Electronic Supplementary Materials for

Towards spatially-organized organs-on-chip:

Photopatterning cell-laden thiol-ene and methacryloyl hydrogels in a microfluidic device

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Supporting Methods: CD4 T cell isolation from PBMC's

RosetteSep enrichment of human CD4+ T lymphocytes.

Contents of a TRIMA apheresis chamber were collected in a 50ml centrifuge tube and diluted with an equal volume of wash buffer (PBS + 2% FBS-heat inactivated). RosetteSep human CD4+ T cell enrichment cocktail (STEMCELL Tech., #15062) was added to the diluted blood at 50µl/ml of the original blood volume. Blood was mixed well and incubated for 20min at room temperature. After incubation, blood was layered over Ficoll-Paque (GE healthcare) for density centrifugation at 1200Xg for 30min with no brake. Enriched CD4+ T lymphocytes were collected post-centrifugation, washed twice and resuspended in wash buffer for counting.

EasySep enrichment of human naïve CD4+ T lymphocytes

Enriched total CD4+ T cells were resuspended in EasySep buffer (wash buffer + 1mM EDTA) at 50 $X10^6$ cells/ml. EasySep human naïve CD4+ T cell isolation kit (STEMCELL Tech., #19555) biotin antibody cocktails were added to the cells at 18µl/ml and incubated at room temperature for 5min to label all cells besides naïve CD4+ T cells. Anti-biotin magnetic beads were added to the cells at 18µl/ml and incubated for 5min at room temperature. Post-incubation, cells were added to a polystyrene round bottom culture tube and placed in an EasySep magnet (STEMCELL Tech.) for 5min. Non-magnetically labelled cells were then collected in a new centrifuge tube, washed once with wash buffer, resuspended in cell culture medium and assessed for purity via flow cytometry.

Supporting Figures

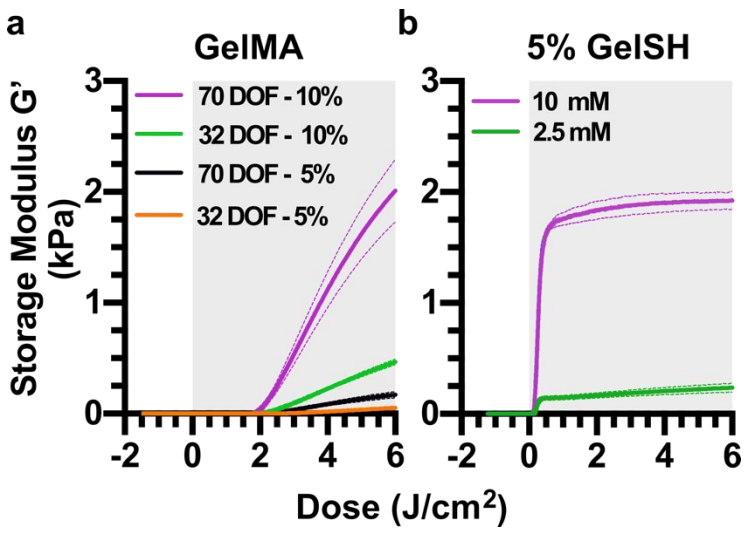


Fig. S1 Initial testing of hydrogel formulation to access biomimetic storage moduli. (a) Rheometry measurements of the storage modulus of the GelMA during *in situ* polymerization under constant light exposure at 50 mW/cm^2. Legend indicates the degree of functionalization (DOF, %) of the gelatin and concentration (% w/v) of GelMA present. n=3. (b) Rheometry measurements of the storage modulus of 5% w/v GelSH during *in situ* polymerization under constant light exposure at 50 mW/cm^2. Legend indicates the concentration of norbornene, where there is 8 mol norbornene per mol PEG-NB. Lines show mean (solid) and std deviation (dashed), n=3 technical replicates. Grey shading indicates the time period when the light was turned on.

