

Supplementary Information

Spatially defined stem cell-laden hydrogel islands for directing endothelial tubulogenesis

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Materials and Methods

Materials

Reagents were purchased from Sigma-Aldrich unless otherwise stated. Human MSCs (Lonza) tested positive for CD105, CD166, CD29, and CD44, negative for CD14, CD34, and CD45 by flow cytometry. Growth factor reduced basement membrane extract (matrigel) was purchased from Trevigen. Human microvascular endothelial cells (hMVECs) were purchased from Cell-systems. EGM-2 growth factor supplemented media was purchased from Lonza.

PEGDA gel fabrication

Polyethylene glycol (PEG-10000 Molecular weight) was modified as described previously¹ to form PEG diacrylates (PEGDA). Briefly, PEG (1 mmol) was dried by co-distillation with toluene 3 times. The dried PEG was then redissolved in dichloromethane (DCM) and toluene (DCM:toluene 5:3). Triethylamine and acryloyl chloride (3 mmol each) were then added under stirring overnight at room temperature. The reaction was filtered and K_2CO_3 (3g) was added under stirring for 1.5 hours. The PEGDA was then filtered, concentrated under vacuum and extracted with diethylether. Lyophilized PEGDA powder was stored at $-20^\circ C$.

Amine groups on proteins were acrylated by the addition of NHS-acrylate (at a ratio of 10:1) for 4 hours in carbonate buffer (pH 9, 1M NaCl)².

To make 2D surfaces, 18mm glass coverslips (Fischer Scientific) were cleaned with ethanol then DI water. The slides were then dried and activated by treatment with 3-(trimethoxysilyl)propyl methacrylate 20% solution in ethanol with 0.3% glacial acetic acid. The slides were then baked at $95^\circ C$ for 1 hour. A 30wt% solution of PEGDA and 50 $\mu g/ml$ acrylated fibronectin and 0.05% 2-Hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone (UV Initiator) was made and 20 μL was pooled between the activated coverslip and a hydrophobically treated glass microscope slide. This setup was placed in a UV crosslinker (Spectronics) and subjected to UV light at an intensity of $\sim 5 mW/cm^2$ for 10 minutes. For 3D gel fabrication, MSCs were trypsinized and pelleted and then resuspended in the gel solution. The gel was formed from solution as described above.

To confirm increased protein incorporation after acrylation, Alexa 546-conjugated fibrinogen (Invitrogen) was acrylated and PEGDA gels were formed with either acrylated or unacrylated fluorescent fibrinogen. After extensive washing, fluorescence was measured across several gels using an InCell analyzer microscope (General Electric) to compare remaining amounts of fibrinogen the gels.

Cell culture and immunofluorescence

MSCs were cultured in low glucose DMEM with 10% fetal bovine serum and 1% penicillin/streptomycin. hMVECs were cultured in EGM-2 (Endothelial Cell Growth Medium) growth factor supplemented media (Lonza). For both cell lines media was changed every 3-4 days and cells were passaged at $\sim 80\%$ confluence.

For immunofluorescence, gels were rinsed with PBS, fixed with 4% paraformaldehyde for 20 minutes and permeabilized with 0.1% Triton-X for 30 minutes. 1% bovine serum albumin was used for blocking. Nuclei and actin were stained with DAPI (1:5000) and Alexa-Fluor 488-phalloidin (1:200), respectively. Paxillin was stained with a primary rabbit-anti-vinculin (ABCAM - Y113) and secondary 555-Alexa fluor goat anti-rabbit antibody (Invitrogen – A-21428). Imaging was done using a Zeiss Axiovert 200M inverted fluorescence microscope.

Vascularization assay

Conditioned media from MSCs was used for a vascularization assay as described previously³. Briefly, thawed matrigel was coated on the bottom surfaces of a 48-well plate and allowed to gel at 37°C for 30 minutes. hMVECs were seeded onto the matrigel at ~15,000 cells/well in serum and growth factor free media and conditioned media from MSCs was added. After 8 hours, tube formation was imaged and quantified using imageJ.

