Rapid casting of patterned vascular networks for perfusable engineered 3D tissues

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Supplementary Methods and Figures

S1. Encapsulation of gradients of cells and immobilized factors

Opposing gradients of cells and immobilized factors (**Supplementary Figure 1**) were fabricated by first positioning a carbohydrate glass lattice in a vertical orientation between two glass slides. Five aliquots of different concentrations of cells and fluorescent beads were created in eppendorf tubes containing a constant concentration of low-melt agarose (see main text) maintained at 37 °C. Cell concentrations ranged from 3e6 cells/mL to 3e5 cells/mL across the aliquots. The aliquots were then serially dispensed into the mold containing the carbohydrate glass lattice which was maintained at room temperature. Delay time between dispensing operations allowed the matrix to partially solidify before the next dispensing operation. After complete gelation (incubation at 4 °C, see main text) the gel was removed from the chamber and processed as described in the main text for uniform gels.

S2. Lentiviral reporters

Lentiviral reporter constructs were generated using Multi-site Gateway recombination (Invitrogen) into a Gateway-compatible version of the third generation lentiviral vector, pRRL [1], in which the CMV sequence was replaced with the R4R2ccdB cassette from pLENTI6 (Invitrogen). Entry clones were generated using the following entry vectors, pENTR5 and pENTR-DTOPO (Invitrogen). cDNAs were constructed from CMV promoter (pCDNA3.1, Invitrogen), destabilized EGFP (pCAG-GFPd2) [2, 3], Gaussia luciferase (pGluc-Basic, NanoLight Technology), EGFP, and IFP-IRES-mCherry (generous gift of Roger Tsien) [4]. pRRL-based reporter lentiviruses were produced in 293T cells co-transfected with the second-generation packaging vectors psPAX2 and pMD2.G (Addgene).

S3. Hepatocyte isolation and maintenance

Rat hepatocytes were isolated from 2-3 month old adult female Lewis rats (Charles River) by collagenase perfusion using methods described previously [6, 7]. Briefly, animals were anesthetized with isoflorane and the portal vein was cannulated. The liver was perfused and digested

with collagenase. Hepatocytes were purified from the digest using Percoll centrifugation and then seeded at a density of 0.5e6 per well of a six well plate adsorbed with 0.14 mg/ml rat tail Collagen-1 (BD Biosciences). The following day, J2-3T3 fibroblasts were added to hepatocyte cultures (ratio of 1:1). Co-cultures were maintained in 'hepatocyte medium' (see below) for 7-10 days prior to encapsulation in perfusable or non-perfusable agarose gels as described in the main text.

S4. Hepatocyte function

Rat albumin in sampled media was quantified by enzyme-linked immunosorbant assay (ELISA) using a rat albumin ELISA kit (Bethyl labs). Urea in sampled media was measured by acid- and heat-catalyzed condensation of urea with diacetylmonoxime to give a colored-product that was measured spectophotometrically (Urea Nitrogen kit; StanBio Labs). For imaging, hepatic hydrogels were sliced into approximately 1 mm slices and then incubated with calcein-AM ("live", 5 μg/ml, Invitrogen) and ethidium homodimer ("dead", 2.5 μg/ml, Invitrogen) for 30 minutes and washed before imaging.