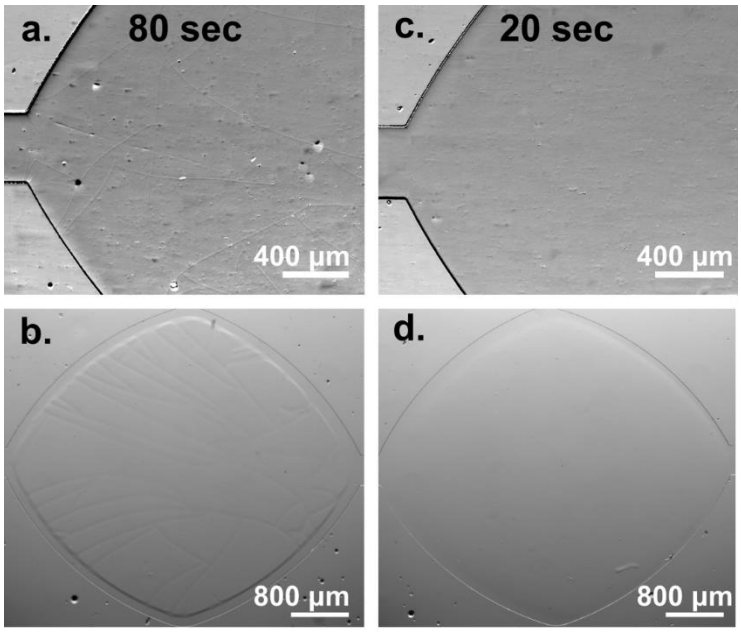


Fig. S2 Optimization of exposure time to air plasma prior to bonding of the microfluidic device. (a) Zoomed-in transmitted light image of PDMS showed cracking after 80 sec exposure to air plasma. (b) On-chip patterned hydrogel (10% GelNB, 3.4 mM LAP, 3.75 mM 4-arm PEG-SH) exhibited defects that were templated by the cracks in the PDMS. (c) Zoomed-in image of smooth PDMS surface after 20 sec exposure to air plasma. (d) Patterned hydrogel without cracks and/or defects on a chip that was plasma-treated for 20 seconds.

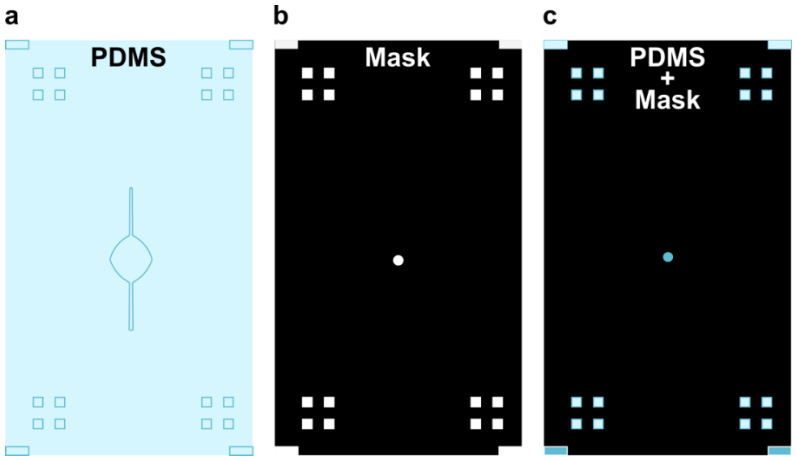


Fig. S3 Alignment of photomask for photopatterning on chip. (a) Schematic of the PDMS layer, which included recessed alignment markers in each corner. Alignment marks were transferred to the PDMS from the SU-8/silicon master. (b) Schematic of a photomask with a transparent central circular feature and transparent alignment markers in each corner. (c) Schematic of PDMS layer aligned with photo-mask. Blue tint from PDMS comes through transparent alignment markers in photomask.

