Supplementary Material (ESI) for Lab on a Chip

Three-Dimensional Photopatterning of Hydrogels using Stereolithography for Long-Term Cell Encapsulation†

Vincent Chan,[ad] Pinar Zorlutuna,[ad] Jae Hyun Jeong,[b] Hyunjoon Kong[b] and Rashid Bashir[acd*]

[a]Department of Bioengineering, [b]Department of Chemical and Biomolecular Engineering, [c]Department of Electrical and Computer Engineering, [d]Micro and Nanotechnology Laboratory, University of Illinois at Urbana-Champaign, Urbana, Illinois 61801 USA

[*]Correspondence:

Rashid Bashir, 2000 Micro and Nanotechnology Laboratory, MC-249, University of Illinois at Urbana-Champaign, 208 North Wright Street, Urbana, Illinois 61801 USA, Tel: (217) 333-3097, Fax: (217) 244-6375, Email: rbashir@illinois.edu

†ELECTRONIC SUPPLEMENTARY INFORMATION

Energy Dose Characterization

Materials and methods

0.1% (w/v) fluorescent Nile Red microparticles (0.7-0.9 µm, Spherotech, Lake Forest, IL, USA) were incorporated into the pre-polymer solution at a 1:1000 dilution. The solution was then pipetted into a custom-made container with a thin cover glass on top, in contact with the solution. Samples were cured in the SLA by writing a cross-hatched pattern using a range of laser energy doses (E_{avg}). The energy doses were controlled by varying the laser beam writing speed in the SLA software. After polymerization, the cover glass was lifted off with the polymerized gel layer attached. The thickness of the cured gel (C_d) was then measured using an inverted fluorescent microscope (IX81, Olympus, Center Valley, PA, USA) and IPLab software (BD Biosciences, Rockville, MD, USA). The measured thicknesses were plotted on a semi-logarithmic graph as a function of energy dose. Using the working curve equation,

$$C_d = D_p \ln \frac{E_{avg}}{E_c} \quad \text{(S1)}$$

where D_p is the penetration depth and E_c is the critical exposure energy, D_p and E_c were calculated with linear regression analysis software (OriginPro 8.1, OriginLab, Northampton, MA, USA).

Results

In order to build complex 3D hydrogels in the SLA with the highest levels of accuracy, two constants, D_p and E_c, need to be precisely determined. The SLA software utilizes these constants in Equation S1† to calculate the energy dose (E_{avg}) required to cure a layer of desired thickness (C_d) of that material. If the

layer is not cured deeply enough, it will not attach to the layer below it, resulting in delamination. If the layer is cured too greatly, it will distort and reduce part accuracy.[1] In this experiment, the energy dose of the laser was varied to obtain a series of gels with different thicknesses. The thickness of each gel was measured by taking advantage of the transparent property of hydrogels and embedding fluorescent microbeads within each sample. These thicknesses were plotted as a function of energy dose on a semi-logarithmic graph to obtain a linear working curve. By rearranging Equation S1† in linear form, it becomes apparent that D_p and E_c can be found by taking the slope and y-intercept of that curve, respectively.

The working curves for 20% (w/v) PEGDA with M_W 700, 3,400, and 5,000 Da are shown in Fig. S1(a)†. The R^2 values, 0.973 for M_W 700 Da, 0.999 for M_W 3,400 Da, and 0.999 for M_W 5,000 Da, indicate that the measurements fit the linear regression model extremely well. The average standard deviation of all the layer thickness measurements was ± 11.9 µm. The D_p values, 0.346, 0.258, and 0.231 mm, and E_c values, 40.89, 11.10, 7.17 mJ/cm^2, for M_W 700, 3,400, and 5,000 Da, respectively, indicate a decreasing D_p and E_c trend with increasing M_W. To test whether these values were accurate, they were inputted into the SLA software, along with user-specified C_d, to make a new series of gels. As an example, the measured thicknesses for M_W 700 Da were comparable to their specified thicknesses (Fig. S1(b)†), showing very little deviation. As shown in Fig. S1(c) and S1(d)†, complex 3D structures prepared from these PEGDA hydrogels were successfully fabricated following the characterization process. For the bottoms-up approach, a second characterization step was performed to determine the amount of volume required to cure a layer of specific thickness. This process is described in Fig. S2†.