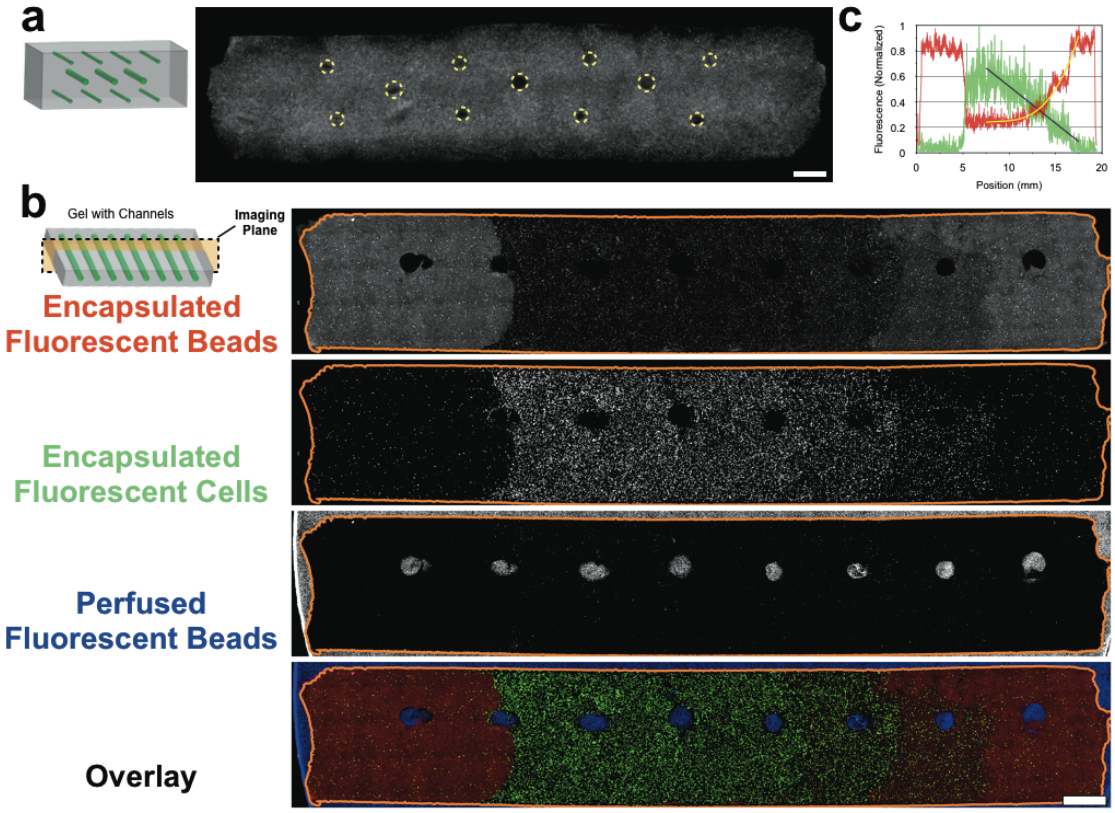
S5. Imaging and Image montage generation

Engineered scaffolds were imaged on an epifluorescence microscope (TE-200, Nikon) or a confocal microscope (LSM 710, Zeiss). Epifluorescence image montages were generated using a combination of automated and manual control point generation and adjustment with autopano-sift-c (2.5.0) and the open-source Hugin software (2010.2.0) to remove spherical aberration and vignetting from each image before adjacent image fusion. Overlays and composite images were created with the open source ImageMagick software. Confocal image montages were automatically generated by microscope control software during image acquisition (ZEN, Zeiss). Fluorescence normalization aided in visualization and discrimination of cell type throughout deep confocal image stacks (**Figure 3c,e,f**) and was accomplished by the open source Enfuse software.

S6. Cell maintenance

Human umbilical vein endothelial cells (HUVECs, Lonza; Basel, Switzerland) and C3H/10T 1/2 cells were maintained in complete Endothelial Growth Medium-2 (EGM-2; Lonza). Human Embryonic Kidney (HEK 293T) cells were maintained in Dulbecco's modified Eagle medium containing 10% fetal bovine serum (FBS), 2 mM L-glutamine, 1000 U/mL penicillin, and 100 mg/L streptomycin at 37 °C/5% CO_2. Hepatocytes were maintained in 'hepatocyte medium' containing DMEM with high glucose (4.5 g/L), 10% (v/v) bovine serum (Gibco), 0.5 U/ml

insulin (Lilly), 7 ng/ml glucagons (Bedford Laboratories), 7.5 μg/ml hydrocortisone (Sigma), and 1% penicillin-streptomycin.



