Supplementary Information

Stereolithographic hydrogel printing of 3D culture chips with biofunctionalized complex 3D perfusion networks

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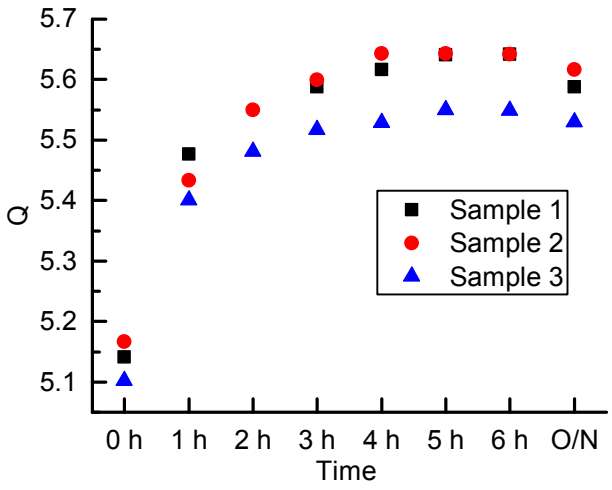


Fig. S1 Time-dependent volumetric swelling ratio of 3D printed solid PEGDA objects immediately after printing (0 h) and after selected times of immersion in DI water (O/N = overnight).

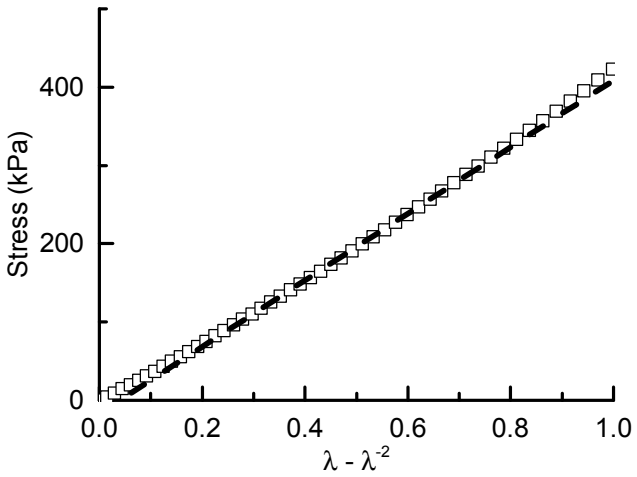


Fig. S2 Uniaxial compression of a 3D printed solid PEGDA cylinder (Ø6 mm; height 5 mm). The measured stress is displayed versus λ-λ^{-2}, where λ is the extension ratio. Linear fitting using ordinate values from 0.2 to 0.8 (dashed line) yields a shear modulus of 0.43 MPa.

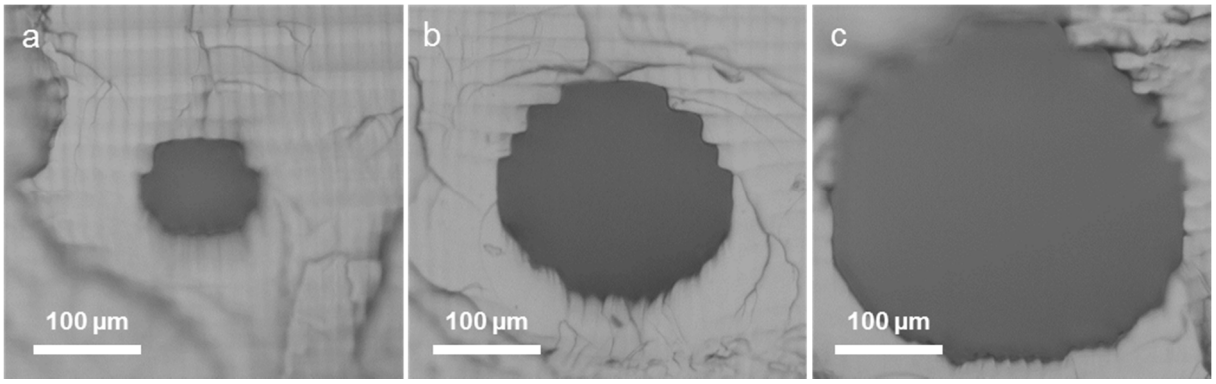


Fig. S3 Optical micrographs of cross-sectioned 3D printed channels of circular design with diameters of (a) Ø100 µm, (b) Ø200 µm, and (c) Ø300 µm.