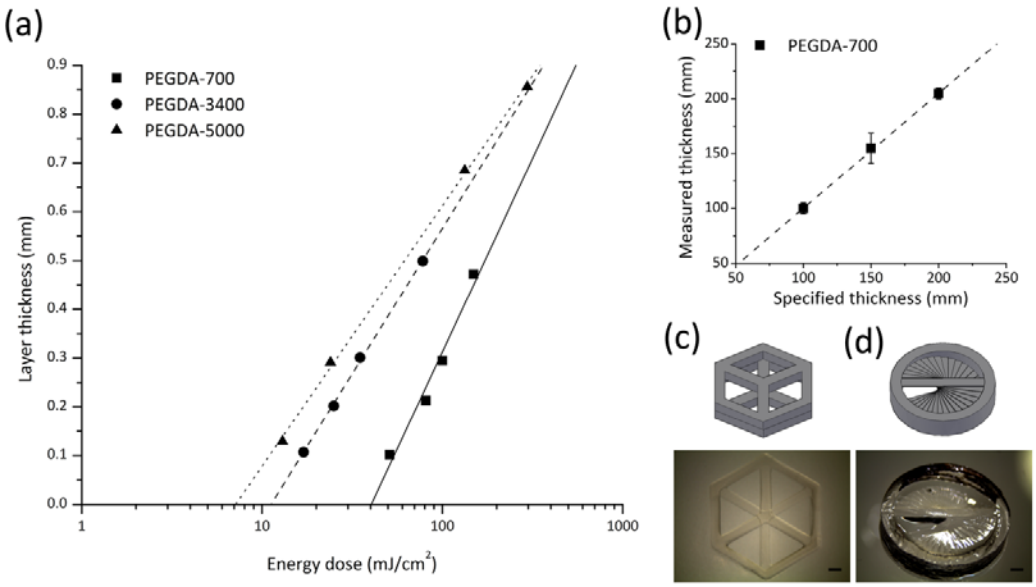

Fig. S1 Fabrication of complex 3D hydrogels in the SLA. (a) Characterization of the laser energy dose required to cure 20% PEGDA hydrogels with M_W 700, 3,400, and 5,000 Da. (b) Example test of D_p and E_c parameters found in (a) for M_W 700 Da. (c and d) CAD drawings and actual images of complex 3D hydrogels prepared from M_W 700 Da and fabricated in the bottoms-up SLA modification. All experiments used 0.5% photoinitiator concentration. Scale bars are 1 mm.