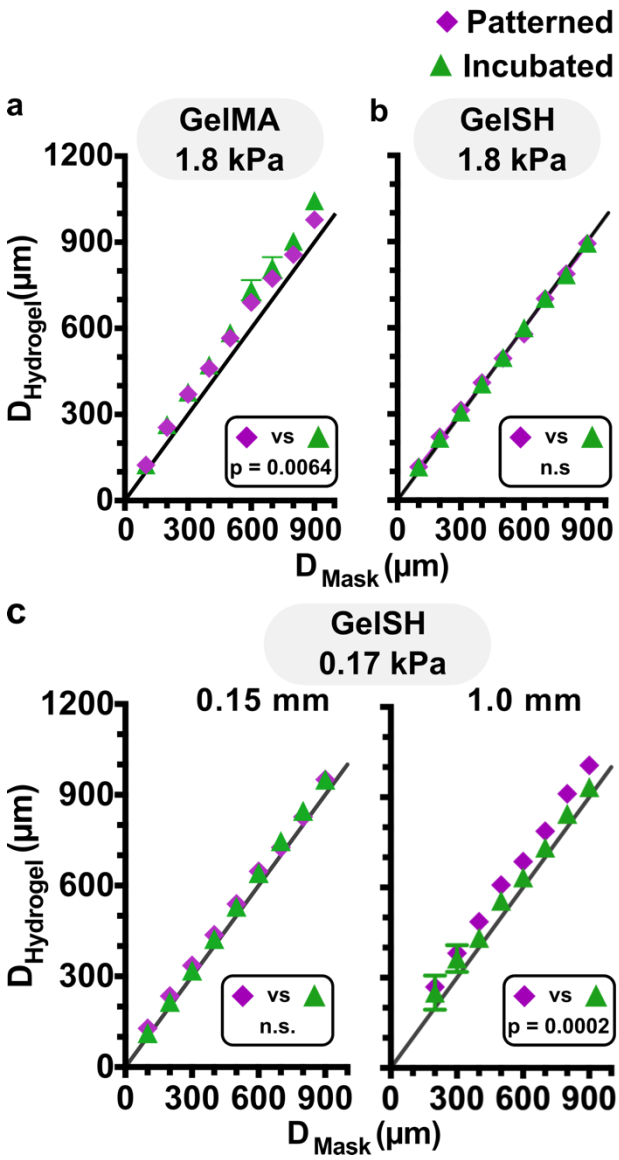


Fig. S4 Assessing the stability of pattern resolution after incubation. (a-c) Quantification of accuracy between the diameter of the design on the photomask and the resulting diameter of the hydrogel region, measured either immediately after patterning ("patterned") or after an additional 30-min incubation and rinse ("incubated"). Symbols and error bars represent mean and standard deviation; some error bars too small to see. Significance obtained by Paired T-test, n.s. indicates $p > 0.05$, in all panels. Black line represents $y = x$. (a) data for 1.8 kPa GelMA hydrogel n = 3, $p = 0.0064$. (b) data for 1.8 kPa GelSH, n = 4, n.s. (e) data for 0.17 kPa GelSH, for microfluidic chips made with (left) a 0.15 mm coverslip, n = 4 chips, or (right) a 1 mm glass slide, n = 3 chips.

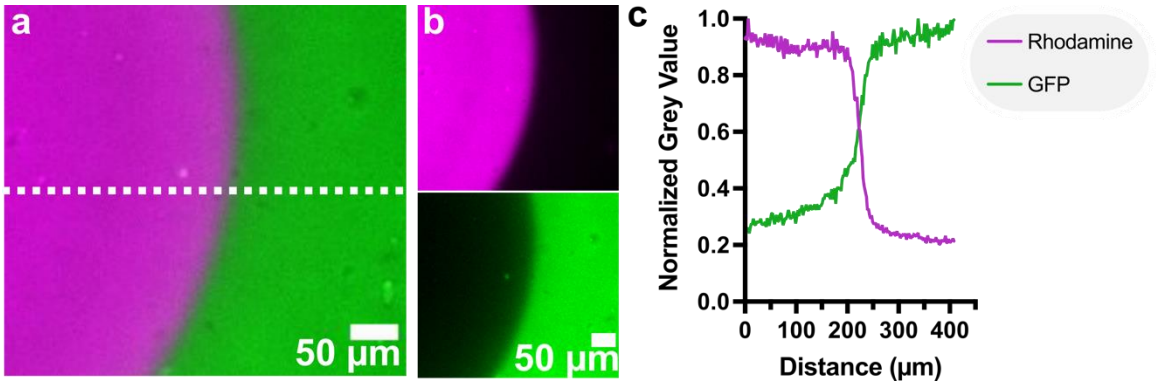
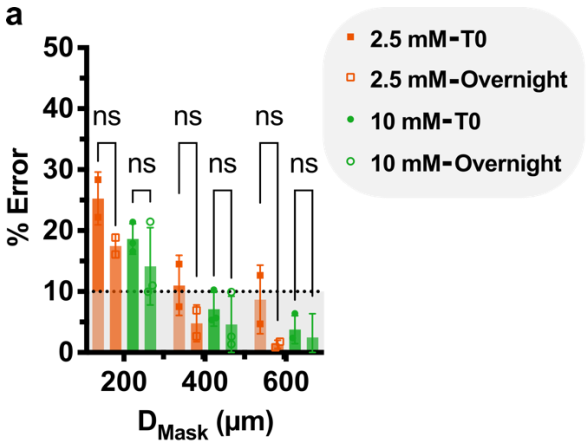


