Electronic Supplementary Material (ESI) for Biomaterials Science.

Supplemental Information

Perfusable cell-laden matrices to guide patterning of vascularization *in vivo*

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Supplementary Methods:

1.1 Quantification of the Degree of Acrylation

Acrylation was confirmed with 1H NMR by measuring the percentage of gelatin's lysine residues with primary amine substitutions.

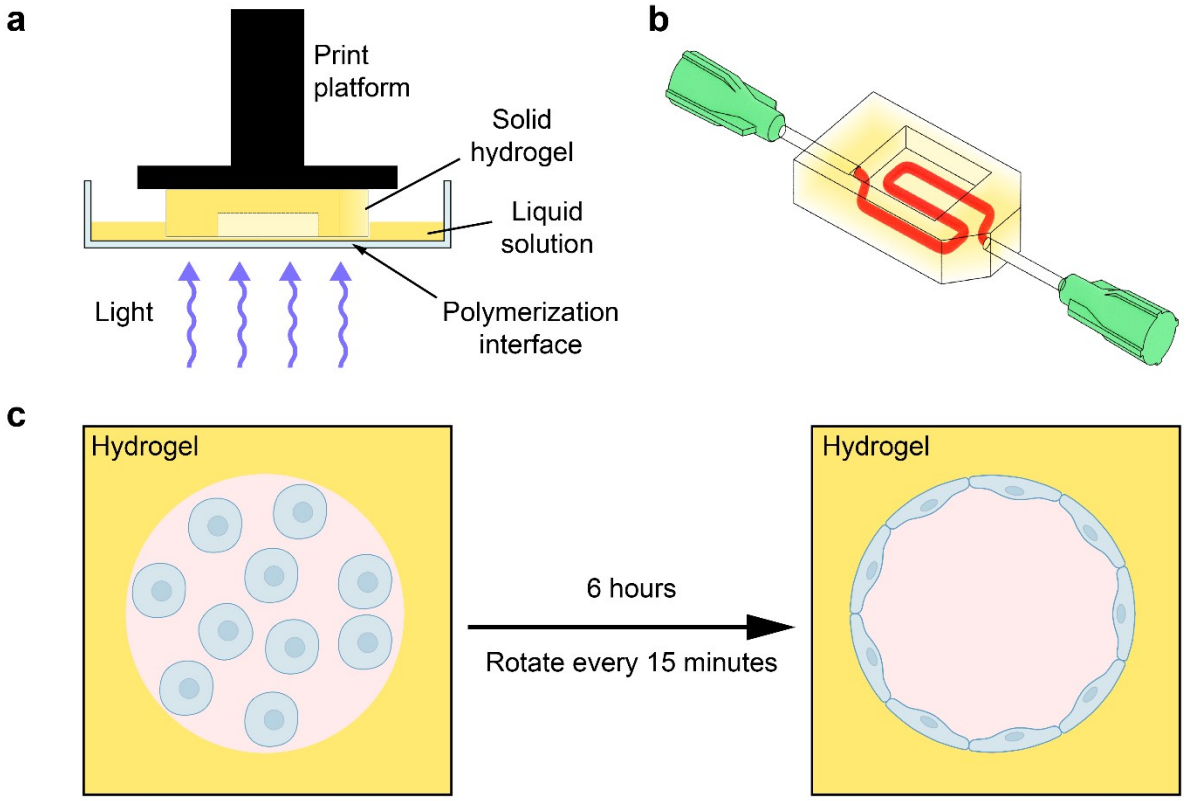
1.2 Photorheology

An AR-G2 parallel plate rheometer was used to determine storage modulus. For each replicate, a 45 µL sample of polymer solution (containing 0.05 wt% Irgacure 2959) was added to the bottom plate and the top plate was lowered to obtain a gap size of 50 µm. The polymer solution was pre-conditioned for 30 s using an angular frequency of 5 rad/s and 10% strain and then exposed to 10 mW/cm^2 of light from a 365 nm lamp.

