Electronic Supporting information

# Replicating 3D printed structures into hydrogels

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**Materials:**

3D printing resin (clear) was purchased from Miicraft, Taiwan. Calcium sulphate hemihydrate, calcium carbonate with 10 $\mu m^3$ crystal size, calcium chloride dihydrate, sodium alginate (medium viscosity), gelatin and alginate lyase were purchased from Sigma-Aldrich. Calcium carbonate with 1~2 $\mu m^3$ crystal size was purchased from BDH (Product no. 275795T). Hydrochloric acid (37%) was purchased from VWR. Genipin was purchased from Guangxi Shanyun Biochemical Science and Technology. Agarose, Dulbecco's Modified Eagle Medium (high glucose), penicillin, streptomycin, fetal bovine serum and life/dead viability kit for mammalian cell were purchased from Life Technologies. 1% suspension of green fluorescent microbeads was purchased from Polyscience.

**3D printing procedures:**

All 3D structures were designed in AutoCAD (Autodesk®) and exported in the file format of .stl. The files were processed by the printer software and the 3D model was sliced into layers. The thickness of each layers was 100 μm and the pattern on each layer was projected to the resin chamber with an exposure time of 7 s per layer. After exposure, the stage of the substrate moved upward for 100 μm to project another pattern to the resin chamber. The 3D master was printed by repeating these steps sequentially.

To clean the uncured resin from the freshly printed master, it was submerged in ethanol bath for 10 min. After air dried the ethanol, the master was post-cured in the provided chamber of the printer for 10 min to increase its mechanical strength.

**Replication 3D printed structure into calcium alginate:**

To prepare 1.625g of the plaster paste, 0.300 g of calcium sulphate hemihydrate was mixed with 0.700 g of calcium carbonate followed by mixing with 0.625 g of de-ionized water. The mixed paste was placed in a centrifugation tube and close packed with the 3D printed structure by centrifugation with 1400 r.c.f. for 30 s. The plaster paste was partially dried in room temperature for 2 h then placed in an 90$^{o}$C oven overnight for complete setting. After taking the mold out of the tube, the mold was heated at 500$^{o}$C in a muffle furnace for 2 h to remove the 3D printed template through combustion. After cooling to room temperature, the cavity was slightly cleaned by a blowgun to remove the loose hanging calcium residues. Then, 0.01M $CaCl_2$ solution was pipetted into the mold with a volume (mL) equal to 25% of the weight of the mold (g). The solution wetted into the mold quickly. Afterwards, 3% (w/v) sodium alginate solution was centrifuged into the mold with 1400 r.c.f. for 60 s. Excessive calcium alginate gel was removed from the outer surface of the mold to expose the $CaCO_3$ surface. Afterwards, the mold was submerged in a 2% HCl solution for 1 h to retrieve the casted alginate. The alginate was further sonicated in a fresh 2% HCl bath for 10 min to remove any calcium particle stuck on the alginate surface. Finally, the alginate was washed with de-ionized water three times, and submerged and stored in de-ionized water.

**Replication 3D printed structure into genipin-crosslinked gelatin:**

Gelatin is a derivative of collagen that forms physical gel at room temperature. Although it is highly biocompatible and cell adhesive, without crosslinking, the gel melts at physiological temperature and soluble in aqueous medium that limited its cell culture application. To crosslink gelatin, glutaraldehyde is the commonly used crosslinker. However, its cytotoxicity is a major concern of its biocompatibility. Instead, genipin is a natural sourced crosslinker that has much lower cytotoxicity compared with glutaraldehyde.[1] Therefore, genipin was used to crosslink the gelatin hydrogel.

