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ADVANCED MATERIALS

Supporting Information

for *Adv. Mater.*, DOI: 10.1002/adma. 201202024

Rapid Fabrication of Complex 3D Extracellular Microenvironments by Dynamic Optical Projection Stereolithography

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Supplementary Information

1. Methods

Synthesis of Gelatin Methacrylate

Briefly, porcine skin gelatin (Sigma Aldrich, St. Louis, MO, USA) was mixed at 10% (w/v) into phosphate buffered saline (PBS; Gibco, Billings, MT, USA) and stirred at 60 ˚C until fully dissolved. Methacrylic anhydride (MA; Sigma) was added to the solution at a rate of 0.5 ml/min until a concentration of 8 % (v/v) of MA was obtained in the gelatin solution. The solution was then stirred for 1 hour at 50 ˚C, followed by a 2x dilution with warm PBS and dialyzed against distilled water using 12-14 kDa cutoff dialysis tubing (Spectrum Laboratories, Rancho Dominguez, CA, USA) for one week at 40 ˚C to remove the unreacted groups from the solution. The GelMA solution was frozen overnight at -80 ˚C and lyophilized in a freeze dryer (Labonco, Kansas City, MO, USA) for one week. Freeze dried GelMA foam was stored at -80 ˚C until further usage.

Freeze dried GelMA macromer was mixed into PBS at a 10% or 15% (w/v) concentration and stirred at 60 °C until fully dissolved. Calcium chloride and sodium carbonate (2g, Fisher Scientific, Fair Lawn, NJ, USA) was added to the GelMA solution and stirred 24 hours until precipitation of sodium chloride, resulting into a solution of GelMA and calcium carbonate microparticles. Photoinitiator (1%(w/v), Irgacure 2959, CIBA Specialty Chemicals, Basel, Switzerland), UV absorber (0.1% (w/v) HMBS, (2-hydroxy-4-methoxy-benzphenone-5-sulfonic acid), Sigma) and solution quencher (0.01% (w/v), TEMPO, Sigma) were added to the solution to allow for photopolymerization and provide efficient cure depth and optimal pattern resolution.

SEM Imaging

The scaffolds were sputter coated with an 8 nm thick Iridium layer using Emitech K575X, and examined in the Field Emission Environmental Scanning Electron Microscope (ESEM).

FEI XL30 ESEM FEG operated at 10 kV using high vacuum mode. For 20% PEGDA scaffolds, we carried out series of ethanol baths (starting concentration from 50% to 100%, 15 min for each step) followed by Critical Point Drying (CPD, Tousimis AutoSamdri 815A), sputter coating, and finally SEM imaging.

Cell Cultures and Staining

NIH 3T3 cell line was purchased from ATCC and cultured according to the protocol provided by ATCC using Dulbecco's Modified Eagle Medium (DMEM, Gibco) supplemented with 10% Bovine calf serum (BCS, Gibco) , and 1% penicillin–streptomycin. Human umbilical vein endothelia cell (HUVEC) were maintained with endothelial growth media 2 kit (EGM-2, Lonza). Both cell types were maintained at 37°C in an incubator with 10% CO_2. Cells were harvested and counted based on the general protocol and then seeded onto the scaffold with their respective growth media. Media were initially changed one day after seeding and then refreshed every other day. The constructs were incubated for 4 days before fixation and staining.

Cells were fixed after 4 days culture and stained for actin and nuclei to probe the cell-material interaction. 4% paraformaldehyde (Electron Microscopy Sciences) was used to fix the cells on the scaffold for 15 minutes at room temperature, and followed by permeabilization with 0.1% Triton X-100 (Sigma Aldrich) in PBS with 2% bovine serum album (BSA, Fisher Scientific) for 30 minutes. Cells were then exposed to 5 units/ml rhodamine pholloidin (Invitrogen) at 37°C for 30 minutes. Nuclei were counterstained with Horchest 33258 DNA dye (Invitrogen).

Confocal Imaging

All Immunofluorescent staining were observed using Olympus FV1000 confocal microscope and analyzed using Fluoview software (Olympus) and Image J software. 10x (0.40 numerical aperture) and 20x (0.75 numerical aperture) lens were used for this work.