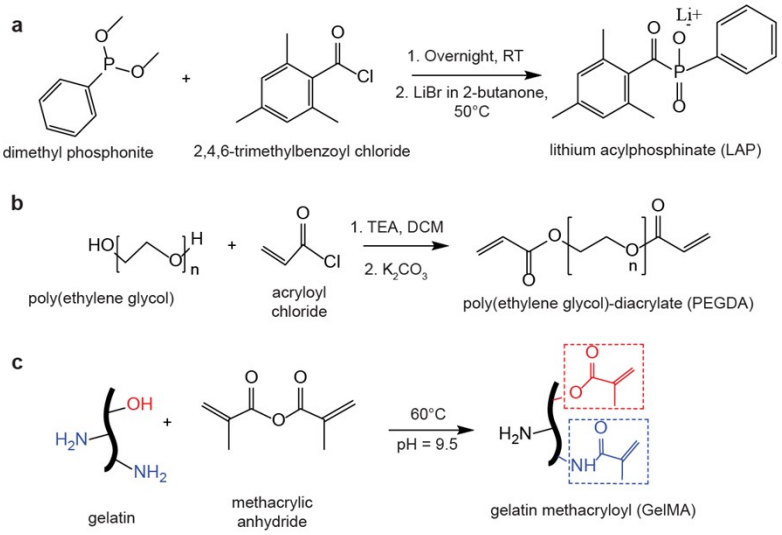
1.3 Polymer Degradation

150 µL droplet hydrogels with varying concentrations of PEGDA and GelMA were synthesized and maintained in 0.2 mg/mL collagenase. Hydrogel wet weight was taken multiple times over a 4-hour duration.

Supplementary Data:



Supplementary Figure 1: Schematic of hydrogel fabrication and endothelialization. (a) Hydrogels are printed using a SLATE stereolithographic 3D printer. Hydrogels are then soaked in sterile PBS (or cell culture media, if hydrogels are in the M-C_CC or M-C_E group) for one day at 37°C, exchanging PBS/media at least twice. (b) M-C_CC and M-C_E hydrogels are catheterized and injected with a suspension of endothelial cells and culture media. (c) After injecting the entire channel with the endothelial cell suspension, hydrogels are rotated every 15 minutes for a total of 6 hours in accordance with an existing protocol (30). This rotation ensures that endothelial cells coat the entire inner surface of the patterned channel. After 6 hours of rotation, cell culture media is flowed through the channel at a rate of 5 µL/min to maintain perfusion culture.



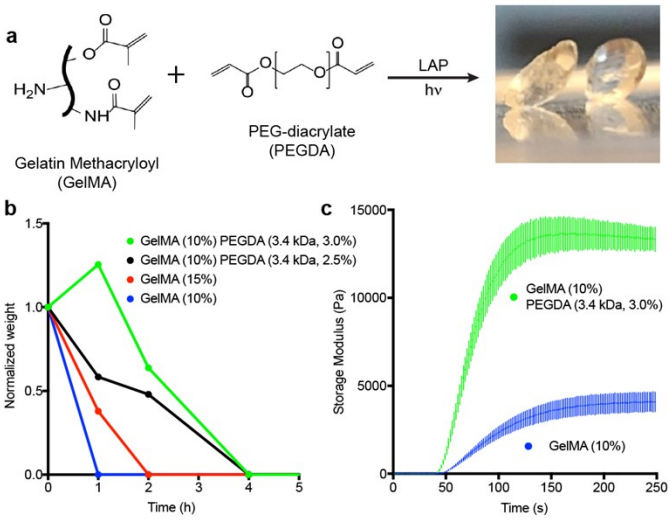
Supplementary Figure 2: Reaction schemes for the synthesis of photoinitiator (LAP) and photoreactive polymers (PEGDA and GelMA). (a) Synthesis of LAP (b) PEGDA, (b) and GelMA.

Polymer	Acrylation (%) (in-house)
PEGDA (3.4 kDa)	88.7 ± 1.3
GelMA	53.4 ± 8.3

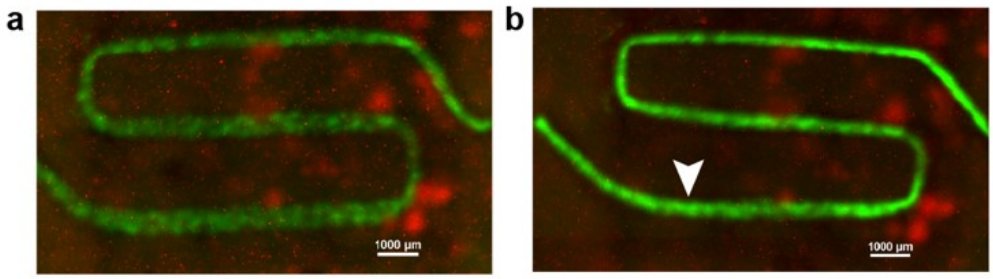
Supplementary Table 1: The degree of polymer acrylation is consistent across multiple batches (n = 3, mean +/- SD).