On the first hand, the plaster mold was firstly prepared as described in previous section. However, instead of loading controlled amount of $CaCl_2$ solution, the plaster mold is submerged in a water bath to fully fill the mold with water. On the other hand, 10% gelatin (w/v) solution was prepared in a 40 ºC water bath. Genipin was dissolved in ethanol (reagent grade, Sigma-Aldrich) to prepare a 20% (w/v) stock solution. The genipin stock solution was added to the gelatin solution to prepare a final gelatin mixture that contained 9% gelatin (w/v) and 1.9% genipin (w/v). The mixture was then pipetted into the cavity of the mold. By cooling to room temperature, the gelatin forms a physical gel. Afterwards, the gel was placed in a humidified chamber for 24 h to complete the crosslinking reaction. At this stage, the gelatin turned into deep blue color that is common for genipin crosslinked protein. Finally, the mold was removed by submerging in 2% HCl bath for 1 h and was sonicated for 10 min in 2% HCl bath.

**Fabrication of 3D microfluidic network embedded agarose:**

Agarose solution, concentrated DMEM and alginate lyase was warmed and mixed to prepare a final gel precursor with 3% (w/v) agarose, 10 U/mL alginate lyase and 1X DMEM. The precursor was poured over the alginate replica and cooled to gel. The agarose was then incubated at 37 ºC for 1.5 h followed by slicing to expose the inlet and outlet of the microfluidic network. The alginate solution was removed by suction through the outlet. For the channel visualization, 1% green fluorescent microbead solution (polyscience) was pipetted into the agarose gel.

**Fabrication of cell-seeded agarose gel with perfusable channel network:**

HepG2 cells were cultured with DMEM, penicillin and streptomycin (100 U), and 10% fetal bovine serum (FBS) in the petri dish. The cells were cultured for 4 days then harvested and suspended in fresh DMEM. The suspension was added to the warm agarose solution and mixed with other components described above to prepare the gel precursor with 3% agarose, 10 U/mL alginate lyase, 1X DMEM and 1 x $10^7$ cells/mL HepG2. After poured on the alginate replica with a trifurcating network structure and gelled by cooling, the cell-seeded agarose was incubated at 37ºC for 1.5 h to reverse the gelation of alginate and reduce its viscosity. Then, the head and tail of the incubated agarose was sliced to expose the inlet and outlet of the microfluidic network. After removing the alginate solution by suction, DMEM (pre-equilibrated with 5.0 % $CO_2$ overnight in the cell incubator) was perfused into the network by a syringe pump at a flow rate of 2 μL/min for 3 days in a 5.0% $CO_2$ cell incubator. To assess the cell viability in the perfused agarose, the agarose was sliced at the middle and stained with

live/dead assay solution. For the control, the agarose precursor was prepared with the same composition and cell density as the agarose used in the perfusion experiment. Instead, the precursor was poured in a PDMS mold without the alginate template. After gelled, the agarose was incubated at 37 ºC for 1.5 h. It was then placed in DMEM bath for 3 days inside the cell incubator. To determine the cell viability at the center of the agarose, it was sliced at the center and stained with live/dead assay. The cell viability was calculated at the edge and center of the gel.

**Cell viability assessments:**

All of the agarose slices were submerged in a live/dead staining solution (2 μM calcein-AM-green and 4 μM ethidium homodimer-1) in the pH-adjusted buffer. The submerged slices were incubated for 30 min at 37ºC and 5.0% $CO_2$. Fluorescence images were acquired with an inverted confocal microscope (Eclipse Ti with D-Eclipse C1, Nikon Instruments). ImageJ was used for merging of green and red fluorescence images and counting the live and dead cells.

## Reference:

1. a. Bigi, G. Cojazzi, S. Panzavolta, N. Roveri, and K. Rubini, *Biomaterials*, 2002, **23**, 4827–4832.