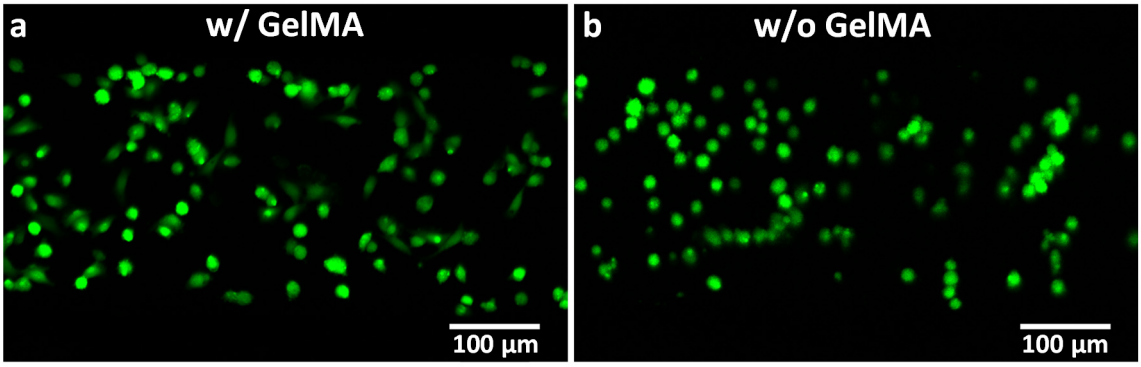


Fig. S4 Fluorescence images of CRL-2922 cells (an endothelial cell line, ATCC) cultured for 4 h at the bottom surfaces of 3D printed PEGDA square channels functionalized with GelMA (a) or without (b). Live cells were stained with calcein AM (green) and seeding density was 5×10^5 cells cm^{-2}. After 4 h of culture, cells started to spread on modified PEGDA hydrogel surfaces while those seeded on non-modified hydrogels retained a spherical shape.

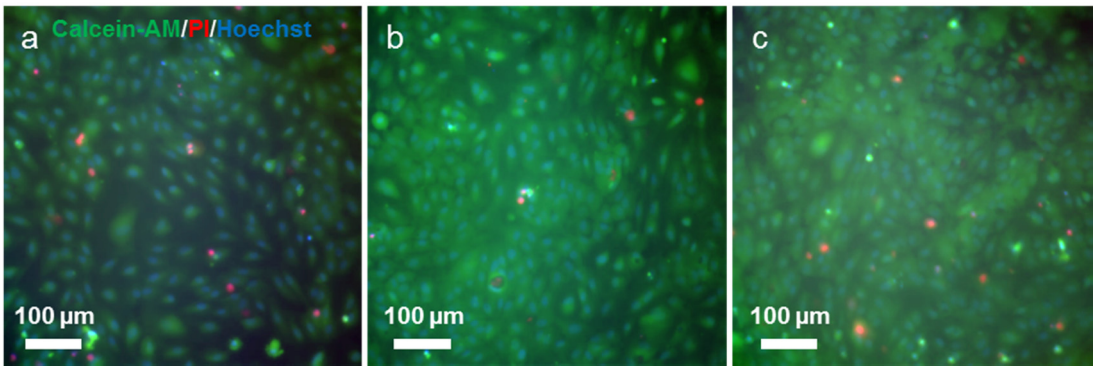


Fig. S5 Representative composite fluorescence images of HUVEC cells cultured for 24 h in 3D printed PEGDA bowl structures functionalized with GelMA. The micrographs show three merged fluorescent channels: live cells (calcein AM, green), dead cells (propidium iodide, red) and nuclei (Hoechst 33342, blue). Seeding densities were (a) 7×10^4 cells cm^{-2}, (b) 1.4×10^5 cells cm^{-2}, and (c) 2.1×10^5 cells cm^{-2}.

