Electronic Supplementary Material (ESI) for Lab on a Chip.

ELECTRONIC SUPPLEMENTARY INFORMATION

Microdroplet Chain Array for Cell Migration Assays

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Materials and methods

Cell culture conditions

Mouse colon adenocarcinoma cell line CT26 (CRL-2638, ATCC, USA), human colon adenocarcinoma cell line RKO (CRL-2577, ATCC, USA), human breast cancer cell line MCF7 (HTB-22, ATCC, USA), and human embryonic kidney cell line HEK 293 (CRL-1573, ATCC, USA) were cultured at 37 °C 5% CO_2 in dulbecco's modified eagle medium (DMEM) (Gibco) with 10% fetal bovine serum (FBS, Hyclone, Logan, USA) and 1% penicillin/streptomycin (Gibco).

Human normal intestinal cell line FHs 74 Int (CCL-241, ATCC) and human bronchial epithelial cell line BEAS-2B (CRL-9609, ATCC) were cultured in RPMI 1640 media (Gibco) containing 10% FBS and 1% penicillin/streptomycin. Besides, the medium of FHs 74 Int also contained 30 ng/mL EGF during culture in the dish. Human normal liver cell line LO2 (ATCC) were cultured at 37 °C 5% CO_2 in RPMI 1640 media with 20% FBS and 1% penicillin/streptomycin. Human embryonic heart tissue cell line HEH-2 (China infrastructure of cell line resource, 3111C0001CCC000177, China) were cultured at 37 °C 5% CO_2 in DMEM with 20% FBS and 1% penicillin/streptomycin. Human breast cancer cell line MDA-MB-231 was cultured at 37 °C in L-15 (Gibco) with 10% FBS and 1% penicillin/streptomycin. Cell density was 10^5-10^6 cells/mL during cell growth, and the culture medium was refreshed every 2-3 days. To get cell suspension for cell migration experiment, cells were digested with 0.25% trypsin/EDTA solution (Genom Biological Medicine Co., Hangzhou, China). The cell suspension was centrifuged and the cell pellet was washed twice with phosphate buffered saline (PBS). Cells were re-suspended by medium according to the demand, namely FBS-free medium

for cancer cells while medium contained 10% FBS and matrigel for normal organ cells. Cell density was measured using a haemocytometer and adjusted according to the requirements of the experiment. In cell migration assays with pattern I, II and IV, the culture medium of cancer cells need to be refreshed by medium contained 0.1% BSA without FBS for starving 12 h before depositing to microchip, after which cell suspension was deposited to target microwells. In cell migration assay with pattern III, cancer cells were suspended by medium contained 0.1% BSA without FBS medium and immediately deposited to target microwells as starving treatment during cell migration.

Generation and characterization of concentration gradient in droplet chain

We used fluorescein isothiocyanate (FITC)-labeled dextran (10 KDa, 0.5 mM) as model sample added into droplet to measure its concentration gradient in droplet chains. Three types of droplet chains with pattern I, II and III were formed using the same procedure as in cell migration assays, except that a 0.5 mM FITC-labeled dextran solution was added into the droplets instead of an actual drug or inducer. Fluorescence images of the droplets in a chain were captured using an inverted fluorescence microscope (Nikon Eclipse Ti-S, Nikon, Tokyo, Japan) with a digital camera. The fluorescent sample concentrations were estimated by analyzing the fluorescence intensities of the captured images using an image analysis software, ImageJ, and using calibration curves obtained from standard solution droplets in the concentration range of 5 nM to 1.0 mM on both sides of the membrane.

