Supplementary Information

Efficient Cell Capture in Agarose-PDMS Hybrid Chip for

Shaped 2D Culture under Temozolomide Stimulation

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1. Cell preparation and staining

Cell culture and preparation of cell suspension

U251, U87 and HUVEC (Cancer Institute & Hospital, Chinese Academy of Medical Science, Beijing, China) were cultured in petri dish with RMIP 1640 culture medium (Mediatech, Inc., USA) containing 10% FBS and 1% penicillin and streptomycin. Cells were trypsin digested by Trypsin-EDTA for 5min, and resuspended by 1640 culture medium to achieve suspension. Then the suspension was condensed by centrifuge. Cell density was confirmed by handheld automated cell counter (Millipore Corporation, USA).

Live/dead staining

0.5µl 4 mM Calcein-AM solution and 2µl 1mM Etdh-2 (Invitrogen) solution were added to 1ml PBS. Then the solution was injected to agarose surface. The agarose gel was preserved in 37\textdegree C for 30min, and then washed by PBS for 3 times. Finally, cell viability was observed by fluorescence microscopy.

DiI, DiO and Hoechst33342 staining

For DiI and DiO (Life Technologies) staining, 5µl cell labeling solution was added to 1ml cell suspension. Then cell suspension was incubated at 37\textdegree C for 30min. After incubation, cell suspension was centrifuged at 1200rpm for 3min, and removed the supernatant. Cells were washed by PBS solution for three times and concentrated to desired density. For Hoechst33342 staining, procedure was the same as DiI and DiO staining, except that 1µl labeling solution was added to 1ml cell suspension.
2. Fabrication of agarose gel microwell array

To ensure integrity of the array and avoid damage to template during separation, agarose solution of 2%wt was used. Higher content would increase the gel rigidity and make it hard to peel off from template, while lower content was unable to provide enough strength after gelation. As shown in Fig. S1, the agarose gel array we fabricated was highly transparent, allowing for the direct observation under microscopy. And the PDMS channel was simply physically attached to the surface of microwell array. We observed that the physical contact of PDMS and agarose provides enough sealing to confine aqua in the predesigned channels without leaking.

![Fabrication of agarose microwell array.](image)

**Fig. S1** Fabrication of agarose microwell array. 1. The SU-8 templates was formed on the silicon wafer as standard lithography protocol and placed in a glass dish. 2. Boiled agarose solution was poured into the dish containing wafer. 3. Place the cast to 65°C incubator and preserve for 30 min. 4. Cooling down the cast at room temperature for 3h. 5. Gelation of agarose gel on SU-8 mold. 6. Cut the casting complex out from dish by surgeon knife. 7. Peel the gel from silicon wafer base. 8&9. Cut the gel into uniform pieces. 10-12. Assemble the PDMS-agarose chip by physically attaching PDMS channel to the gel surface. 13. Sampling by direct pipette transfer.
Fig. S2 (A) PDMS-agarose chip fabrication and assembling. (B-D) Side, front and vertical views of the chip layout.
3. Continuous observation of cell culture on agarose gel array

HUVEC, U87 and U251 cells were localized on the different regions on microwell array and cultured for 2 days. The agarose gel provided good biocompatibility, and cell survival was witnessed after 2 days’ culture. Due to the surface modification of fibronectin or other adhesive factors on dish culture, cells can easily attach to the surface and exhibit specific morphology with pseudopods. However, in hydrogel culture, due to the lack of adhering site, cells exhibited the spherical morphology.

![Continuous observation of different cell types for 48h on the same agarose microwell array (observation area was not fixed). Scale bar: 100µm.](image-url)
4. Comparison of cell capture performance on arrays of different materials
PDMS microwell array was compared with agarose gel arrays. We found the microwell occupancy on PDMS substrate was significantly lower than agarose gel. And on agarose substrate, the cell occupancy was nearly 100% if the cell density of infused suspension was high enough.