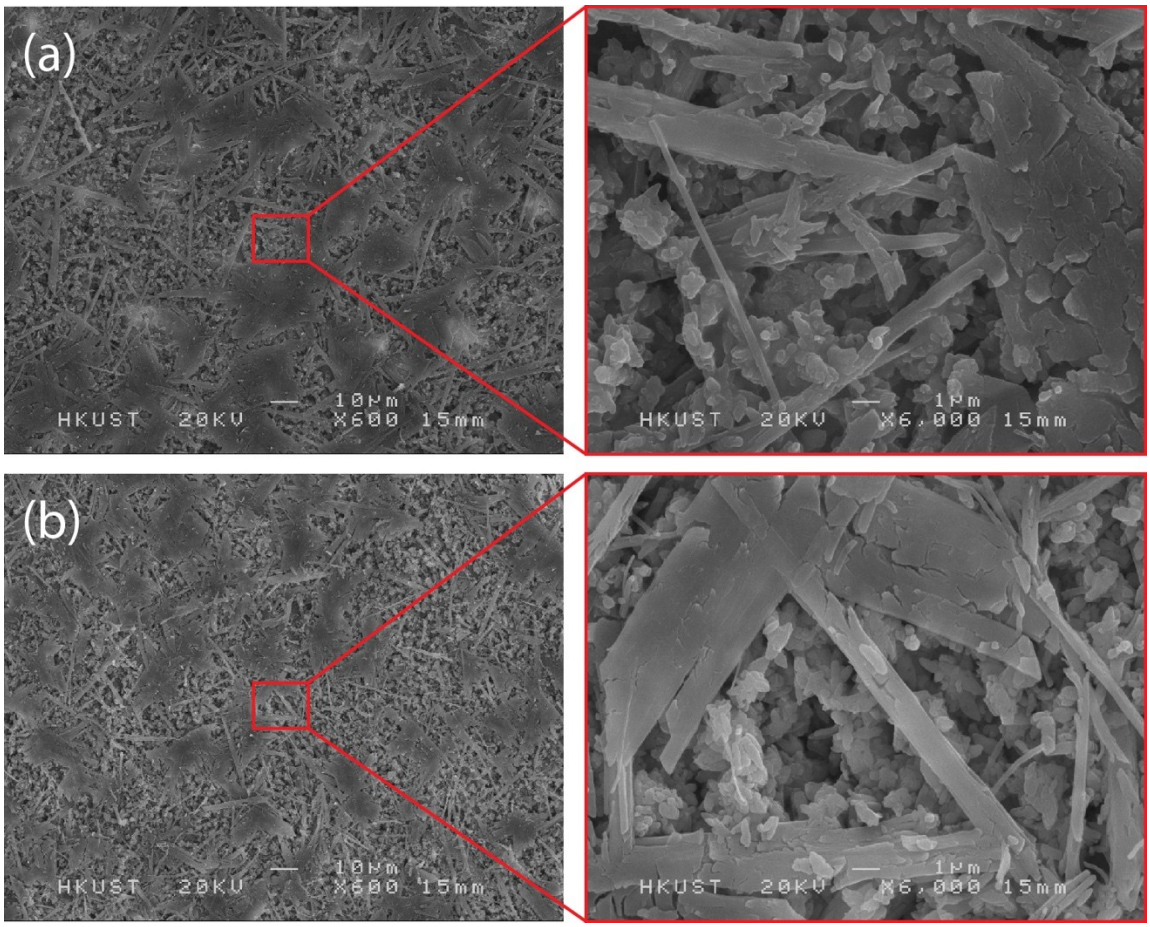


Figure S-1. The SEM image of the plaster mold before **(a)** and after **(b)** the heat treatment (500 ºC for 2h). The needle and plate crystals are $CaSO_4$, while the smaller crystals are $CaCO_3$.