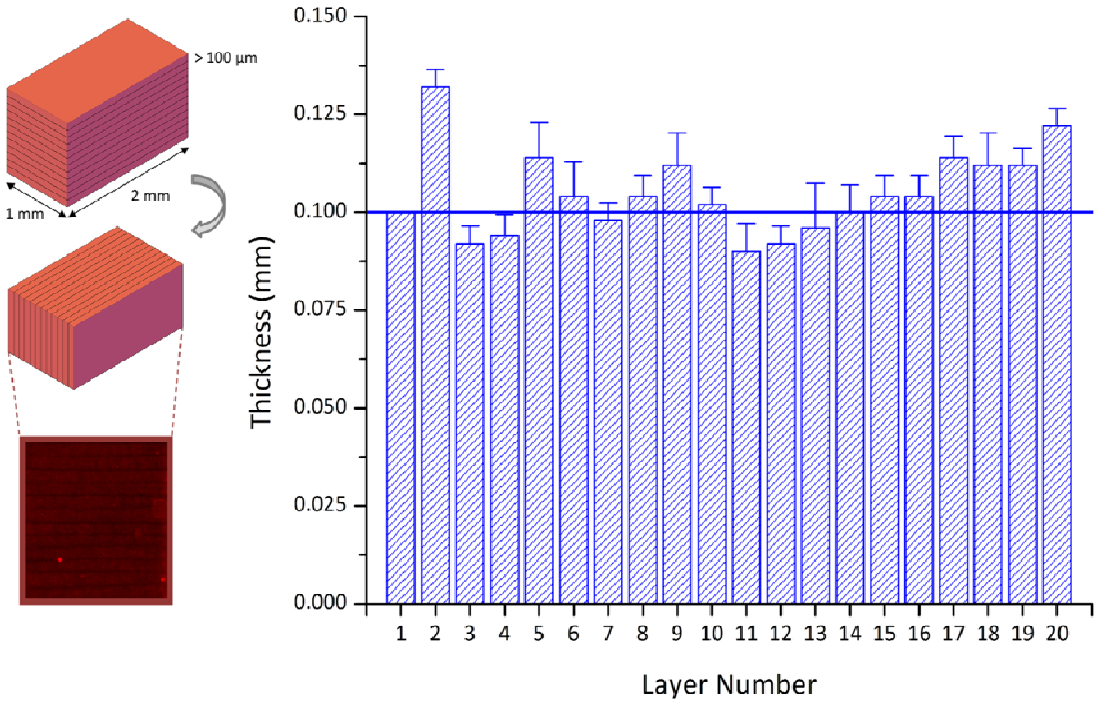

Fig. S2 Volume deposition characterization. For all multi-layer experiments utilizing the bottoms-up approach, the thickness of each layer was, on average, 100 µm. In order to do this, the volume had to be calibrated precisely for each layer due to surface tension between the walls of the container and the pre-polymer solution. 1 X 2 mm blocks were built in the SLA with embedded fluorescent microbeads. Each block had up to 20 layers. The thickness of each layer was measured by tipping the block on its side and visualizing in fluorescence microscopy. ImageJ analysis software was used to determine the thickness. The volume was then adjusted until the average of all layers reached 100 µm. All values are mean ± standard deviation of n = 10.

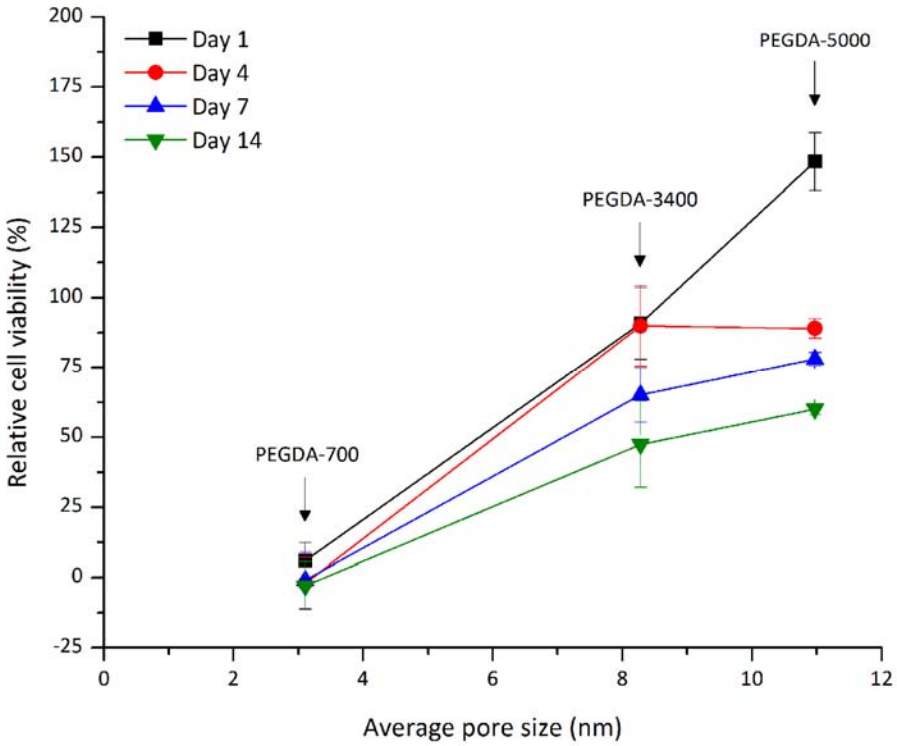

Fig. S3 Effect of average pore size on cell viability. The relative cell viability and proliferation generally increased with increasing average pore size. It is well-known that larger pore sizes increase the diffusion of oxygen, nutrients, and waste into and out of the cell-embedded gels. Larger pore sizes also remove the photoinitiator compounds and its free radical by-products out of the gel quicker, thereby increasing initial cell viability. Additionally, cell spreading and protein synthesis/secretion is improved in higher chain length gels, even in the absence of adhesion peptides.

