Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information

Nonlinear 3D Projection Printing of Concave Hydrogel Microstructures for Long-Term Multicellular Spheroid and Embryoid Body Culture

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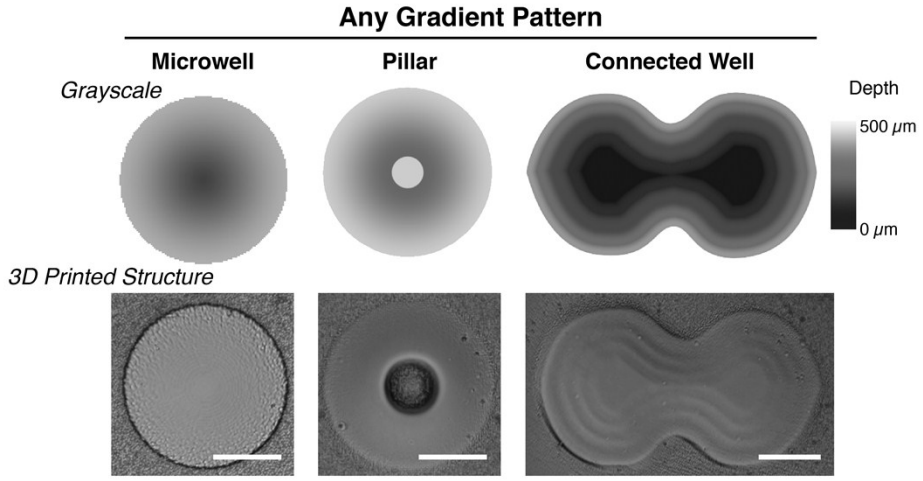

Figure S1. Any gradient pattern can be designed and subsequently printed using this process, adding to its versatility in fabrication design.

