Supporting Information

Visible Light Induced Electropolymerization of Suspended Hydrogel Bioscaffolds in a Microfluidic Chip

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1. Radical polymerization process

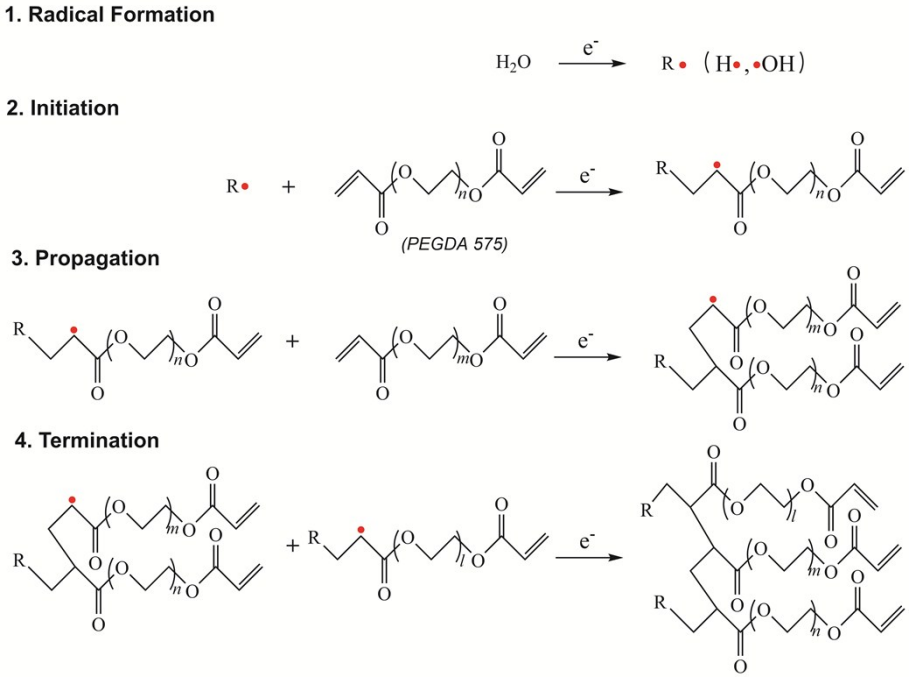

Figure S1. Free radical polymerization of PEGDA hydrogels: radical formation, chain initiation, chain propagation, and chain termination.

We chose PEGDA as a typical hydrogel material because of its outstanding biocompatibility and flexible functionality. The PEGDA monomer contains carbon-carbon double bonds, which served as active reaction centers to produce polymer chains. As shown in Figure S1, firstly, activated H and/or OH radicals are formed via the electrolysis of water molecules depend on the photosensitive semiconductor layer used. Then, those reactive radical species attack a monomer molecule via its carbon-carbon double-bond, producing a new active center on the vinyl carbon of the PEGDA molecule chain and triggering chain initiation. This process is repeated many times over as more PEGDA molecules are added to the reactive centers of the original molecule, thus creating crosslinked PEGDA macromolecular chains. This process is called intramolecular chain propagation (m=n). Attacking and crosslinking also occur between different separate crosslinked PEGDA macromolecules, thus forming larger macromolecules with a higher degree of crosslinking. This process is called intermolecular chain propagation (m≠n). At any time, the polymerization reaction may be terminated by the combination of two radical centers from the same or different molecules. Finally, the crosslinked hydrogel fully occupied the light-defined interface area by replicating the designed optical pattern and expelling incoming precursor molecules for further polymerization. Fortunately, the crosslinked hydrogel structures are water absorbent and swollen to some extent, allowing delamination from the interface

layer and transfer to the bulk-solution region, which will free the interfacial sites for new reactions to occur. This continual interface-confined polymerization process formed a continuous polymerizing-delaminating-polymerizing reaction-loop, which produces various interesting hydrogel microstructures under different polymerization-to-delamination rates.