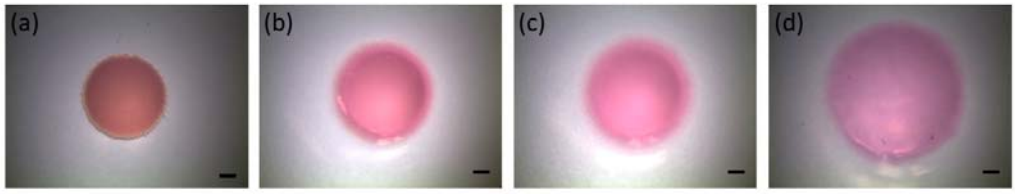

Fig. S4 Swelling of laser-polymerized PEGDA hydrogels as a function of M_W. 20% PEGDA hydrogels with M_W (a) 700, (b) 3,400, (c) 5,000, and (d) 10,000 Da were used to make disks with a diameter of 5 mm in the SLA. The gel disks were incubated in culture medium for 24 hours at 37^{o}C before imaging in the stereomicroscope. The diameter of the gel disks increased as a function of increasing M_W. Scale bars are 1 mm.

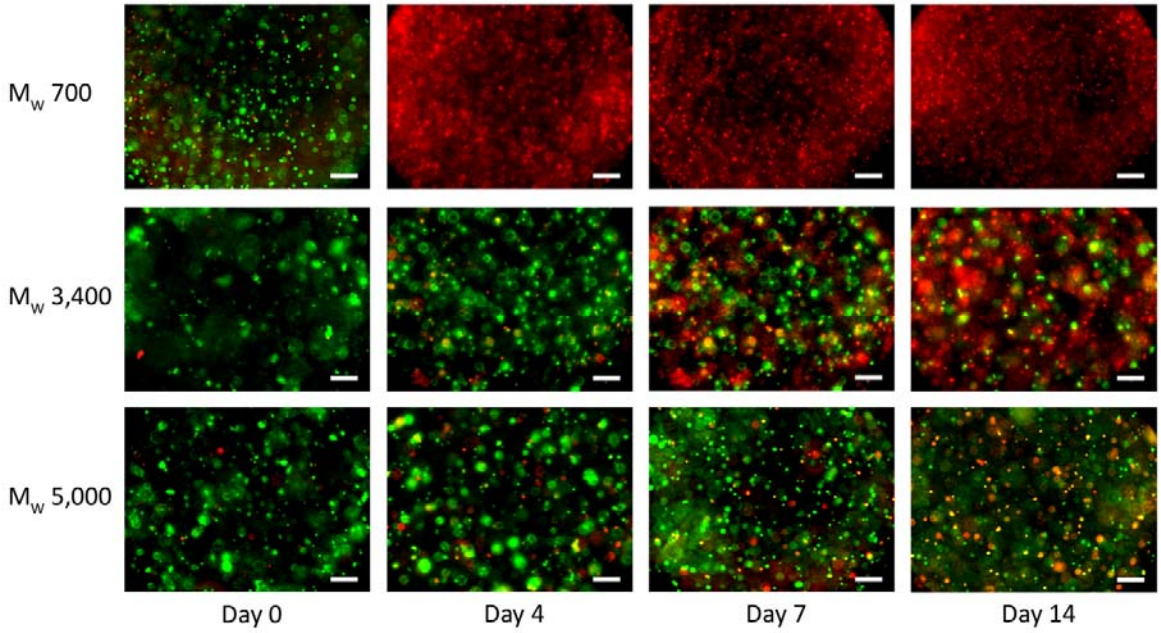

Fig. S5 Qualitative LIVE/DEAD staining of laser-polymerized PEGDA hydrogels. NIH/3T3 cells were encapsulated in laser-polymerized 20% PEGDA hydrogels at a density of 1 X 10^6 cells/mL and cultured over 14 days. Viability was evaluated at 0, 4, 7, and 14 days using calcein AM and ethidium homodimer in fluorescence microscopy. Green indicates live cells and red indicates dead cells. Scale bars are 200 µm.

1. H. Nguyen, J. Richter, P. F. Jacobs, On Windowpanes and Christmas Trees: Diagnostic Techniques for Improved Part Accuracy, in Proc. 1st Eur. Conf. Rapid Prototyping, ed. P. M. Dickens, University of Nottingham, Nottingham, 1992, pp. 133–161.