Polymer	Storage Modulus (Pa)
3.4 kDa PEGDA (20% w/v)	64,962 ± 4,091
GelMA (5% w/v)	688 ± 179

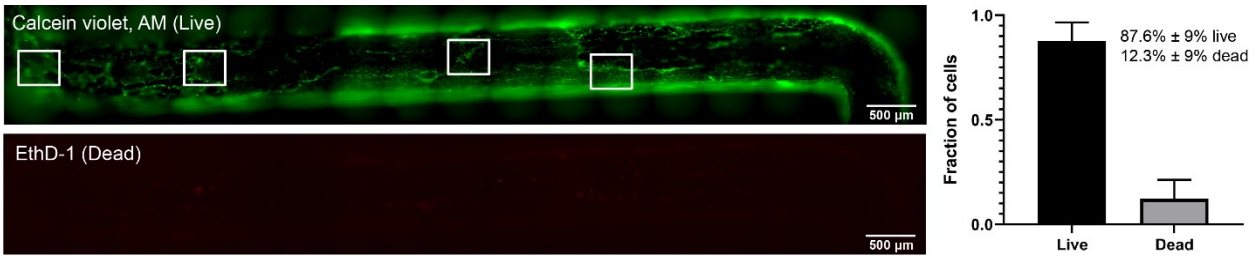
Supplementary Table 2: Analysis of polymer mechanics demonstrates low batch-to-batch variability n = 3, mean +/- SD).



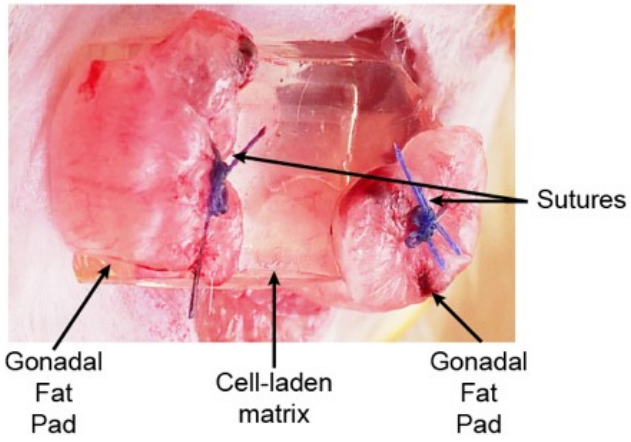
Supplementary Figure 3: Photopoylmerized hydrogels with tunable physical and enzymatic degradation properties. (a) GelMA and PEGDA were photocrosslinked with LAP to form 150 µL droplet hydrogels. (b) Varying concentrations of GelMA and PEGDA resulted in tunable degradation kinetics in 0.2 mg/mL collagenase (n = 1 per group). (c) The addition of PEGDA greatly enhanced hydrogel storage modulus and allowed improved handling (n = 9 per group, mean +/- SD).



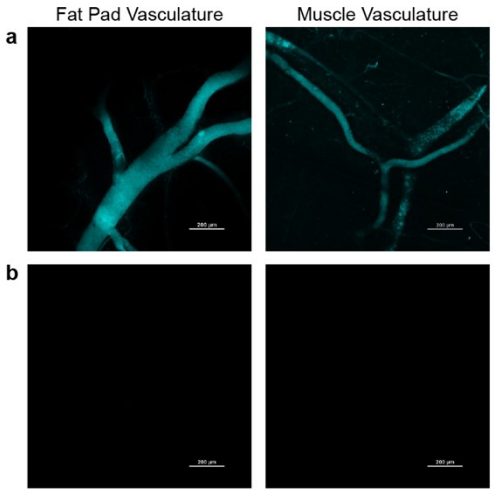
Supplementary Figure 4: Contraction of collagen cords in vitro over one day. (a) On day 0, GFP HUVECs and hMSCs resuspended within 2.5 mg/mL collagen are injected into the channel of matrices with bulk RFP HUVECs and hMSCs (scale = 1000 µm). (b) After one day, the GFP HUVECs and hMSCs are seen to contract the collagen matrix, forming a collagen cord (white arrowhead; scale = 1000 µm).