2. 3D hydrogel grids

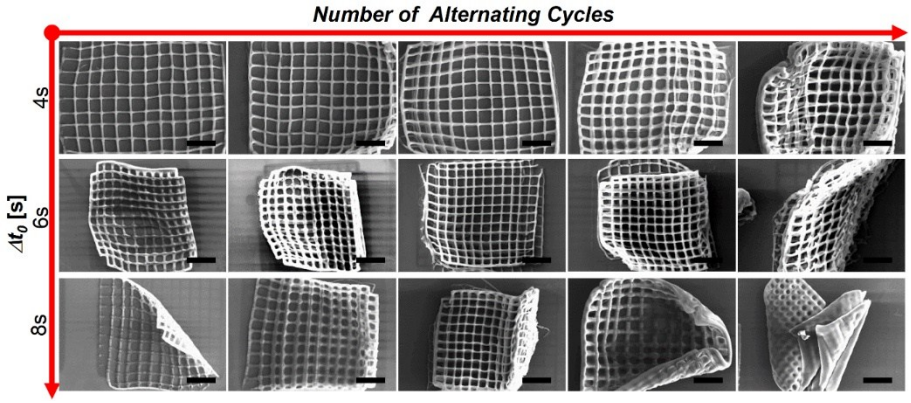

Figure S2. 3D hydrogel grids fabricated with different alternating time interval Δt_0 values and alternating cycles. All scale bars are 50 μm.

As shown in Figure S2, with increased alternating time interval, hydrogel sheets delaminate and fold from the substrate, which will disrupt the integrity of the whole structure. Additionally, at a certain Δt_0, increasing the number of alternating cycles increases the hydrogel grid printing layers, thus creating thick hydrogel grids.

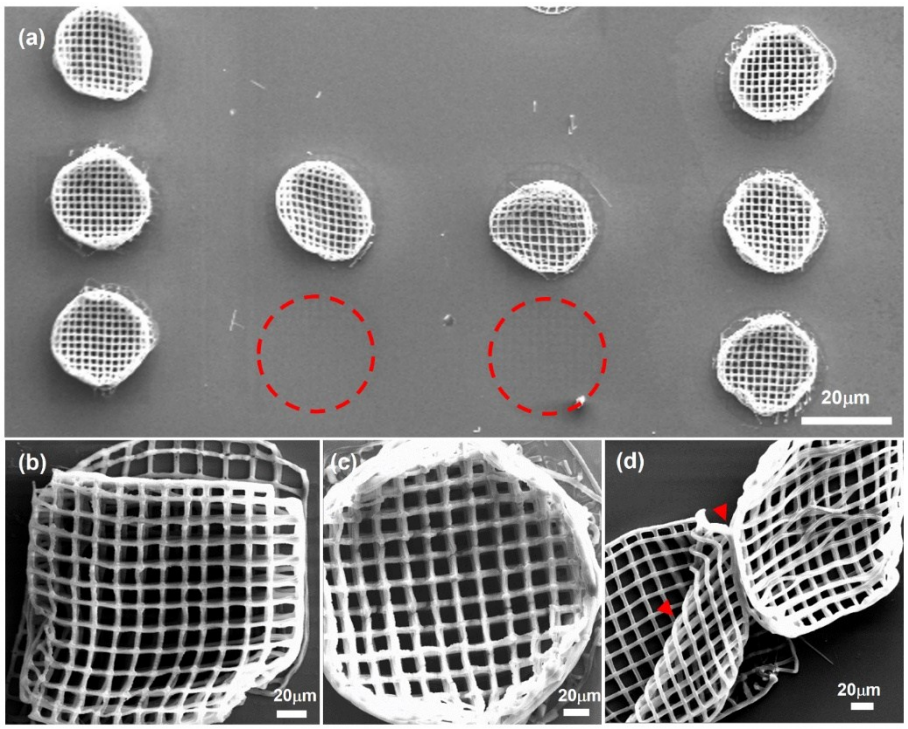

Figure S3. 3D hydrogel grid arrays fabricated using the VLEP system. (a) Pie-shaped 3D hydrogel grid array. The red-dashed circles denote hydrogel grids that washed away after the experiment. (b) and (c) show two different shapes of the hydrogel grids. (d) A broken 3D hydrogel grid caused by increasing the alternating interval time Δt_0. The red triangle markers denote the delamination sites.