Cell migration assay

For the basic cell migration assay, droplet chains with two droplets in each chain (pattern I) were used (Fig. 1c1). Before the cell migration experiments, 500 nL inducer droplets containing matrigel with 20% FBS (v/v) and 500 nL control droplets without the inducer were first sequentially formed in microwells on the underside of the chip membrane. In the inducer droplet, a 20% FBS concentration higher than the commonly-used 10% in traditional petri-dish experiments was adopted to generate the FBS concentration gradient. After a 30-min incubation at 37 °C for matrigel curing, different cell suspension droplets containing CT26, RKO, MCF7 or MDA-MB-231 cells in a serum-free medium with a final cell density of 5×10^5 cells/mL were generated on the membrane upside. The chip device was placed in the incubator for 24 h to perform cell migration experiments. After the migration experiments, the cells in the droplet chain array were fixed with 4% paraformaldehyde and stained with 0.1% crystal violet (Solarbio, Beijing, China), rinsed with deionized water, and imaged by a microscope (CKX41, Olympus, Tokyo, Japan). An image for whole droplet in each droplet chain was first taken for counting total number of cells on both sides of the membrane in each droplet. Due to the good transparence and thin thickness of 20 μm for the PC porous membrane, cells on both the upside and underside of the membrane in each droplet could be observed in one image (see Video S1 and Fig. 2b1). Then, the upside PDMS layer of the chip was revealed from the membrane, and the cells on the upside surface of the membrane were wiped off. The cells remained on the underside surface of the membrane after passing through the membrane micropores in each droplet were imaged with the microscope, and the number of cells migrated through the membrane to the underside was counted using the image. The migrating percentage in each

droplet chain was calculated with the data of total cell number and migrated cell number. After the experiments, the PC membranes with the fixed cells stained by dyes could be long-term preserved as specimens.

In the cell migration assay with competitive microenvironment, droplet chains with pattern II were used (Fig. 1c2). For each droplet chain, two 800 nL droplets of matrigel with 20% FBS (droplet 1) and without FBS (droplet 2) as control were first formed on the underside of the membrane, and then a middle droplet (droplet 3) of 800 nL starving cancer cell suspension (CT26, RKO, or MDA-MB-231 cells) was formed on the upside of the membrane (Fig 3a). After droplet generation, the chip was installed as shown in Fig. 1d, pattern II to perform cell migration. After 24-h migration experiment in the incubator, similar method as in basic cell migration assay was adopted for cell fixation, staining, imaging and counting.

The biomimetic chemotaxis assay used droplet chains as pattern III (Fig. 1c3 and 1d). In each droplet chain, droplet 2 of matrigel on the underside of the membrane was first formed. Then, droplet 1 and 3 containing 50% FBS and cell suspension in culture medium, respectively, were formed on the upside of the membrane. After two days' culture, the culture mediums in droplet 1 and droplet 3 were replaced with new medium for rebuilding the FBS concentration gradient. After another two-day incubation, the cells in droplets were fixed and stained. A different data processing method, equidistant line method (Fig. 3f), was used in biomimetic chemotaxis assay by first using equidistant lines to plot out subregions in droplet 2 to obtain both cell number and migration distance data characterized by the subregions (Fig. 3f) of the migrated cells in droplets. The region in droplet 2 non-overlapping with droplet 3 was divided to 10 equi-distance subregions with equi-distance of adjacent lines. Both the results of cell

numbers and cell migration distances could be obtained by counting cell numbers in the 10 subregions (Fig. 3g).

The multi-organ cell co-culture and cell migration assay were performed with pattern IV with flower-like droplets (Fig. 1c8 and 1d). Five petal droplets containing organ cells as HEH-2, LO2, FHs 74 Int, BEAS-2B, and HEK 293 cells in matrigel were formed in five 1.2 mm diameter petal microwells on the underside of the membrane, respectively. Before depositing cell suspension to the microwells, 400 nL matrigel prepared with organ cell optimal medium (matrigel, 1.1 mg/mL; DMEM medium with 10% FBS for HEH-2 and HEK 293 cells; 1640 medium with 10% FBS for LO2, FHs 74 Int, and BEAS-2B) were added into corresponding petal microwells, and allowed to be cured in the cell culture incubator at 37 °C for 30 min. Then, five organ cell suspensions with volume of 300 nL, cell density of 3×10^6 cells/mL and matrigel concentration of 1.1 mg/mL were added to the five petals, respectively. In the control group, petal droplets contain the same mediums as in the organ cell experiment but no organ cells. After the generation of the petal droplets, the chip was turned over, organ cells in droplets were cultured for 12 h in the hanging droplet mode. A central droplet of 2 μL containing ca. 2000 metastasis cancer cells was formed in the microwell (2.4 mm diameter) on the upside of the membrane, forming the flower heart droplet. After 24 h culture, the migration of cancer cells in the central droplet to different petal droplets were evaluated with the similar method as in basic cell migration assay.