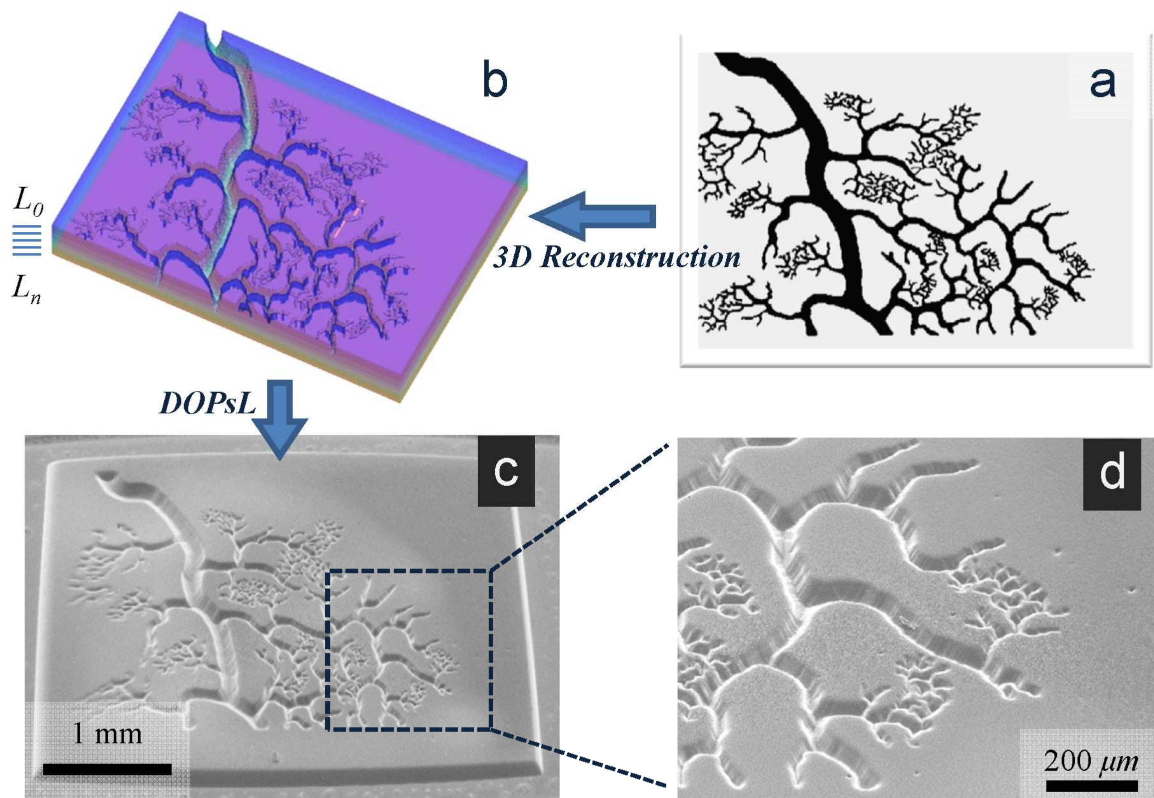

Figure S1: Fabrication of (biomimetic) vascular structures. a, a 2D image is generated, **b**, the image is converted to a 3D model built using CAD technology, **c**, the 3D scaffold is fabricated after exposure to UV-light during the DOPsL procedure, **d**, a closeup view of the fabricated structure.