As shown in Figure S3, differently shaped 3D hydrogel thick structures are fabricated by our method. All

these 3D hydrogel microstructures could be transferred to other place without any damage as indicated by the red-dashed circle, which shows a clear substrate after transferring.

3. Critical size of dynamic multi-layered electropolymerization

The planar dimensional size (width and length) of a solidified hydrogel pattern is mainly determined by the size of custom-designed illumination pattern that projected on the α-Si:H layer and affected by its clarity (in-focus or defocus). Besides, parameters such as frequency and magnitude of external applied voltage and concentration of precursor solution that determined the polymerization speed could also affect the size of hydrogel pattern to some extent. The third dimensional size (height or thickness) of solidified hydrogel pattern is mainly determined by the combination of polymerization speed and cure time. Considering the layer-by-layer fabrication manner of our proposed VLEP method, the critical feature size includes minimum line-width, maximum length, and critical thickness of a single visible hydrogel layer.

Take a basic line-shaped hydrogel pattern for example, the critical feature sizes include line-width, length, and thickness (height). Based on our experimental results (Figure 5 in revised manuscript), the averaged minimum line-width is around 3 um, which is obtained under 1 pixel-width line-shaped light projection. This value is determined by the intrinsic resolution of our projector (1 pixel equals 2.7 um). As for the length, the largest length is determined by the size of overall illumination field, which is defined by the condenser lens under the microfluidic chip and above the projector. For our 50X condenser lens, the diameter of the overall illumination field is about 1100 um. So the theoretically largest length of a one-shot fabricated hydrogel line is about 1 mm. But, this is not the limit. If necessary, the 3D adjustable platform could be programmed to automatically move with a path to extend the length of patterned hydrogel.