Patterning MSC Islands

Patterned islands of MSCs encapsulated in PEGDA were formed by sandwiching MSCs suspended in PEGDA gel solution (with initiator and acrylated fibronectin) between 2 slides, one hydrophobic and one treated for gel attachment using 20% (3-(trimethoxysilyl)propyl methacrylate solution in ethanol. This sandwich was then placed in contact with a photomask that is transparent where encapsulated MSCs are desired. UV light is shined through the mask using a mask aligner onto the PEGDA so that the initiator is activated only at the transparent areas in the mask. After 10 minutes of UV exposure, the excess PEGDA solution is washed away.

To assess the shapes of the islands, we encapsulated 5%(v/v) FITC-labeled 1 μ m beads (Invitrogen - F-8823) inside the PEGDA islands and these were imaged with a Carl Zeiss LSM 700 confocal scope. Z-stack images were used to make profiles of the islands

In order to confirm MSC patterning, MSCs were incubated for 30 minutes with cell tracker green CMFDA dye (Invitrogen) as per manufacturer's instructions prior to encapsulation. The labeled cells were pelleted and excess cell tracker disposed of before encapsulation. MSCs in islands were visualized by fluorescently imaging the PEGDA islands.

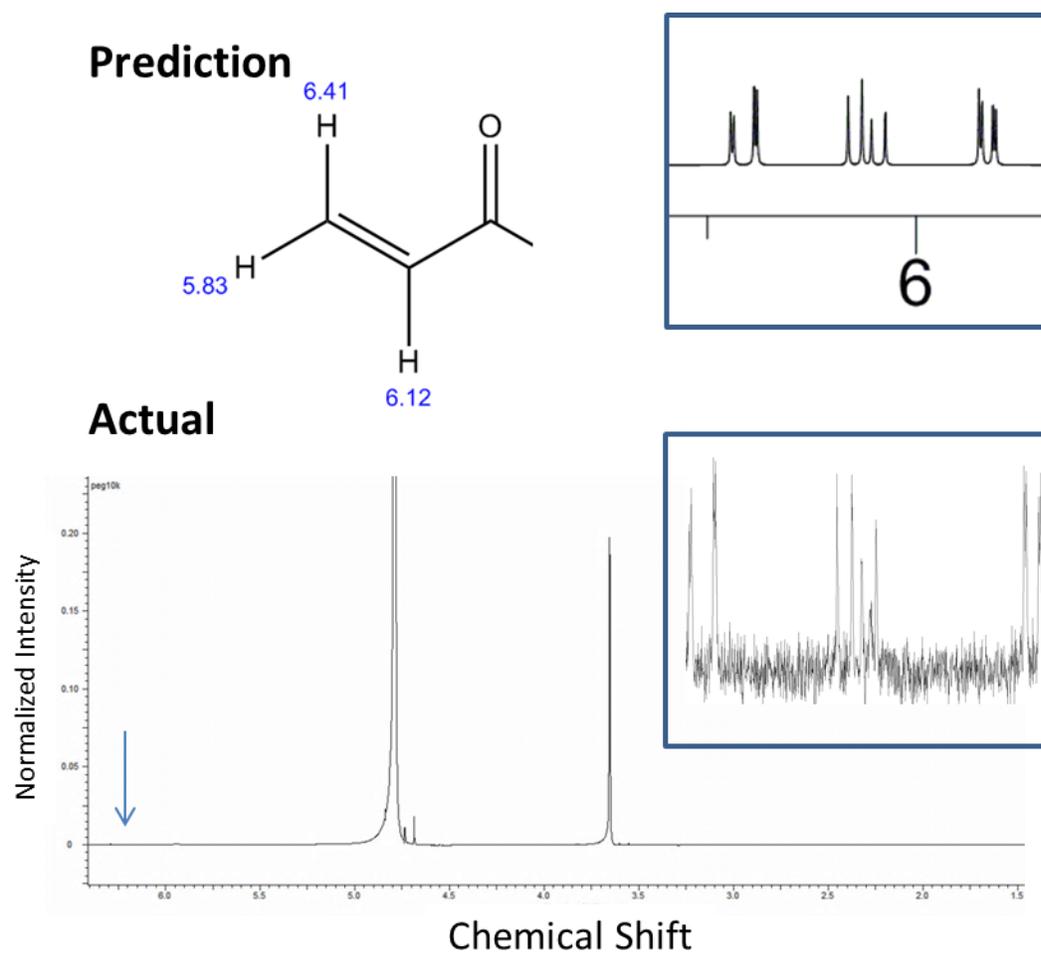
MSC-hMVEC co-culture

For MSC and hMVEC co-culture, MSC encapsulating PEG islands are formed as described above and then ~50 μ l of matrigel is placed on top of encapsulated MSC islands and sandwiched with another hydrophobic slide. After 30 minutes in the incubator, the matrigel solidifies and the gel is detached and hMVECs are seeded on top of the matrigel. The co-cultures are kept with a 50:50 mixture of MSC and hMVEC media. Images of the surface were taken after 8-24 hours of co-culture.