Fig. S5 Boundary of two sequentially patterned hydrogels. (a) Zoomed-in composite image and (b) individual channels of boundary from image on Fig. 4b. Image collected on a widefield microscope (Zeiss Axiozoom). White dotted line shows position of linescan. (c) Linescan of measured intensity in Rhodamine (magenta) and GFP (green) channels from Fig. S5a. Linescan data were normalized by dividing by the max intensity in each channel.

a



b

Macromer	Formulation	Expected G' (kPa)	Feature Size (µm)		
	[NB]		600	400	200
5% GelSH	10 mM	1	100%	100%	100%
	2.5 mM	0.1	80%	80%	80%

Fig. S6 Accuracy and stability of cell-laden micropatterned features. (a) Calculated percent error of each feature versus the target diameter from the photomask design, for 5% GelSH hydrogels with 2.5- or 10-mM NB linker concentration. Feature diameter was measured immediately after patterning (T0) and again after overnight (12 hr) culture under fluid flow. The dotted line was drawn arbitrarily at 10% error, and grey area shows values that fall in that region. Bars represent mean and standard deviation. Significance obtained by Two-way ANOVA, n.s. indicates $p > 0.05$. (b) Stability of patterned hydrogels after overnight perfusion. Reported values are % of patterned features that remained anchored on the chip, as opposed to dissolved or rinsed away, after overnight perfusion. N=9 features for 10 mM chips and N=6 features for 2.5 mM (3 features/chip).

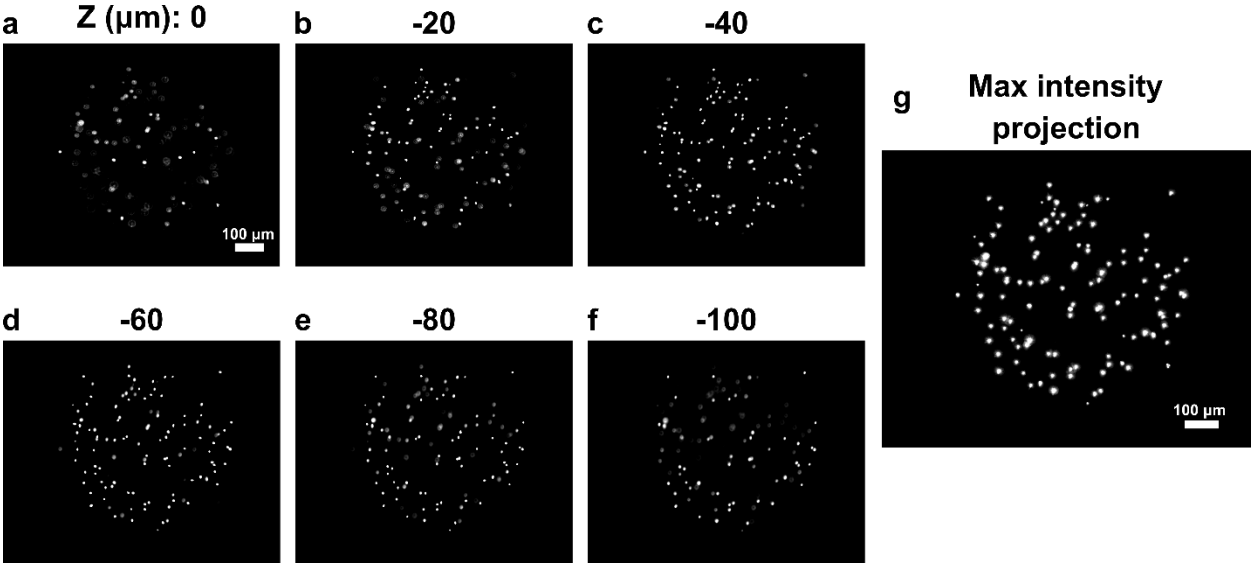


Fig. S7 Cell distribution in the z-direction in photopatterned gels and method of analysis by max intensity projection. (a-f) A representative cell-laden photopatterned gel was imaged at 112x magnification, at depths varying from 0-100 μm. NHS-rhodamine labeled human primary T cells were in-focus in different locations in each image, indicating that cells were distributed throughout the gel and not in a monolayer. Z: 0 μm is near the top of the chamber, near the PDMS layer and Z: -100 μm is near the bottom of the chamber, near the glass layer. Images were obtained with the Zeiss AxioZoom microscope. (g) A max-intensity projection was generated from the 13 images spanning 130 μm. In-focus cells from each focal plane are presented together, which simplifies cell counting. (Images are representative of n=3 chips)