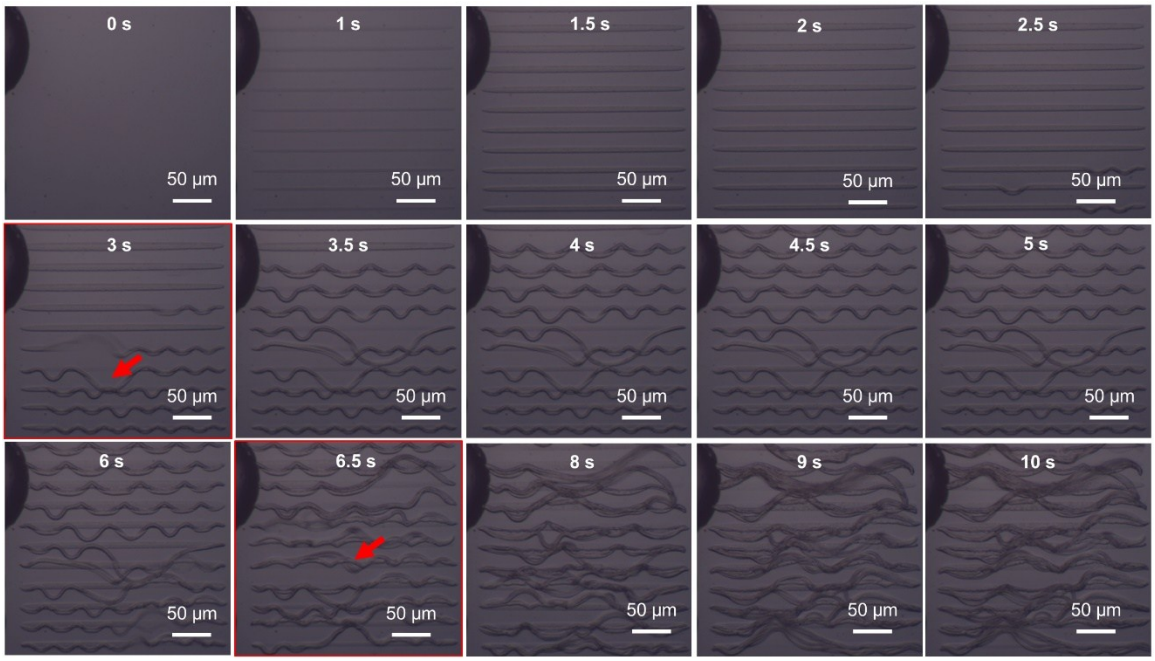

Figure S4. Investigation on the delamination time-point of a visible single hydrogel layer. Snapshots of optical images showing the real-time evolution of line-shaped hydrogel patterns formed in the photosensitive microfluidic chip. The delamination of first visible single hydrogel layer occurred at ~3 s. The delamination of second visible hydrogel layer occurred at ~6.5 s.

We investigated the delamination time-point of a visible single hydrogel layer by recording the printing process of hydrogel line-shaped patterns with 12 frames per second. As we can see from the video snapshots in Figure S4, the first hydrogel layer delamination occurred at ~2.5 s to ~3 s (calculated by the video frames). The second hydrogel layer delamination occurred at ~6.5 s. And after the delamination of first several layers,

the subsequent delamination speed is a little faster. Please note that the delamination time-points of all the hydrogel line arrays have some variations because of the non-uniformed illumination power of projected light. As a result, the averaged time for the delamination of a single hydrogel layer is about 3 s.