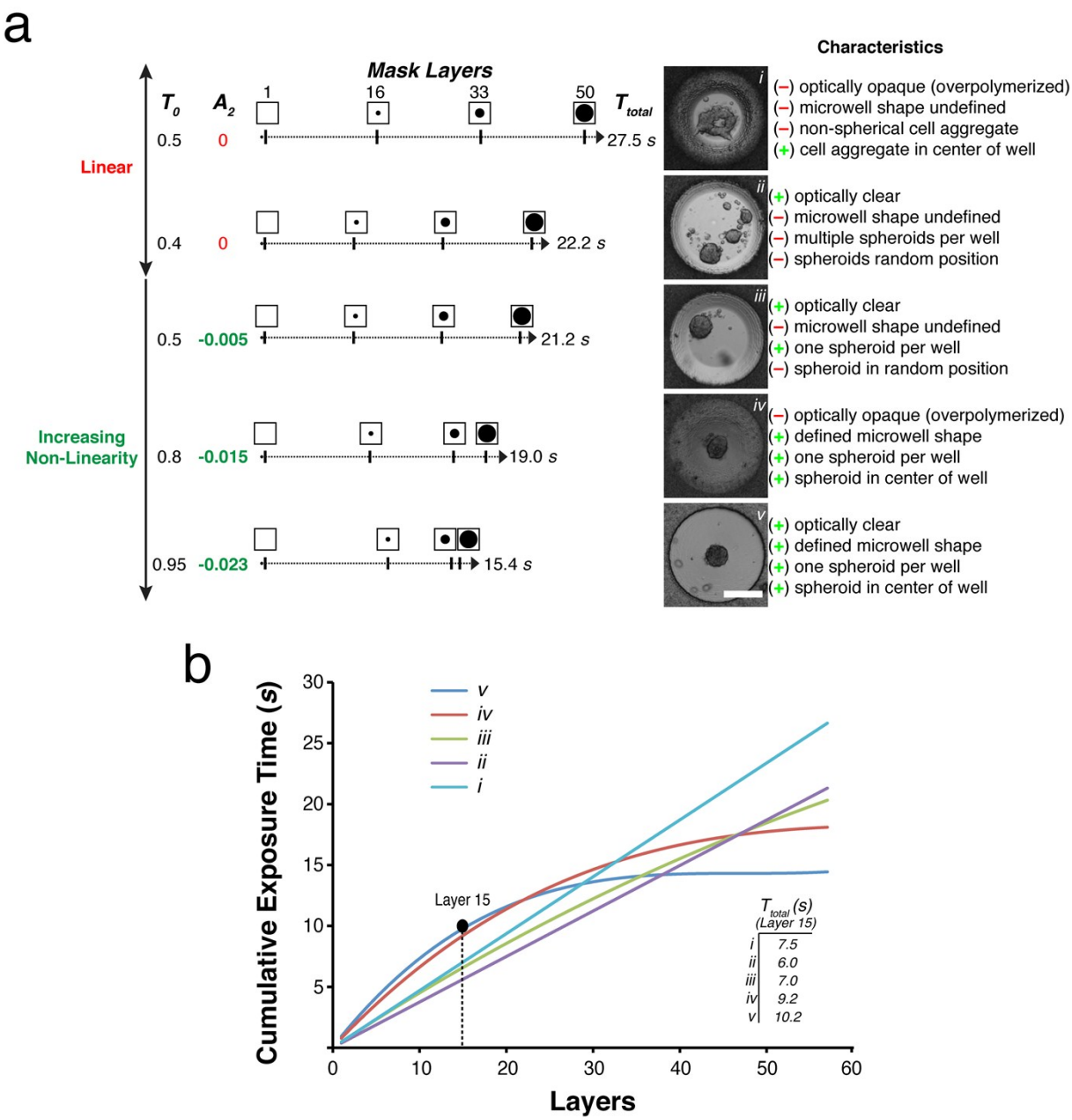

Figure S2. Characterization of nonlinear projection printing. (a) Varying the fabrication parameters (T_0 and the nonlinear factor, A_2) to achieve an optically clear microwell that allows for single 3D cell spheroid growth in the middle of the concave hydrogel (*i* to *v*). The resulting microwells and day 3 spheroid culture are shown on the right. b) Graphical representation of the cumulative total exposure time at different layers in the optical mask

series. The first 15 layers have a blank pattern, exposing the entire prepolmyer solution to UV light and acting as the base of the structure. T_{total} values at layer 15 are displayed in the inset.

