Supplementary Information

3D-printing Enabled Micro-assembly of Microfluidic Electroporation System for 3D Tissue Engineering

Qingfu Zhu, Megan Hamilton, Bryan Vasquez, Mei He

a Department of Chemical and Petroleum Engineering, Bioengineering Program, University of Kansas, Lawrence, Kansas, USA
b Department of Chemistry, University of Kansas, Lawrence, Kansas, USA

Investigation of cell membrane electro-permeabilization and propidium iodide delivery.

3D cells were seeded on chip according to the previously described 3D culture method. After 24 hours after cell seeding, the culture medium was replaced by low conductivity medium and 20 µL of 10 µg/mL propidium iodide solution (PI, sigma-Aldrich, USA) was added on the top of the cell culture. After 30 min incubation, the electroporation was performed. To investigate the influence of electric field scanning on the PI delivery, four parallel experiments were conducted in terms of different electric field directions i.e. single direction, two cross directions, three directions and four directions. The total pulse duration was 32 ms for each experiment. The transfected cell cultures were transferred from chip to a 1.5 mL centrifuge tube. Without removing the upper layer cell medium, the gel was mechanically disrupted thoroughly by pipetting up and down. The mixture was centrifuged at 600 g for 6 min. To break colonies, 100 µL accumax™ solution (Sigma-Aldrich, USA) was added and incubated for 5 min at 37 °C. The results have been evaluated by spinning the cells on glass slide followed with fluorescence microscopy analysis.

3D cell culture and study of growth rate

The peptide hydrogel matrix (PepGel) was used as the scaffold with a peptide gel concentration of 0.2% for 3D cell culture. The 2D cells growing at a confluency of 80-90% were re-suspended by 0.25% trypsin/EDTA (Sigma-Aldrich) solution and centrifuged for 5 mins at 250 g-force for seeding into peptide
hydrogel. Cells were prepared with the same cell seeding density in peptide hydrogel for monitoring the growth rate. The initial cell seeding density is $1.5 \times 10^5$ /mL. For counting the 3D cultured cells at certain duration (6 hours after seeding, 2 days, 3 days, 5 days, 6 days and 8 days), pipet up and down of culture for several times to break up hydrogel and suspend the spheroids, and then transfer to 15 mL tube. Rinse well the culture with 2 mL of media twice and transfer to the same tube. Mix 8 mL more media into the tube well. Centrifuge at 250 g-force for 5 minutes. Use micro-pipette at 50 µL to remove all media from cell pellet but not to disturb the cell pellet. Resuspend the cell pellet in 50 µL Trypsin and mix well for 5 minutes, and then add 50 uL of Trypsin inhibitor in the mixture. Re-measure the total volume if possible (usually the volume will be 110 uL from residual media and cells). Add an equal amount of Trypan blue and use 10 uL to count cells (Cell counting chamber, Fisher).

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**Fig. s2.** Time-dependent HeLa cell on-chip 3D culture. Cell culture conditions according to the method were described in the experimental part. Bright-field microscopic images a to f represent the cells cultured for 0 day (6 hours after seeding), 2 days, 3 days, 5 days, 6 days and 8 days, respectively. The initial seeding density is $1.5 \times 10^5$ cells/mL. The scale bar is 80 µm. 3D cultured cells are counted using the method described above along different culture durations for calibrating and estimating total cell numbers and growth rate (calibration curve in right hand).

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**Fig. s3** The bright-field microscopic images showing the characteristic morphology of 3D cultured Hek-293 cells and HeLa cells, which is significantly bigger than their single cells with different morphology.
Confocal imaging of 3D cells in scaffolding matrix

3i/Olympus Spinning Disk Confocal Inverted Microscope with CUBE 405nm and Sapphire 488nm lasers. Images were captured using 40X 1.3NA UPlanFL N Oil Objective, a Hamamatsu Flash 4.0 v1 CMOS camera and the SlideBook 6.0 Imaging Software. All image acquisition were 3D capture with 0.340 microns step size in Z and Binning 1x1. Then, a 2D image was generated representing the maximum Z projection intensity of the 3D image using SlideBook 6.0.

Cells with gel matrix rinsed with warm (37°C) HBSS/ 0.01% sucrose for 5 minutes, then fixed with 2% PFA diluted in HBSS/ Sucrose, and rinsed again with HBSS/ Sucrose. All cells were stained with DAPI nuclear stain (10µM) diluted in HBSS and added Vectashield Mounting Media and put into Ibidi confocal well plate. Note that the matrix caused pretty high background, due to light diffraction from the gel matrix during the z scanning. We used VisiKol® clearing solution (visikol.com, per vendor’s instruction) before imaging to clear the background. Images were taken as the 2D images representing the maximum Z projection intensity of the 3D image using SlideBook 6.0.

![Confocal images showing different depths and locations of 3D cultured HeLa spheroids](image.png)

**Fig. s4.** Montaged confocal images showing the different depths and locations of 3D cultured HeLa spheroids, indicating the good growth and dispersion of 3D HeLa spheroids.