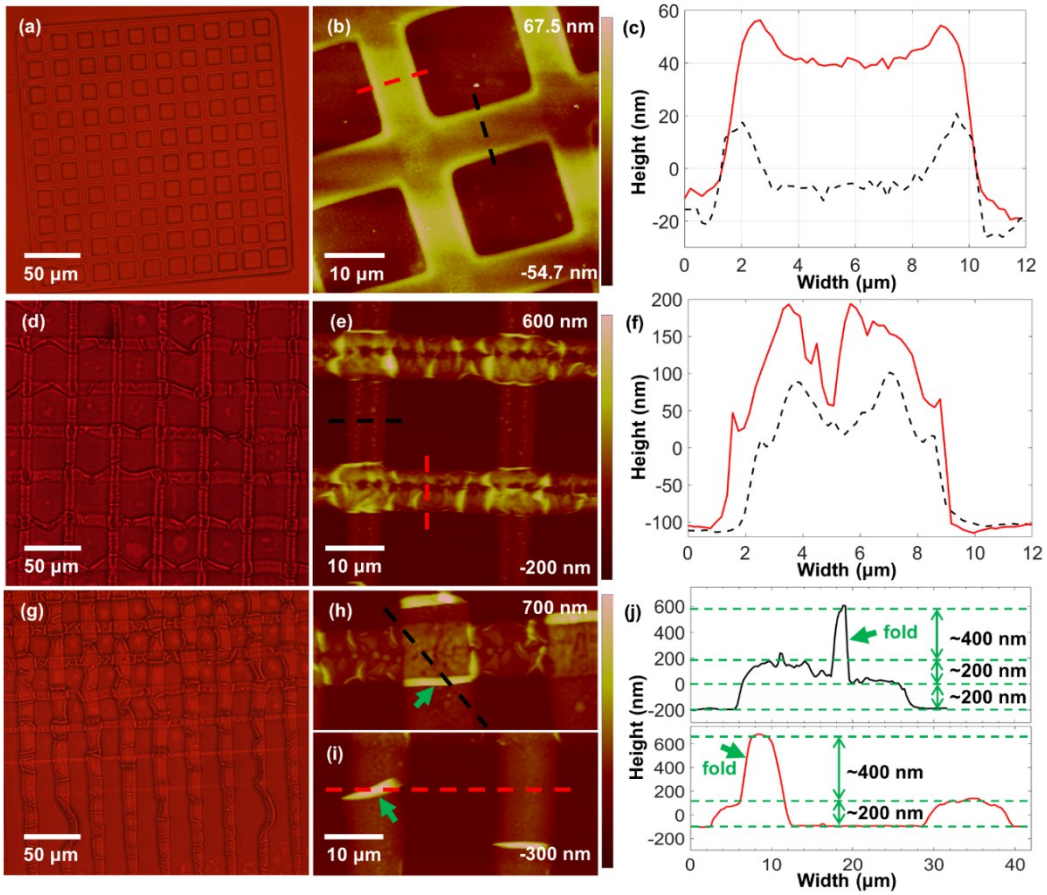

Figure S5. Investigation on the critical thickness of a hydrogel thin layer. All of the hydrogel patterns are printed at crossover time of 1s and 10 V_{pp}/1 kHz. (a) Hydrogel grids printed for 3 s, during which 2 s for vertical lines and 1 s for horizontal lines. (b) AFM height image of a basic cell of hydrogel patterns in (a). (c) Cross-section profiles of hydrogel lines according to the red and black dashed-lines shown in (b). (d) Hydrogel grids printed for 5 s, during which 2 s for vertical lines and 3 s for horizontal lines. (e) AFM height image of a basic cell of hydrogel patterns in (d). (f) Cross-section profiles of hydrogel lines according to the red and black dashed-lines shown in (e). The horizontal hydrogel lines are delaminated from the substrate and wrinkled to form some folds. (g) Hydrogel grids printed for 6 s, during which 3 s for vertical lines and 3 s for horizontal lines. (h) and (i) AFM height image of a basic cell of hydrogel patterns in (g). (j) Cross-section profiles of hydrogel lines according to the red and black dashed-lines shown in (h) and (i). All hydrogel lines are delaminated from the substrate and wrinkled to form some folds (green arrows). The critical thickness of a hydrogel layer is around 200 nm.

According to above investigations of the delamination time-point of hydrogel layers, we designed a series of experiments to study the critical thickness of a single hydrogel layer by controlling the printing time. We set the alternating time as 1s, which means that the light illumination of horizontal lines last for 1 s (12 frames per second) and then changed into vertical lines illumination for 1 s and then continuously repeated. As we can see from Figure S5, (a) to (c) show the height of a thin layer of hydrogel cured for 3 s, during which 2 s for vertical hydrogel lines and 1 s for horizontal lines. The AFM characterized heights of vertical line and horizontal line are ~50 nm and ~20 nm, respectively. Apparently, this hydrogel pattern has not delaminated from the substrate. In FigureS5 (d) to (f), the vertical line is cured for 2 s and the horizontal line is cured for

3s, and the heights are ~120 nm and ~200 nm, respectively. From the AFM image in (e), the horizontal line has some bright wrinkles (larger height) which represent for the folded thin hydrogel layer. The thin flexible hydrogel film delaminated from the rigid substrate and wrinkled into folds to release the mismatched inner stress. In Figure S5 (g) to (i), both vertical and horizontal lines are cured for 3s and have a height of ~ 200 nm according to the cross-section profiles in (i). From the upper profile (black-colored) in (i), the height of the cross-point is the plus of height of both layers. The green arrows represent the folds of hydrogel film produced by hydrogel wrinkling. (Note: the non-uniform line-width at bottom area in optical image (g) was caused by unclear defocused light pattern projection.) From above investigation we can estimate that the critical thickness of a visible delaminated single hydrogel layer is about 200 to 300 nm.

As a summary, the critical feature sizes of our VLEP system are: minimum line-width of ~3 um, maximum length of 1 mm, and critical thickness of a single visible hydrogel layer of 200 ~ 300 nm.

