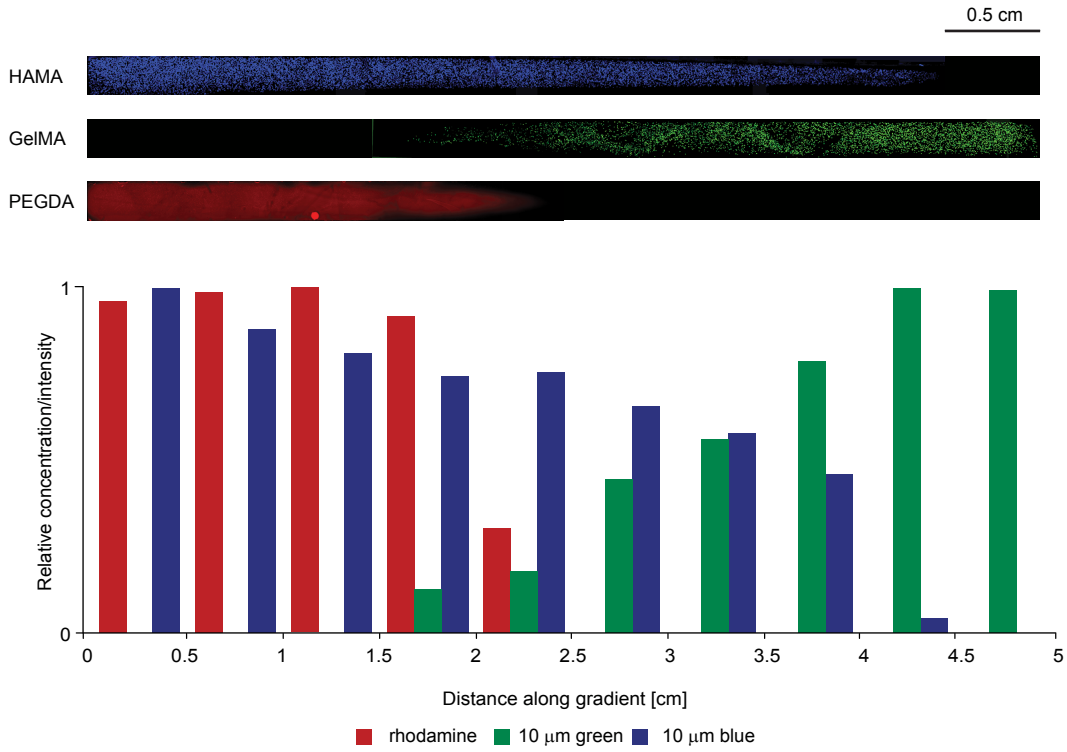

Figure S4. Triple layer gradient hydrogel with soluble and microsphere gradients in an open channel device. Stitched 10X microscope images of rhodamine gradient in 20% PEGDA 2000 in the bottom layer, a 10 µm green fluorescent microsphere gradient in 5% GelMA in the middle layer and a 10 µm blue fluorescent microsphere gradient in 1% HAMA in the top layer. Bar chart characterizes the centerline relative intensity in each interval for the bottom layer and the centerline microsphere concentration in each interval for the middle and top layers. Intensity levels of displayed microscope images have been increased to visualize particles. Periodic patterns due to nonuniform lighting in stitched microscope images.