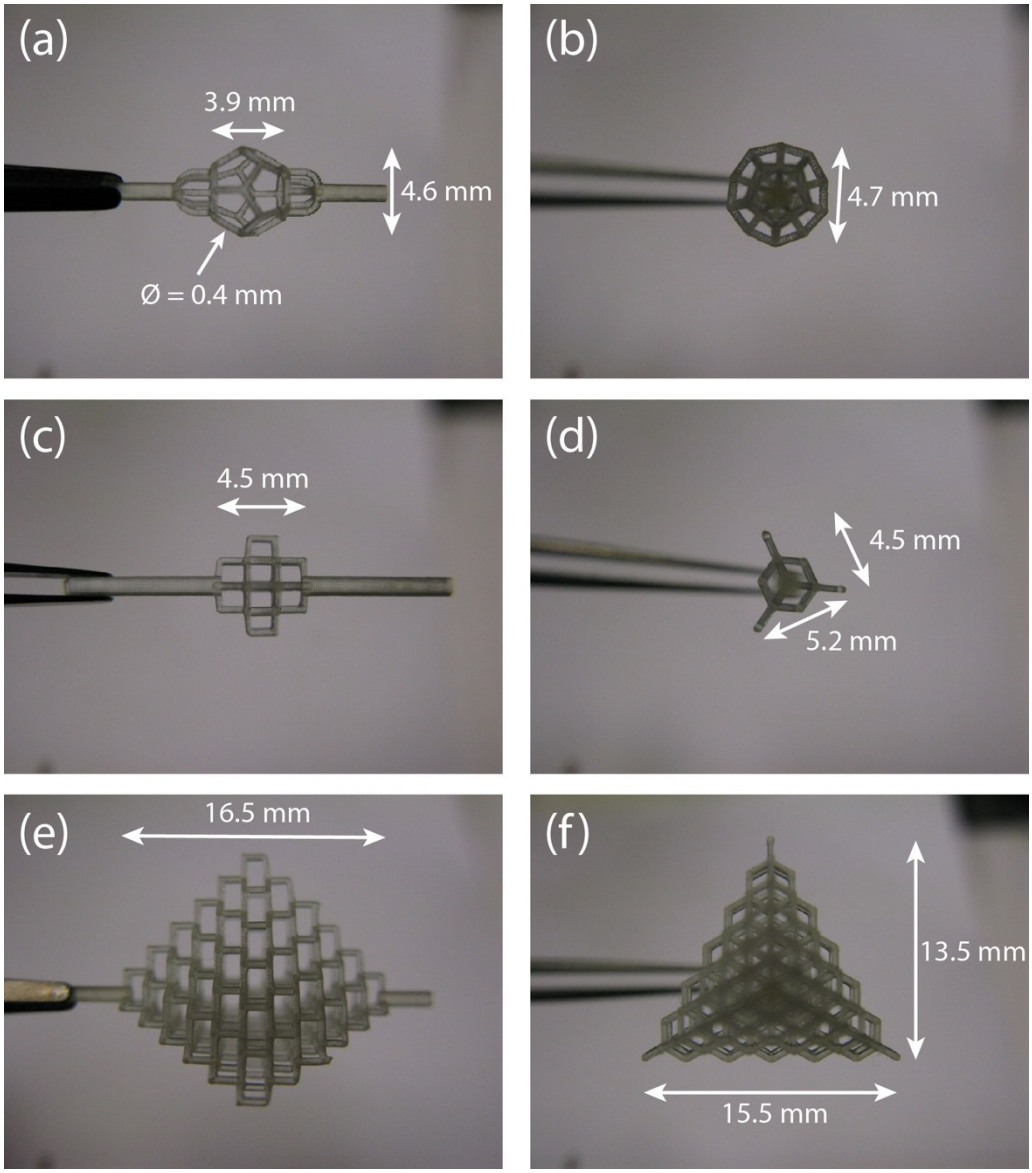


Figure S-2. Detailed dimension of the 3D printed masters. The diameter of the arms in all of the masters are 400 µm as shown in (a). Dodecahedron, side view **(a)**, front view **(b)**. Trifurcating network, side view **(c)**, front view **(d)**. Scaled trifurcating network, side view **(e)**, front view **(f)**.