4. Viability of cells co-cultured with 3D hydrogel bioscaffolds

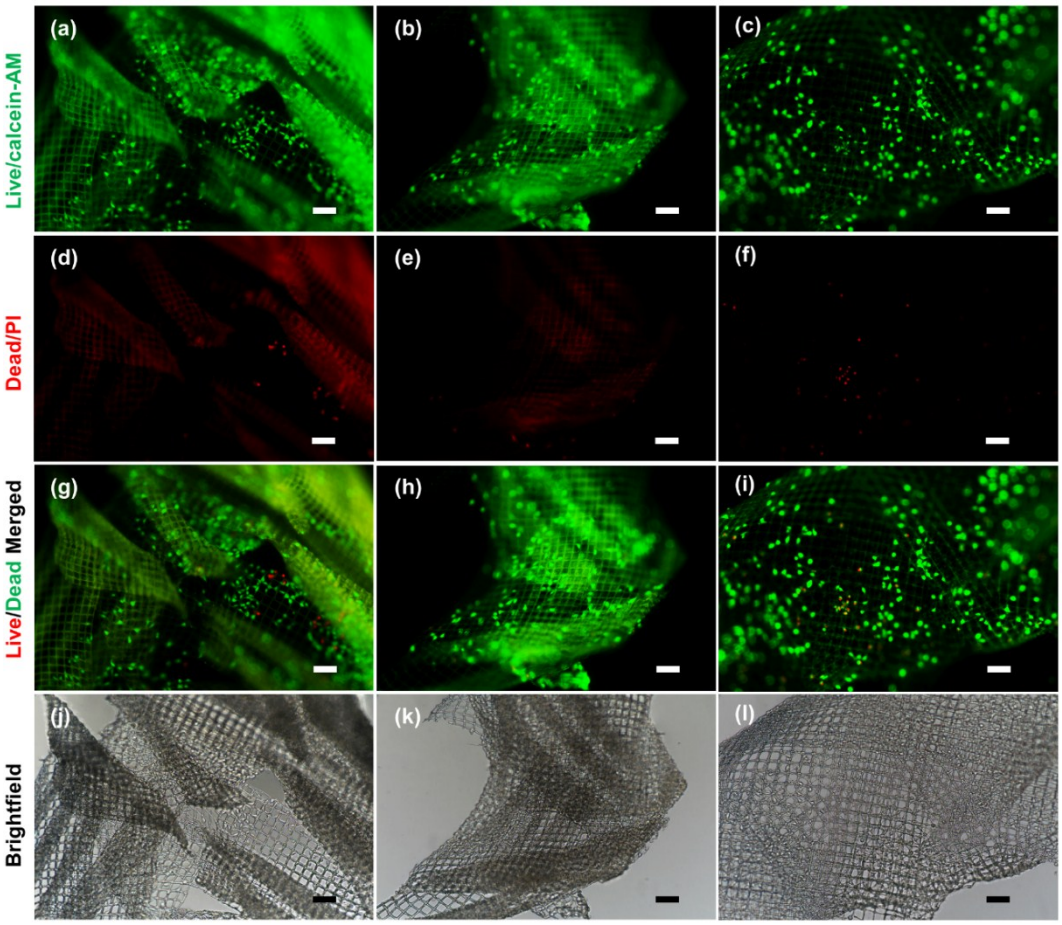

Figure S6. Living/dead fluorescent image of Calcein-AM/PI co-stained L929 cells co-cultured within a 3D fibronectin-treated PEGDA bio-scaffold after 24 h. (a) to (c) Alive cells were stained by green calcein-AM. (d) to (f) Dead cells were stained by red PI. (g) to (i) Merged live/dead fluorescent images. (j) to (l) Bright field optical images of L929 cells co-cultured with stacked hydrogel networks. All scale bars represent 100 um.