References

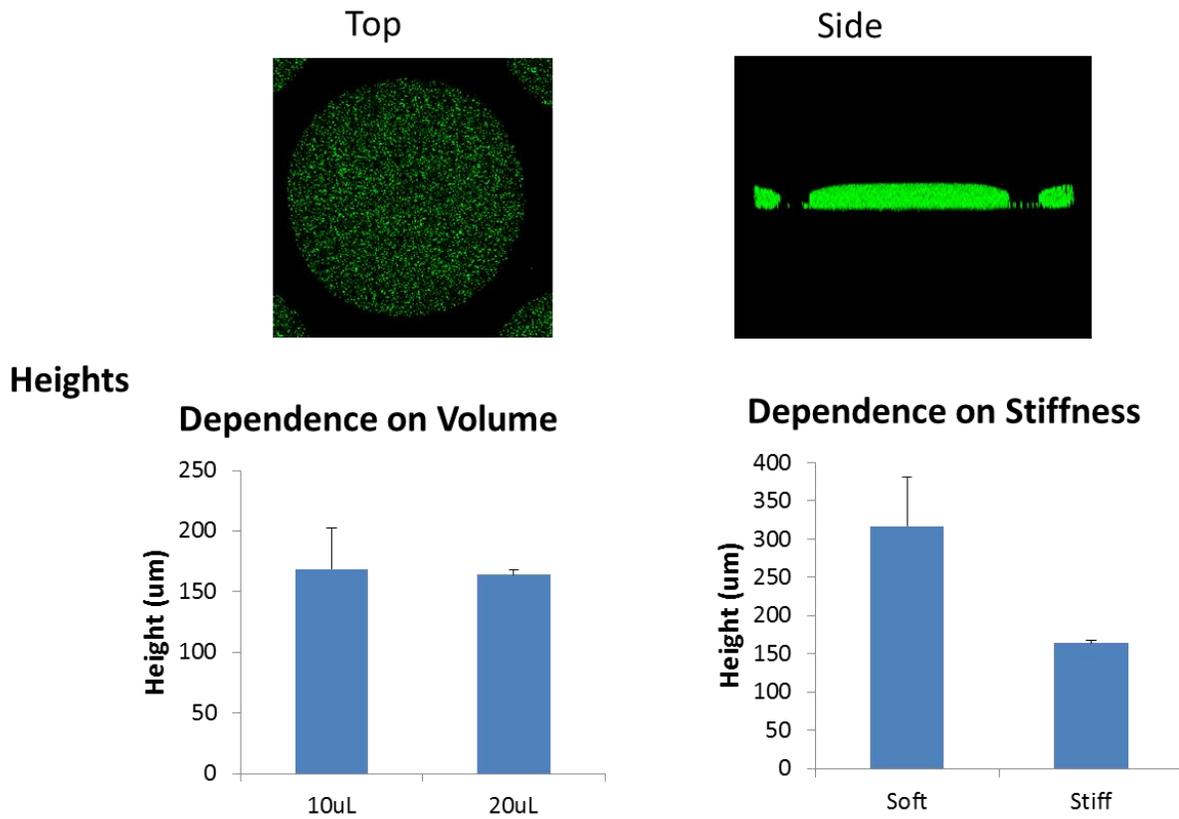
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- (2) Shoemaker, S. G.; Hoffman, a. S.; Priest, J. H. *Appl. Biochem. Biotechnol.* **1987**, *15*, 11–24.
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Figure S1



NMR confirmation of PEG modification into PEGDA shows an acrylation of ~85%-95%.

Figure S2



PEGDA island height can be varied by changing the gel crosslinking density but not by changing the amount of gel solution prior to polymerization.