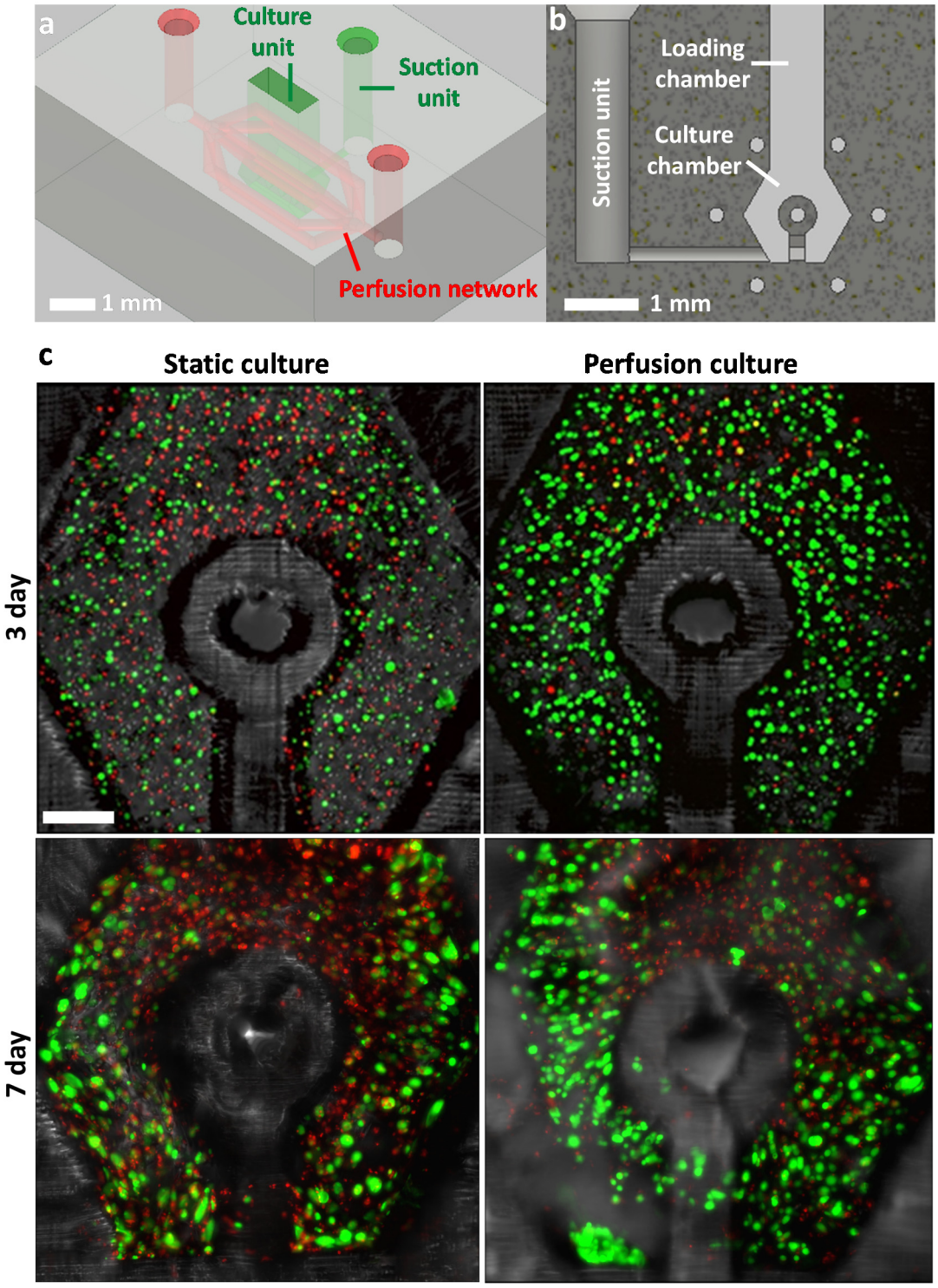


Fig. S6 (a) Design of a culture chamber with 6 surrounding and one central traversing vascular channels and (b) a corresponding cross-sectional view. A loading chamber is incorporated right above the culture chamber to simplify the cell loading procedure by enabling cell loading through pipetting rather than perfusion. A suction unit composed of a perfusion channel connected to the culture chamber is intended to completely remove liquid medium by suction before cell loading. (c) Confocal fluorescence

micrographs of cross-sectioned slices of the culture chamber in printed 3D culture chips showing the live HT-29 cells (calcein-AM, green), the dead cells (propidium iodide, red) and the PEGDA construct outline (transmitted light, gray) after a culture period of 3 and 7 days in the presence and the absence of medium perfusion through the channel network. The scale bar is 200 μm.

Experimental sections

Cell culture

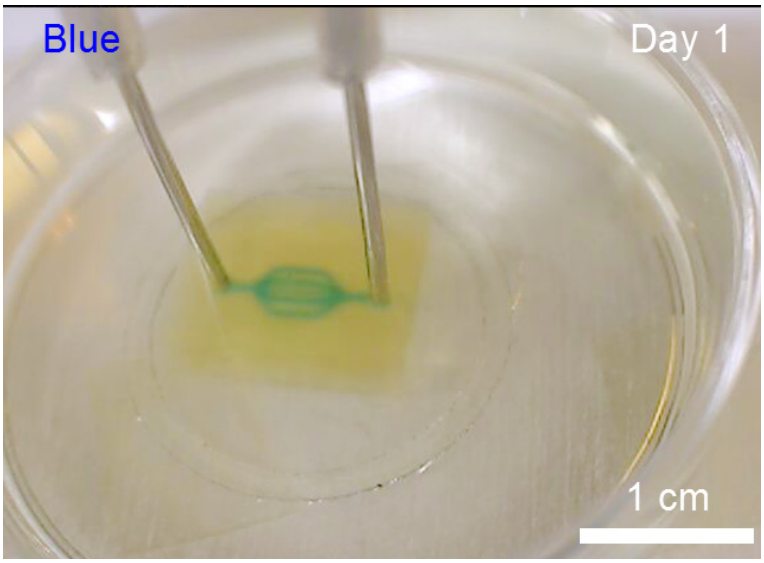
Human colorectal adenocarcinoma cells (HT-29) (ATCC, HTB-38, Rockville, MD, USA) were cultured in Dulbecco's modified Eagle's medium with high glucose (DMEM, Biowest) supplemented with 10% v/v fetal bovine serum (FBS, Sigma-Aldrich) and 1% v/v penicillin-streptomycin (P/S, Sigma-Aldrich). Cells were incubated at 37 ^{o}C in a humidified atmosphere with 5% CO_2. Cell cultures were passaged according to the vendor's manual.