The viability of the L929 cells co-cultured in the net-like hydrogel frameworks after 24 h is accessed by using a fluorescent live/dead staining kit of green Calcein-AM and red Propidium iodide (PI). Figure S6 (a) to (c) and (d) to (f) show the live (green) and dead (red) cells in fluorescence, respectively. The merged fluorescent images in (g) to (i) show great viability of cells co-cultured in differently arranged hydrogel scaffolds, which is above 85 % from statistical estimation in Figure S7. From the bright field optical image of hydrogel net-like

scaffolds we can see that the hydrogel net-like layers could be folded up to form more complex 3D frameworks with intertwined hydrogel backbones, which could form microscale pores and gaps for nutrients and waste exchange in thick hydrogel modules.

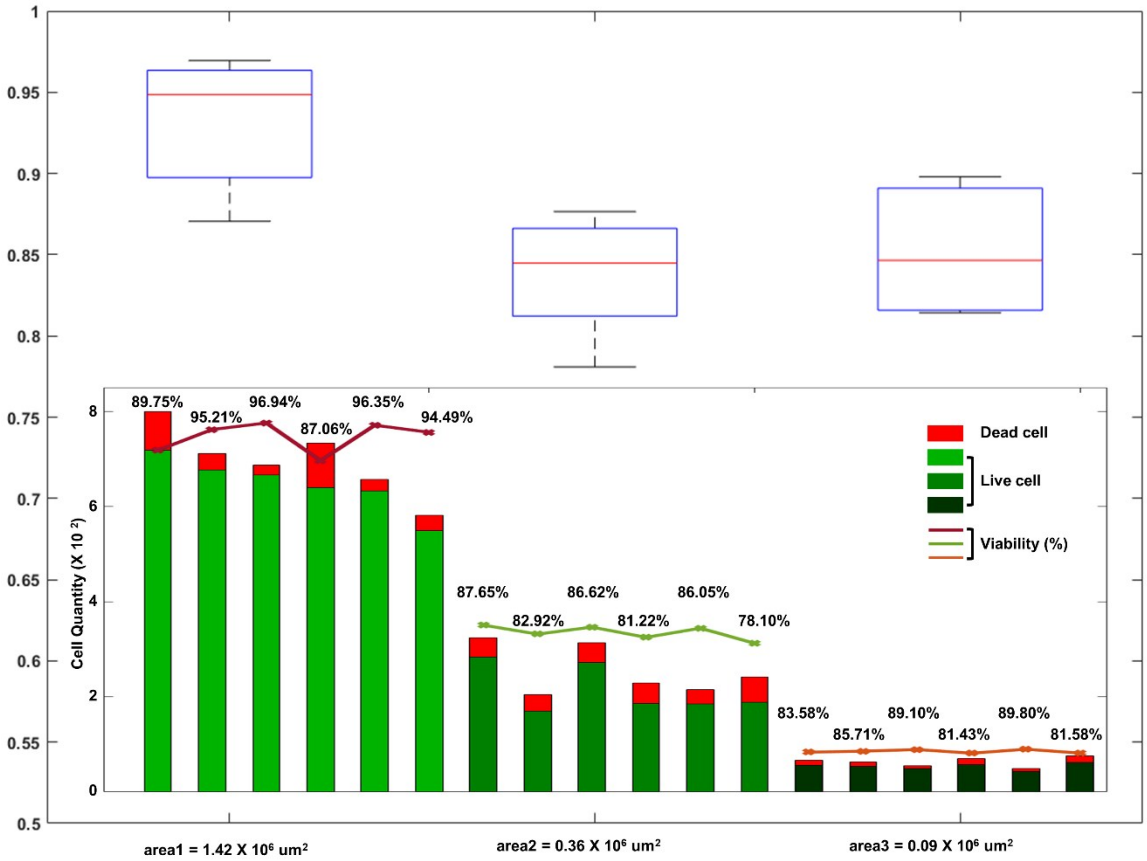

Figure S7. Statistical results of the viabilities of L929 cells seeding on net-like hydrogel scaffolds with different areas. Inset shows the detailed statistical data, there are 6 samples in each group with different area.

5. Videos

Video S1. Fabrication and peeling off of multilayered hydrogel grids.

Video S2. Fabrication and delamination of 1D hydrogel lines.