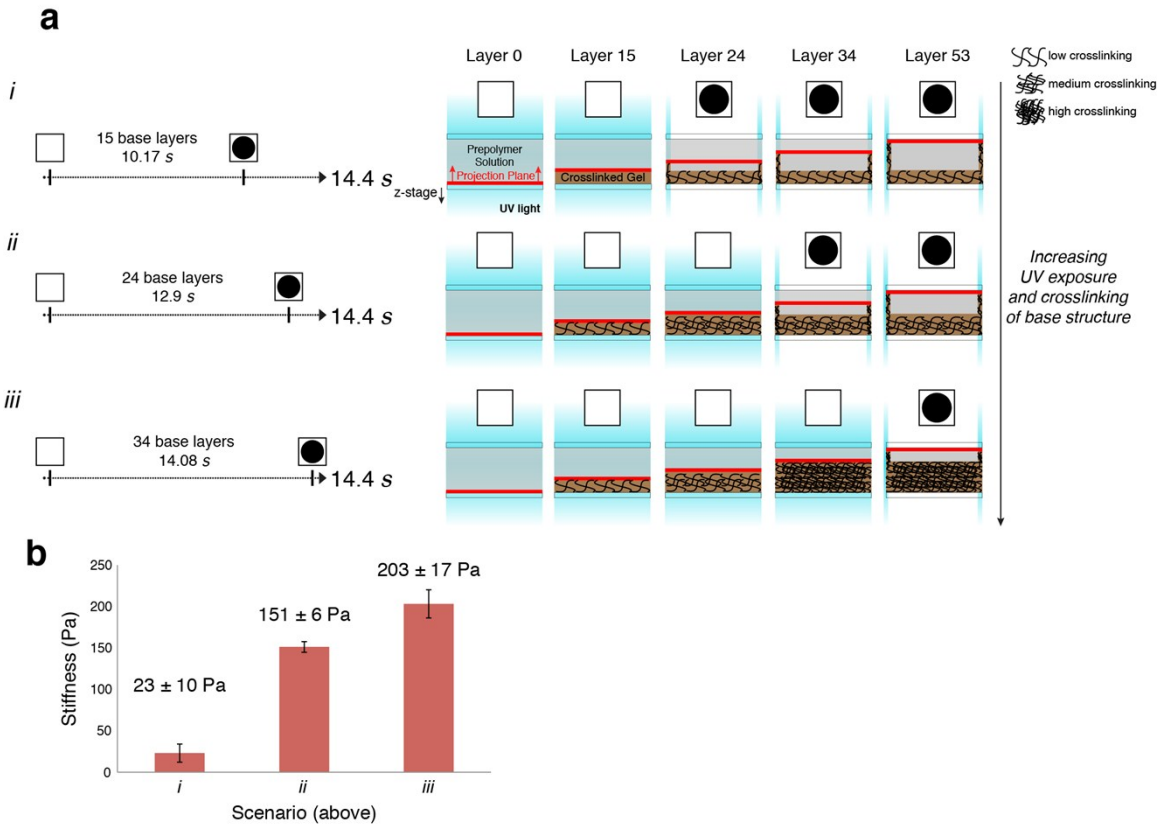

Figure S3. Flat hydrogels exposed to different base layers in the 3D printing process. (a) Schematics showing three scenarios for different base layers and exposure times and (b) the resulting stiffness profiles from atomic force microscopy measurements.

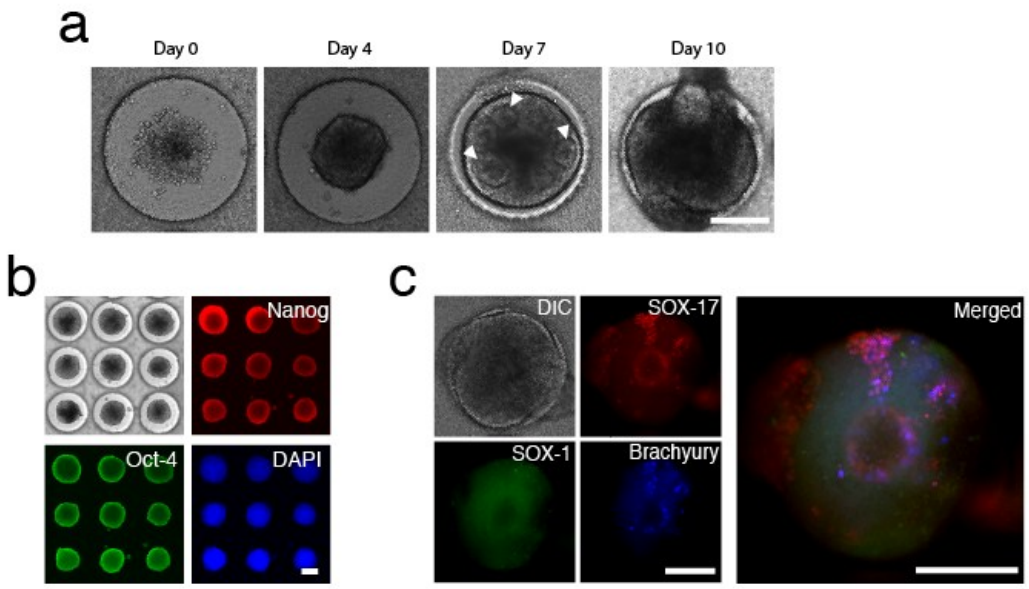

Figure S4. iPSC EBs formed from initial seeding density of 400 k mL^{-1}. (a) Timelapse images of EB formation with iPS cells over 10 d. Arrows indicate areas of intra-organoid cavities, visual markers of differentiation. (b) Immunofluorescent staining of pluripotent markers Nanog and Oct4 at day 3. (c) Immunofluorescent staining at day 10 of the three germ layers – ectoderm (SOX-1), endoderm (SOX-17), and mesoderm (brachyury) in concave hydrogels. All scale bars = 200 µm.