Supplementary Figure 1: Multilayer vascular channels and complex cellular and immobilized factor gradients in engineered tissues. **(a)** 3D multilayer architectures were fabricated in cellularized gels. Here, 10T1/2 cells expressing EGFP were encapsulated in fibrin (40 mg/mL) at 2.5e6 cells/mL and then cross-sectioned for epifluorescent cell imaging and image-montage generation. Perfusable channels are highlighted (dotted yellow lines). Scale bars = 1 mm. **(b)** Complex gradients of immobilized factors (fluorescent beads, red) and cells (expressing EGFP, green) can be fabricated in a single scaffold independently from the channel architecture (fluorescent beads, blue). The gel boundary is outlined in orange and images represent a maximum intensity projection of a 256 μm z-stack automated montage. Scale bar = 1 mm. **(c)** Line plots of normalized fluorescence across the construct from **(b)** indicates that step, linear, and exponential gradients of cells (green) and immobilized factors (red) were readily generated in a single perfusable engineered tissue construct. Trendlines for the linear and exponential regimes (purple line, cells; yellow line, beads) showed good correlation with these gradients (R^2 for linear cell gradient = 0.75; R^2 for exponential bead gradient = 0.93). Here, cells were encapsulated along with immobilized fluorescent beads as a model factor but this technology should be readily translated to immobilized gradients of adhesive peptides, proteins and growth factors, or the ECM itself. Scale bar = 1 mm.

COMSOL Model of Nutrient Uptake in Vascular Hydrogels (model nutrient = O_2)

Assumptions

- Steady-state
- No v change in Θ
- Fully developed flow
- Symmetry everywhere
- Flux and concentration matching
- D constant
- Uptake is governed by Michaelis-Menten kinetics
- Use oxygen as model nutrient

Governing Equation

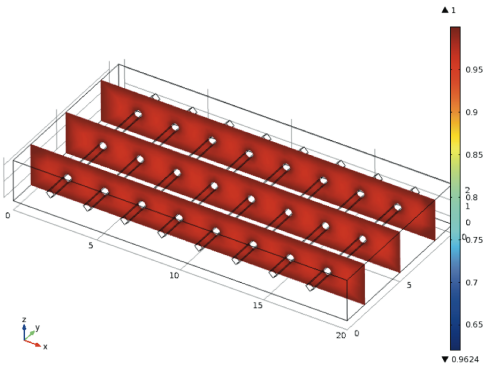
$$\frac{\partial C_i}{\partial t} = D\nabla^2 C_i - \underline{\upsilon}\cdot\nabla C_i + R$$

in Hydrogel

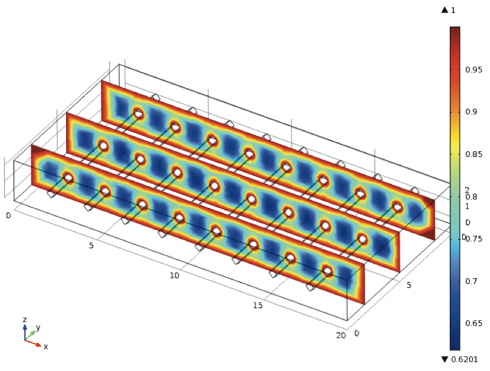
$$\frac{\partial C_{HYD}}{\partial t} = D_{eff}\nabla^2 C_{HYD} + R$$

$$0 = D_{eff}\left[\frac{1}{r}\frac{\partial}{\partial r}\left(r\frac{\partial C_{HYD}}{\partial r}\right) + \frac{\partial^2 C_{HYD}}{\partial z^2}\right] - \frac{v_{\max} C_{HYD}}{K_M + C_{HYD}}$$

Low Uptake Rate (Low Cell Concentration)



High Uptake Rate (High Cell Concentration)



Supplementary Figure 2: Finite element model of nutrient consumption by encapsulated cells in the interstitial space of perfusable hydrogels (using oxygen as a model nutrient). The model was implemented in COMSOL by starting with the governing mass transport equation, inputting the listed assumptions to simplify, and applying to the given hydrogel geometry with accompanying boundary conditions to solve. Exploration of the model in COMSOL indicates that at low nutrient uptake rate (corresponding to low cell concentrations), the nutrient concentration is high everywhere in the gel, while at high nutrient uptake rate (corresponding to high cell concentrations), nutrient concentration is high at the gel perimeter and radially around perfused channels. The nutrient concentration profile observed in this model is consistent with the empirical data presented in **Figure 4**.

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