Electronic Supplementary Material (ESI) for Lab on a Chip

-- A novel 3-D bio-microfluidic system mimicking in vivo heterogeneous tumour microstructure reveals complex tumour-stroma interactions

1. Benign breast epithelial cells MCF-10A-GFP and epithelial type breast cancer cells MCF-7 were seeded and co-cultured in an MSACM assembly at a ratio of 1:1. The MCF-7 cells were stained with the CellTracker™ Orange CMRA Dye (C34551, Invitrogen) right before they were seeded in the collagen chamber for fluorescent observation. As an epithelial type breast cancer cell line, MCF-7 is not as malignant as the MDA-MB-231. However, the formation of MCF-10A lumen structure was still disturbed by MCF-7. As shown in Fig. S1A-B, although there were weak connections among the MCF-10A cells, these cells were still dispersed randomly in the microchamber, without obvious aggregation. It indicates that similar to the MDA-MB-231, MCF-7 also disturbs the MCF-10A aggregation.

The expressions of E-cad in the cell lines of MCF-7, MCF-10A, and MDA-MB-231 have been measured with reverse transcription-polymerase chain reaction (RT-PCR) in 2-D. The results have been analyzed and normalized to the MCF-7 expression. Thus the E-cad relative expressions in MCF-7, MCF-10A, and MDA-MB-231 are 1, 5.089, and 0.0188, respectively.

The E-cadherin expression was also tested and analyzed with the immunofluorescent method. The result is shown in Fig. S1C. Different from the MDA-MB-231, MCF-7 expresses E-cad. In the co-culture system of MCF-10A and MCF-7, the E-cad expression of MCF-7 was still normal, but the E-cad expression of MCF-10A was down-regulated dramatically, even much lower than the expression in MCF-7, which also indicates the disruption of MCF-10A cell aggregation by the MCF-7.
Figure S1. Co-culture system of MCF-10A-GFP and MCF-7. (A) Fluorescent image of part of the microchambers array in the MCF-10A-GFP (green) and MCF-7 (red) co-culture system, showing dispersed MCF-10A distribution. (B) Confocal image showing the cell distribution in a single microchamber. (C) Representative images of the cells morphology and E-cad expression in a single microchamber.

2. Fig. S2A&B show the PDMS substrate of the MSACM, and PDMS stamp for micromoulding the microchambers on the collagen surface. Mechanical pressure was applied to assemble the MSACM. A plexiglass jig was designed and fabricated to clamp the chip. Fig. S2C&D are, respectively, the top and bottom images of the plexiglass jig. Once the microfluidic chip was fabricated and assembled with the cover glass, the sealed chip was put into the sterilized jig. The position of the collagen platform needed to be aligned with the open window in the center of the jig bottom. Then the jig top was aligned and assembled to the jig bottom, fixed with the screws on the four edges of the jig. There were eight medium wells in the jig top, corresponding to the inlets for the medium microchannels. Cell culture medium was injected into the microfluidic system via the medium wells, which were also used for stocking extra medium to avoid evaporation, and acted as the source and sink for gradient formation, as shown in Fig. S2E.
Figure S2. Images of the MSACM and the fabricated plexiglass jig.

3. This figure shows how the 3-kDa FITC-Dextran concentrations at various positions change with time in the MSACM system.
Figure S3. The 3-kDa FITC-Dextran concentration changes as function of time. The results at positions of 0 mm, 2.5 mm, 5 mm, 7.5 mm, and 10 mm away from the source microchannel are indicated by the black, red, blue, magenta, and green curves, respectively.

4. The fabricated microfluidic system with cells was observed under microscope for 24 hours continuously. Time-lapse images were taken every 1 hour. During the observation, the cells were cultured in an on-stage incubator to maintain the cell culture condition with the temperature of constant 37 °C and with 5% CO₂. As shown in Fig. S4, the time-lapse images of the same microchamber indicated that in the MCF-10A mono-culture system, the MCF-10A cells formed aggregation of a lumen-like structure in 8 hours after being seeded into the microchamber. In the following time, the cells were in a dynamic state but still kept aggregated, with the shape and position of the aggregation keeping changing. The MCF-10A cells in the co-culture system with MDA-MB-231 were, however, always in a dispersed single cell state, during their dynamic movement process.

![Table of Time-lapse Images](image)

Figure S4. Time-lapse images for cell movement in the MCF-10A monoculture and co-culture systems.