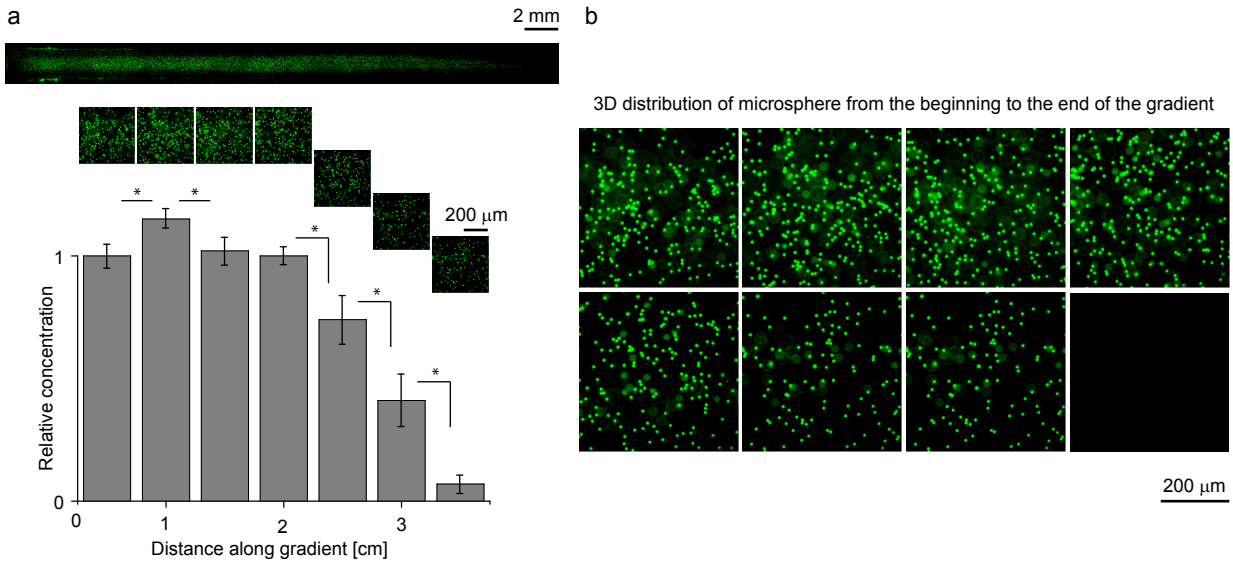

Figure S5. Microsphere gradient in 1% HAMA in open channel device. (a) Stitched 2X microscope images. Bar charts are based on normalized and laterally averaged microsphere from 10X microscope images captured in each interval. Error bars indicate the standard deviation over three repetitions. 10X zooms are sub-images cropped from 10X microscope image. * indicates that the mean difference between a pair of bars in a bar chart were statistically significant ($p < 0.05$) based on one-way analysis of variance (ANOVA) testing.(b) Microspheres in an out of focus in the 10X zooms indicate the 3D positioning of the microspheres.

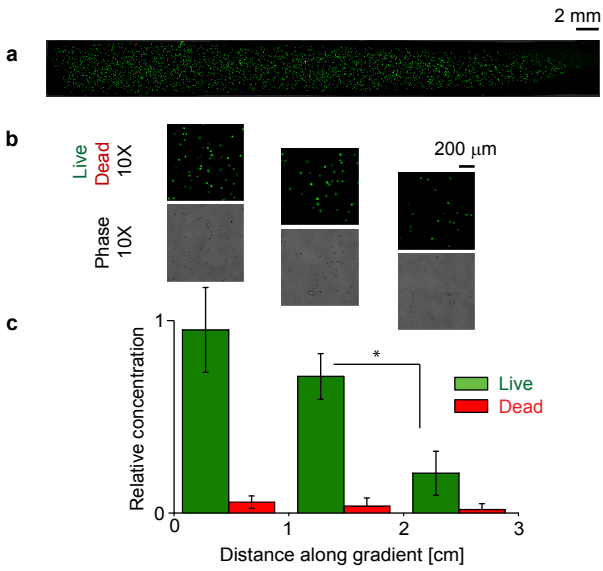

Figure S6. Cell concentration gradient in 5% GelMA in open channel. (a) Stitched 2X microscope images. (b) 10X zooms and (c) bar chart characterize the live/dead cell counts in each interval. * indicates that the mean difference between a pair of bars in a bar chart were statistically significant ($p < 0.05$) based on one-way analysis of variance (ANOVA) testing.