Fig. S4 Bright field photographs of microwell array of different materials and cell capture fluorescence images (A, B: PDMS; C, D: agarose gel; cell density 1.0×10^7/ml). Cells were stained by Calcein-AM before array captured. Scale bar: 200µm.
Microwell structure provided protection for cells inside on both PDMS and agarose substrates, and after washing only the microwell docked cells remained. There are two major differences between PDMS and agarose substrates in cell capture. Firstly, cells on PDMS substrate did not exhibit significant distribution into microwells, and there were many cells settling outside of the wells. Secondly, for PDMS substrate, it was found that cells originally settling in microwells could also be washed away. This result could be due to the PDMS microwells sparsely occupied by only one or two cells, while in agarose substrate, multiple cells crowded into one microwell. Therefore cells in PDMS microwells may not fully contact with the bottom or the side walls but loosely floating among wells, which make the dislodgment easier.

Fig. S5 Images of HUVEC capture on PDMS and agarose substrate before and after washing. Cells were stained by Calcein-AM before array captured (cell density 1.0×10^7/ml). Scale bar: 200µm.
5. Fabrication of arrays with various well diameters and spacings

Arrays with different diameter and spacing in range from 15µm to 40µm were fabricated. And the photos were listed as following. We then studied the influence of diameter, spacing, cell suspension density on cell capture performance. We found that as long as the cell suspension density was high enough, wide combinations of diameter and spacing could reach 100% cell occupancy.

Fig. S6 Bright field images of agarose microwell array with different diameters. (A): 15µm; (B) 25µm; (C) 35µm. Images of cell capture on agarose microwell with different diameters. (D) 20µm; (E) 25µm; (F) 30µm. Scale bar: 200µm.
Fig. S7 Cell capture by microwell arrays with different diameters and spacing. HUVEC were used as model cells, and the suspension density was set as $1.25 \times 10^7$/ml (A) diameter 15µm, spacing 20µm; (B) diameter 15µm, spacing 30µm; (C) diameter 15µm, spacing 40µm; (D) diameter 20µm, spacing 40µm; (E) diameter 25µm, spacing 40µm; (F) diameter 30µm, spacing 40µm. Cells were stained with Calcein-AM. Scale bar: 200µm.
Fig. S8 Fluorescence images of cell capture on microwell array. HUVECs were used as model cells and stained with Calcein-AM. Cell density was kept the same in each column, and diluted by 2 folds in neighboring columns from left to right; microwell diameter was kept the same in each row, and increased from 15µm to 30µm by step of 5µm from top to bottom. Scale bar: 200µm.
6. Cell capture efficiency study

Cell density gradients of HUVEC and U251 were established to study the cell capture efficiency. Cell density was diluted by 2 folds in sequence from con.1 to con.4. Diameter of the microwells was 30µm with 80µm in depth and the spacing between two neighboring wells was 50µm. For U251 con.4 was set as $1.698 \times 10^6$/ml; for HUVEC con.4 was $1.849 \times 10^6$/ml.

![Fig. S9](image-url) Cell counts in one microwell against successively diluted cell densities. (A) U251 and (B) HUVEC.
We found that capture efficiency was gradually increased as the cell suspension went diluted for both cell types. This is reasonable because when the cell density was too high, the excessive cells would settle outside the microwell and be washed away.

**Fig. S10** Comparison of cell capture percentage (cell captured/ cell introduced) between (A) U251 and (B) HUVEC.
7. Study of temozolomide stimulation on U251 and HUVEC culturing

Effect of temozolomide (TMZ) on cell culturing was studied with U251 and HUVEC culture on petri dish. The two cell types were cultured alone respectively for 2 days. TMZ concentration gradient of 0, 300, 600, 900µM was established. We found that when increasing the TMZ content, U251 cells proliferation was inhibited, which could be told by the reduced cell density and increased cell death in Fig. S11. For HUVEC, the TMZ significantly depressed the formation of vascular networks as shown in the reduced vascular lumen-like structures of Fig. S11.

![Fig. S11](image)

**Fig. S11** Effect of temozolomide stimulation on HUVEC and U251 viability after 48h culture (HUVEC and U251 were cultured alone on Petri dish). Scale bar: 200µm.