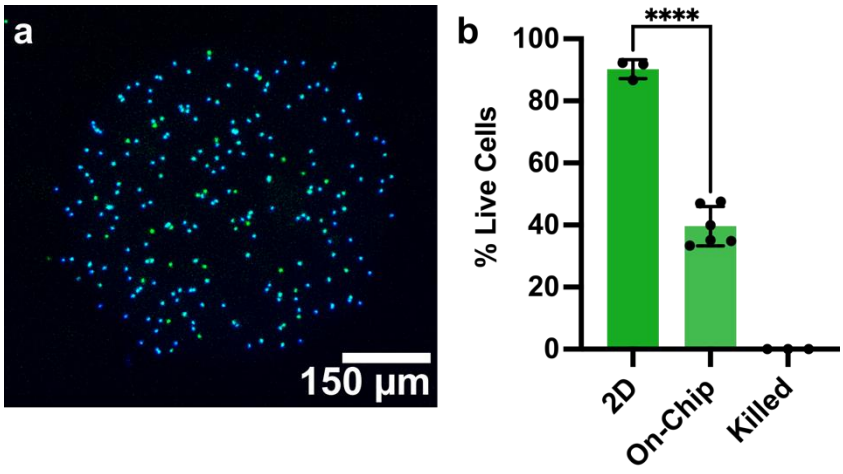


Fig. S8 Viability of patterned T cells after 24 hr culture under fluid flow. (a) Representative image of patterned 3D culture after viability staining with Calcein-AM (green) and DAPI (blue). (b) Quantification of the viability of patterned CD4+ T cells in 5% GelSH with 10 mM NB hydrogels as a function of feature dimensions after overnight culture under continuous fluid flow, versus off-chip (2D) controls (****$p \leq 0.0001$ One-way ANOVA with Tukey's multiple comparisons).

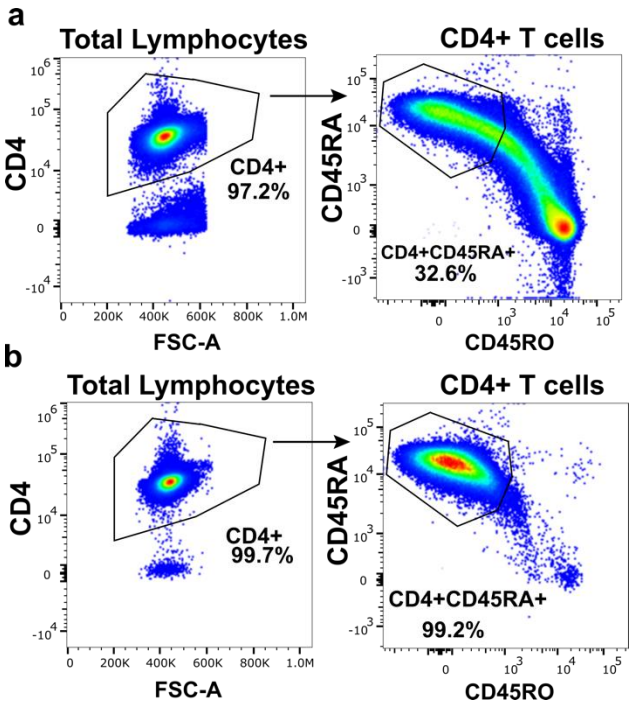


Fig. S9 Representative characterization of isolated human naïve CD4+ T lymphocytes. Naïve CD4+ T cells were isolated using a two-step enrichment process from TRIMA collars obtained from healthy donors. Purity of isolated naïve CD4+ T cells was determined using flow cytometry after surface staining of lymphocytes with antibodies against CD4, CD45RA, and CD45RO. (a) Phenotype of the T cell population after RosetteSep™ and Ficoll-Paque density centrifugation, showing enrichment of total CD4+ T lymphocytes. (b) Phenotype of the T cell population after isolation from enriched total CD4+ T cells using EasySep™ negative selection kit, showing high purity of naïve CD4+ T lymphocytes (CD4+CD45RA+CD45RO-)**.**