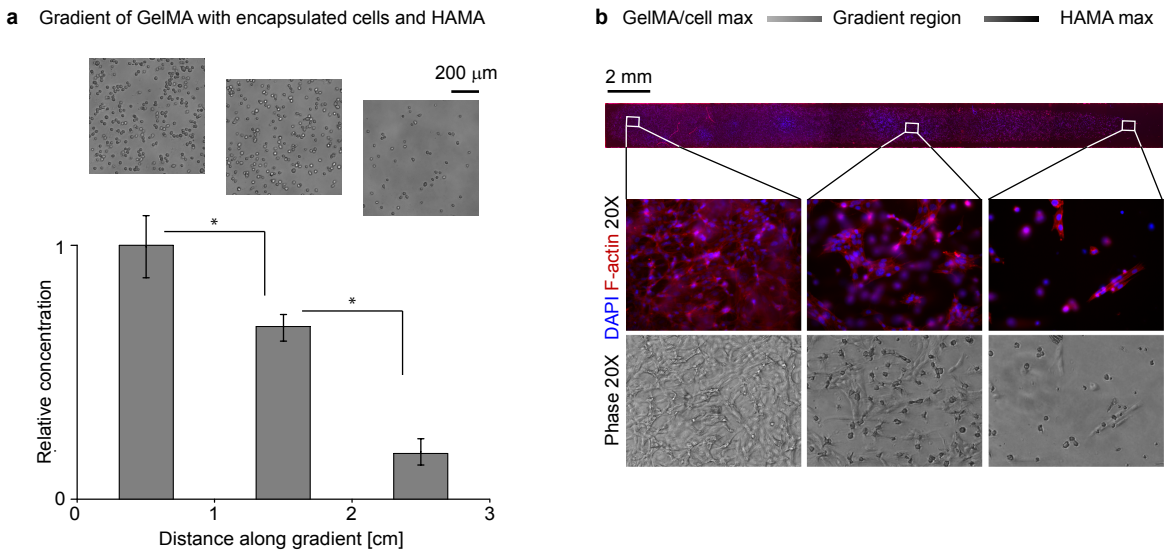

Figure S7. Encapsulated cells in GelMA - HAMA biomaterial gradients in open channels. (a) Bar chart and 10X zooms characterize the centreline concentration of cells encapsulated in 3D. * indicates that the mean difference between a pair of bars in a bar chart were statistically significant ($p < 0.05$) based on one-way analysis of variance (ANOVA) testing. (b) Gradient biomaterial exhibited a cell spreading gradient, visualized by staining cells for F-actin (phalloidin) with a nucleic counterstaining (DAPI). Stitched 2X images show the overall cell gradient profile, with white boxes to indicate the relative positions of the 20X images along the open channel, from left to right.

Supplementary Table S1. Drop and pre-wet constituents for making each layer in our multi-layer gradient hydrogels. Layers are ordered in the manner they were created, the bottom layer (layer 1) to the top layer.

Figure	Layer	Pre-wet (15 µl)	Drop (7.5 µl)
2a	1 (bottom)	1% HAMA	1% HAMA + 0.1% rhodamine
	2 (top)	5% GelMA	5% GelMA + 0.1% fluorescein
2b	1 (bottom)	1% HAMA	1% HAMA + 10 µm blue microspheres
	2 (top)	5% GelMA	5% GelMA + 10 µm green microspheres
2c	1 (bottom)	20% PEGDA 2000	20% PEGDA 2000 + 0.1% rhodamine
	2 (middle)	5% GelMA	5% GelMA + 10 µm green microspheres
	3 (top)	1% HAMA	1% HAMA + 10 µm blue microspheres
S4	1 (bottom)	20% PEGDA 2000	20% PEGDA 2000 + 0.1% rhodamine
	2 (middle)	5% GelMA	5% GelMA + 10 µm green microspheres
	3 (top)	1% HAMA	1% HAMA + 10 µm blue microspheres

Supplementary Video S1. High-definition (HD) video was captured of gradient generation in an open channel device. The open channel was pre-wet with 20 µl of 5% GelMA with blue food dye in PBS. The droplet added to the pre-wet channel was 10 µl of 5% GelMA with red food dye in PBS. The video was cropped and converted to MP4 format.

Supplementary Methods I. Methods and Materials

The materials and methods key to the new open channel device are listed in the main text. Methods and materials common to the current and past projects,[1] such as cell culturing and imaging, are listed below.

Materials: Hydrophobic WX2100 spray (Cytonix Corp., Beltsville, MD); pre-cleaned microscope glass slides (Thermo Fisher Scientific Inc., Waltham, MA); poly(ethylene glycol-dimethacrylate) (PEGDM, MW 1000) (Monomer-Polymer & Dajac Labs, Trevose, PA); photoinitiator (PI) 2-hydroxy-1-[4-(hydroxyethoxy)phenyl]-2-methyl-l-propanone (Irgacure D2959, Ciba Specialty Chemicals Inc., Florham Park, NJ); gelatin, methacrylic anhydride, 3-(trimethoxysilyl) propyl methacrylate, (Sigma-Aldrich Inc., St. Louis, MO); sodium hyaluronate (avg. MW 53 kDa, Lifecore Biomedical Inc., Chaska, MN); green fluorescent polymer 10 µm microspheres (1 wt% solids, Duke Scientific Corp., Palo Alto, CA); Live/Dead® and phalloidin (Alexa Fluor 594) stains (Invitrogen Corp., Carlsbad, CA); 4,6-Diamidino-2-phenylindole (DAPI, Vector Laboratories Inc., Burlingame, CA). NIH-3T3 cells were cultured in Dulbecco's modified Eagle medium (DMEM, Invitrogen Corp., Carlsbad, CA) supplemented with 10% fetal bovine serum and 1% penicillin–streptomycin (Invitrogen Corp., Carlsbad, CA) in a 5% CO2, 37 °C incubator. Gelatin and HA were methacrylated by standard chemical procedures to produce gelatin methacrylate (GelMA)[2] and hyaluronic acid methacrylate (HAMA).[3] All other reagents and tissue culture components were purchased from Sigma-Aldrich Inc. (St. Louis, MO) unless otherwise noted.