Long-term perfusion culture

The perfusion culture setup consisted of a medium reservoir, a multi-channel peristaltic pump, a sample incubator, a heated circulating water bath and a gas supply unit. An external supply of 95% air and 5% CO_2 was used to maintain constant pH of culture medium in both the medium bottle and petri dishes containing the printed 3D culture chips. The medium reservoir was composed of two glass bottles with holes drilled in their lids for tubing, one for culture medium and the other filled with sterilized water for humidifying the gas mixture before entering the medium bottle in order to minimize evaporation of medium. A 0.22 μm filter was connected to the end of the gas tubing to avoid contamination. A water bath was used to keep both the medium and humidifying bottle warm. Printed chips were kept inside a microscope stage incubator. A heated circulating water bath was used maintaining the incubator temperature. The gas mixture also passed through the heated circulating bath before entering the incubator. Before perfusion culture started, all the tubing was perfused by a large amount of 70% ethanol, followed by sterilized water using a peristaltic pump (Watson-Marlow, 205U). The surfaces of all equipment, including water bath, sample incubator, the pump and the outer surfaces of the tubing were wiped with 70% ethanol

prior to performing the experiment. Printed 3D culture chips immersed in DMEM culture medium supplemented with 2% v/v penicillin-streptomycin, along with all the glass bottles were sterilized by UV-C illumination for 20 min (254 nm, UV sterilization cabinet, Cleaver Scientific). Prior to cell loading, sterilized chips were transferred to a laminar flow bench after immersion in DMEM medium overnight to exchange the water in the hydrogel for culture medium. Cell-laden GelMA solution was prepared by mixing warm (37 °C) LAP-containing GelMA solution in DPBS with an equivalent volume of HT-29 cells suspension to reach final concentrations of 100 mg mL^{-1} GelMA, 1 mg mL^{-1} LAP and 1×10^7 cells mL^{-1}. Before cell loading, the remaining liquid medium in the culture chamber was completely removed by suction using a syringe connected to the needles as well as micro-tubing. The culture chamber was finally loaded by dropwise pipetting cell-laden GelMA solution into the chamber, followed by 2 min of UV-A illumination (330-380 nm, peaking at 365 nm, 18 mW cm^{-2}). Needles that were pre-filled with culture medium were then inserted into the channel connectors in the printed chips and the perfusion rate was set at 70 μL min^{-1}. Cells cultured in equivalent chips without medium perfusion through the channel network were used as controls. After culture, samples were sectioned by manual cutting. The generated cross-sectional slices were stained with calcein-AM (2 μg mL^{-1}, Invitrogen) and propidium iodide (PI, 2 μg mL^{-1}, Sigma-Aldrich) for 1 h and then characterized by confocal laser scanning microscopy using excitation at 488 and 555 nm.

Movie S1. Perfusion using red- and blue-dyed liquids of 3D printed PEGDA object with two separate channels (cross-section 300×300 μm^2) immersed in water (3× speed).



Movie S2. Long-term perfusion of a 3D printed PEGDA object containing a multi-furcated channel network (each channel of cross-section Ø~200 μm) immersed in a water-filled petri dish with change between dyed liquids (blue, red, and undyed liquid) every day. The dye diffuses from the channel volume through the diffusion-open PEGDA matrix and establishes a stable color (concentration) gradient near the object's perimeters within the first 12 h of each change of dyed liquid. The gradient remains over the 7 days captured in the video, via daily exchange of the surrounding water bath that would otherwise become saturated with the diffusing dyes.

Movie S3. Perfusion of 3D printed PEGDA object with a dual planar channel geometry (cross-section 200×200 μm^2).

Movie S4. Perfusion of 3D printed PEGDA object with a triple 3D channel geometry (cross-section 200×200 μm^2).

Movie S5. Perfusion of 3D printed PEGDA object with a spiral channel geometry (cross-section 200×200 μm^2).