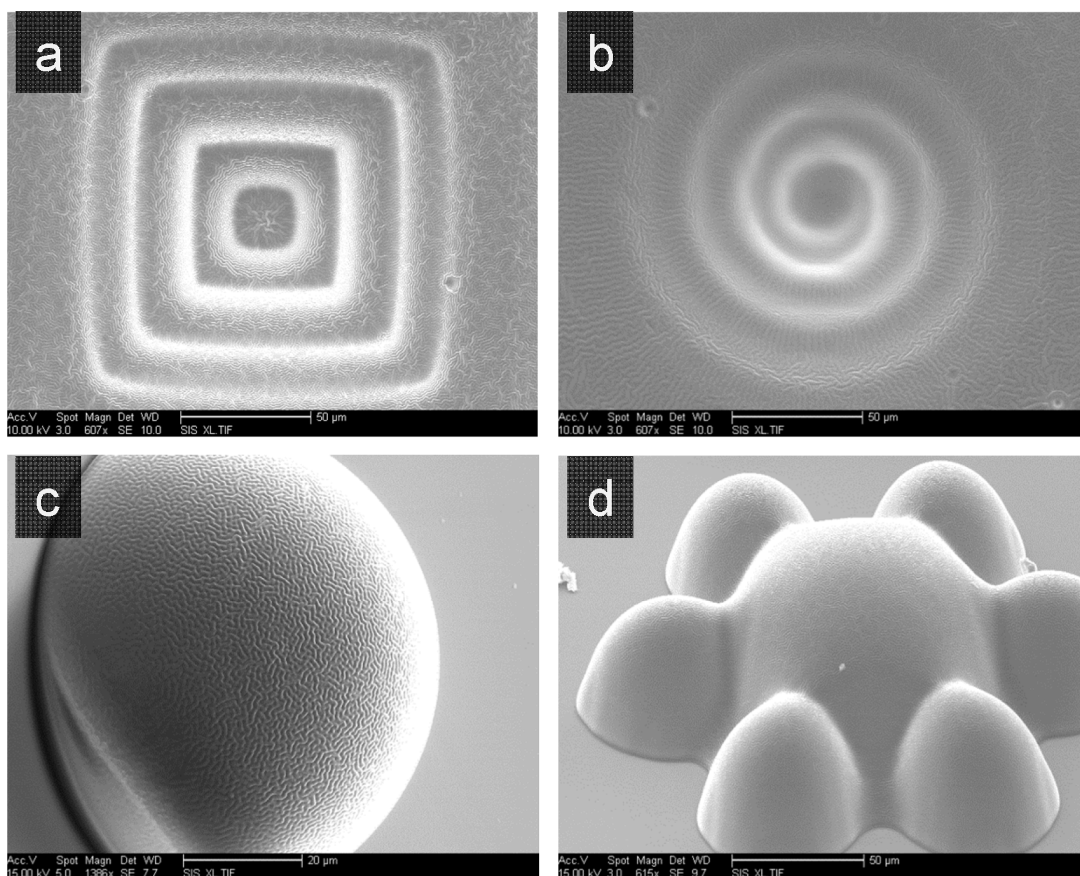

Figure S2: Magnified SEM Images of the Fabricated 100% PEGDA Microarchitectures.
a, stepwise well; **b**, spiral well; **c**, hemisphere; **d**, flower-like.

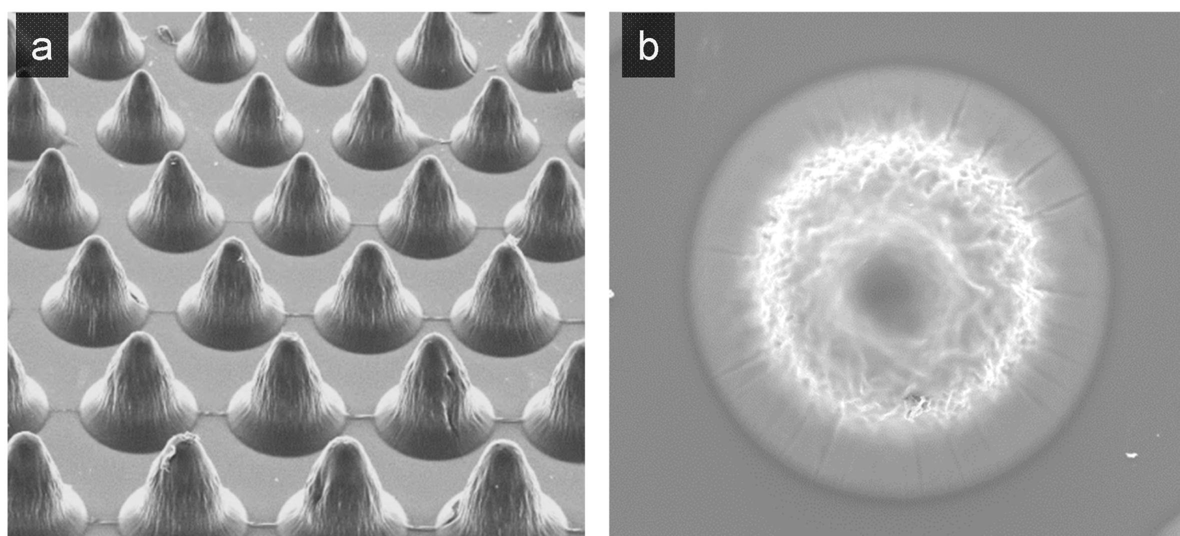

Figure S3: SEM Images of the Dehydrated 20%-PEGDA Hemisphere Microstructures.
a, Angled view of dehydrated hemisphere arrange; **b**, top view of a dehydrated hemisphere.