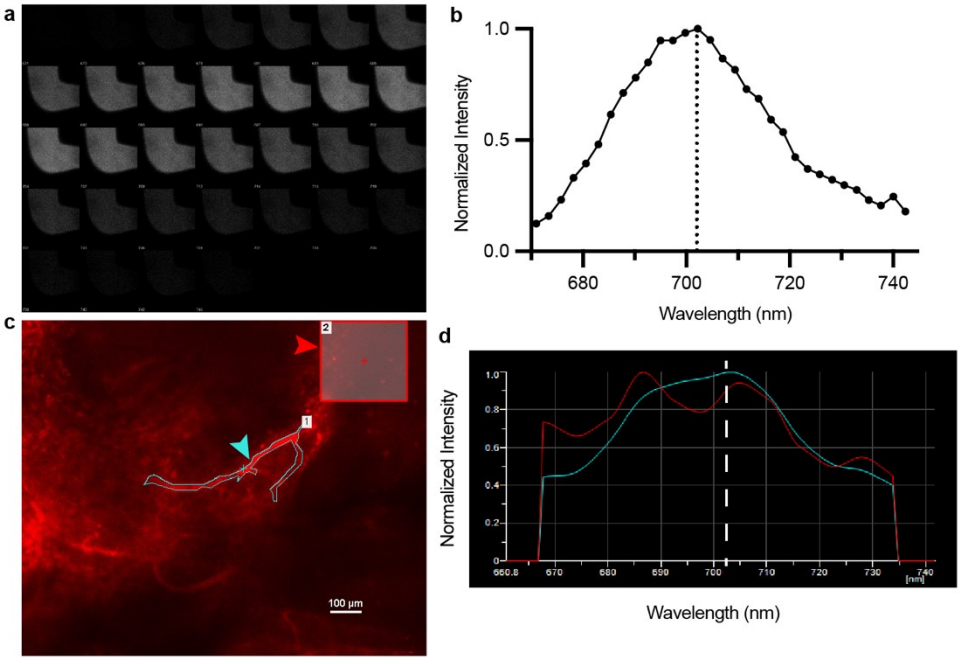
Supplementary Figure 5: Over 85% of perfusion-cultured HUVECs remain viable in patterned matrices after seven days of perfusion: An acellular matrix with an endothelialized serpentine channel, perfused at five µL/min for seven days, was stained for cell viability. We prepared the Calcein violet, AM solution (ThermoFisher Scientific) at one µM and one µL per mL of the DEAD (Ethidium Homodimer-1) component of the LIVE/DEAD imaging kit (Invitrogen, R37601) in PBS. The staining solution was injected into the channel and incubated for 30 minutes at 37 °C before imaging (Nikon Eclipse Ti epifluorescence microscope). Four regions of interest were randomly selected to manually count live and dead cells for a cell viability output.



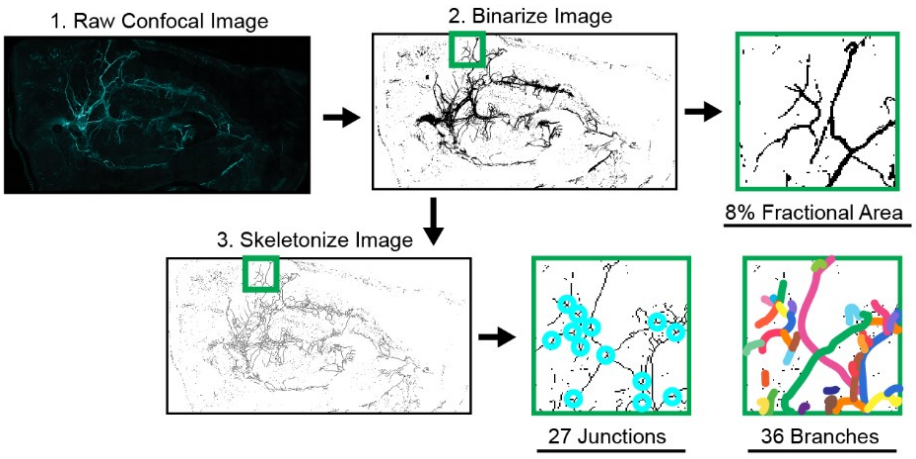
Supplementary Figure 6: **Gonadal fat pad implantation of cell-laden matrices.** Surgical photograph of implanted matrix sutured to the left and right gonadal fat pads.



Supplementary Figure 7: *In Vivo* Testing of Far-Red Dextran. (a) Vessels in the fat pad and muscle tissue of mice that received an intravenous injection of CF680 Far-Red dextran. (b) Vessels in the fat pad and muscle tissue of mice that did not receive an intravenous injection of CF680 Far-Red dextran. (scale = 200 μm).



Supplementary Figure 8: CF680 dextran spectral fingerprint. (a) CF680 spectral fingerprint identified within an acellular matrix with CF680 dextran injected into the perfusable lumen. (b) Quantification of emission fluorescent spectrum in acellular matrix demonstrates peak emission at 702 nm. (c) Identification of vascular fluorescence region (blue arrowhead) and background region (red arrowhead) within retrieved cell-laden matrix after i.v. CF680 dextran administration to mouse (scale = 100 μm). (d) Quantification of spectral fingerprints in the vascular region (blue trace) compared to the background region (red trace).



Supplementary Figure 9: Image Analysis Pipeline to Identify Key Vascular Parameters. Confocal imaging of intravenous fluorescent dextran illuminates vascular networks in the matrices (Confocal). This image with 16-bit color depth is first binarized with ImageJ (Binarize). The binary image indicates the location of the vasculature (black pixels) compared to the background (white pixels). This binary image is quantified to identify the fraction of blood vessels (black pixels) to total pixels in the image. The binarized image is thus quantified by the fractional area of the gel occupied by the vasculature. Then, the binarized image is skeletonized with ImageJ. The number of junctions, branches, and total branch length (not shown here) can be quantified using the ImageJ Skeletonize plugin. Junctions and branches in sample ROIs are shown below.