Gradient protocol: The concentration gradients were produced in the open channel as described in the main text. The open channel was plasma treated prior to each use; no effect on the hydrophobic region was observed. When pipetting fluid onto the open channel, the angle between the centre axis of the pipette and the centerline of the stripe was kept less than 20°. Following droplet addition, the gradient solution in the open channel was allowed to stand for a prescribed amount of time while diffusive mixing smoothed the gradient profile vertically and laterally. During operation, the device was kept in a covered humid Petri dish

(with wet towel) to avoid evaporation. Agitating the device could cause fluid flow and distort the gradient; agitation was avoided until analysis or crosslinking were complete.

Fluorescence imaging: Fluorescence images were captured with a Kodak Gel Logic 100 Imaging System with optimal exposure times and zoom.

Flow speed experiments in Fig. S3: The gradient protocol was carried out with pre-wet solutions containing 1X Dulbecco's Phosphate Buffered Saline (DPBS) solution with 20% (w/v) PEGDM 1000. Droplets of Trypan blue solution containing the same % PEG as the pre-wet solution were pipetted onto one end of the filled open channel. Subsequent fluid motion was recorded by digital camera at 60 frames per second (fps) and the dye tip position was measured in successive frames with Matlab.

Fluorescent dye gradients in Fig. S3: Gradients of fluorescent dye were generated by pre-wetting a single layer open channel with 20 μL PBS and adding a 10 μL droplet of the same solution plus 0.1 % fluorescein dye. The centerline profiles were extracted and processed with ImageJ and Matlab.

Microsphere gradients in 1% HAMA in Fig. S5: The gradient protocol was carried out by pre-wetting an open channel with 40 μL of 1% HAMA dissolved in PBS, and then adding a 20 μL drop of the same solution plus 0.1% (w/v) of a solution containing 10 μm diameter green fluorescent microspheres, diluted 20 times from the stock solution. Images were captured along the length of the channel with an inverted fluorescence microscope (TE- 2000-U, Nikon, Melville, NY) with 2X and 10X objectives, and quantified with Matlab. The 10X images were taken every 0.5 cm along the stripe, starting at approximately 0.25 cm from one end of the channel.

Cell concentration gradient in 5% GelMA in the open channel in Fig. S7: The gradient protocol was followed by pre-wetting the open channel device with 15 μl 5% GelMA and 0.5% PI (w/v) in DPBS, and then adding 5 μl drop of 5% (w/v) GelMA and 0.5% PI (w/v) in DPBS containing NIH-3T3 cells (5×10^6 cells/ml). 1 min after droplet addition, the precursor solution was photocrosslinked by 30 s exposure to UV light (wavelength 360-480 nm, power 6.9 mW cm^2), placed in DMEM, and incubated at 37 °C for 4 hours. The hydrogel was then stained with a Live/Dead® assay and imaged with fluorescence microscope. The quantification protocols were the same as for the microsphere gradients.

Cell spreading gradient in a gradient hydrogel in Fig. S7: The gradient protocol was followed by pre-wetting an open channel device with 15 μl of 0.5% (w/v) PI and 1% (w/v) HAMA in DPBS, and then adding a 5 μl drop containing NIH-3T3 cells (5×10^6 cells/ml), 0.5% (w/v) PI and 5% (w/v) GelMA in DPBS. The gradient precursor solution was allowed to stand for 1 min and was then photocrosslinked by 15 s exposure to UV light (wavelength 360-480 nm, power 6.9 mW cm^2). The device was placed in DMEM and incubated at 37 °C for 1 day. To visualize cell spreading, the gradient hydrogel was fixed with 4% paraformaldehyde and stained with phalloidin and DAPI according to manufacturer protocols to visualize F-actin filaments and cell nuclei, respectively. Overlapping 2X, 10X and 20X phase and fluorescence images were captured by inverted fluorescence microscope. Cell counts were extracted from 20X phase images of the stripe captured at day 0 (i.e. following crosslinking) using ImageJ and Matlab.

Data analysis: Statistical significance was determined by balanced one-way analysis of variance (ANOVA). For all statistical tests, the level of significance was set at $p < 0.05$.

References

1. M. J. Hancock, F. Piraino, G. Camci-Unal, M. Rasponi and A. Khademhosseini, *Biomaterials*, 2011, **32**, 6493-6504.
2. J. W. Nichol, S. T. Koshy, H. Bae, C. M. Hwang, S. Yamanlar and A. Khademhosseini, *Biomaterials*, 2010, **31**, 5536-5544.
3. J. A. Burdick, C. Chung, X. Jia, M. A. Randolph and R. Langer, *Biomacromolecules*, 2004, **6**, 386-391.