Statistical analysis

All quantitative data for each condition was repeated four times using four cell droplet

chains unless stated otherwise. Mean ± SD values were compared using Student's t-test, and $P<0.05$ was taken as the minimum level of significance.

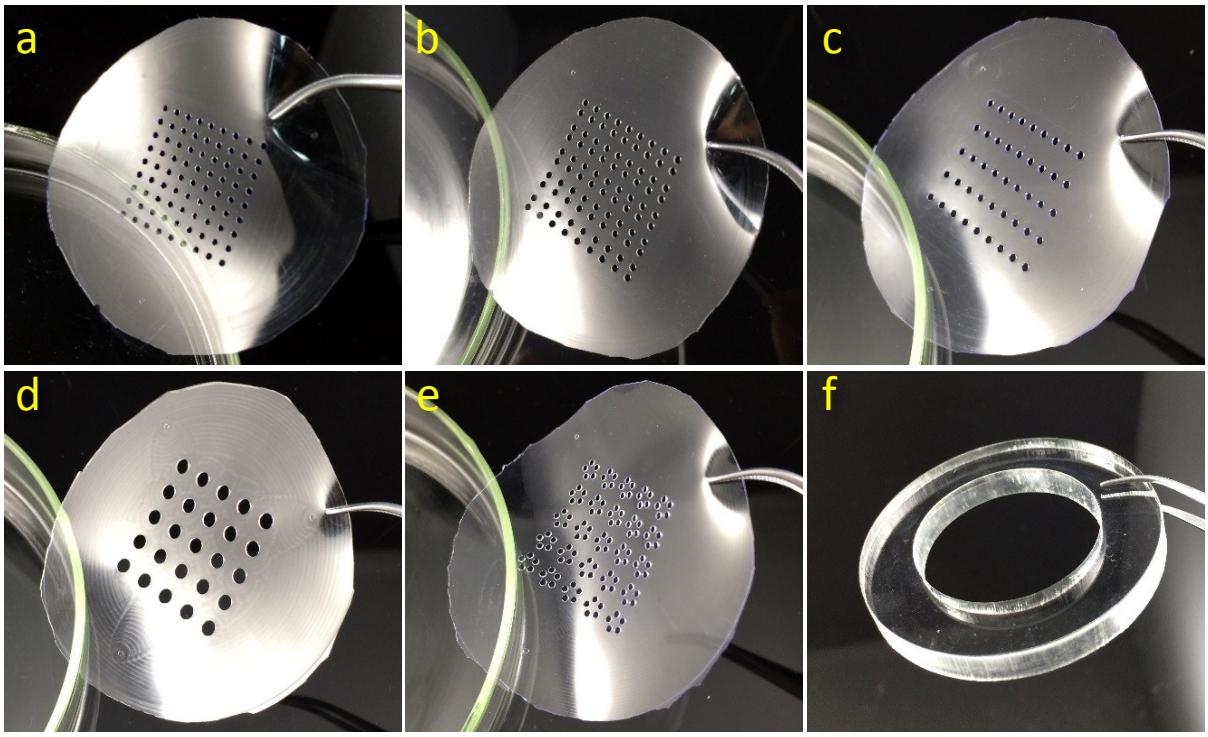

Figure S1. Typical images of elements used in microchip of three-dimensional microdroplet chain array. (a-e) Five PDMS layers with different through hole configurations. Two PDMS layers of (a) are used in building pattern-I droplet chain array. PDMS layers of (b) and (c) are used to build pattern II or III droplet chain array. PDMS layers of (d) and (e) are used in building pattern IV droplet chain array. (f) Image of polymethyl methacrylate (PMMA) ring with 30 mm i.d. and 50 mm o.d, fabricated by a laser engraving machine.

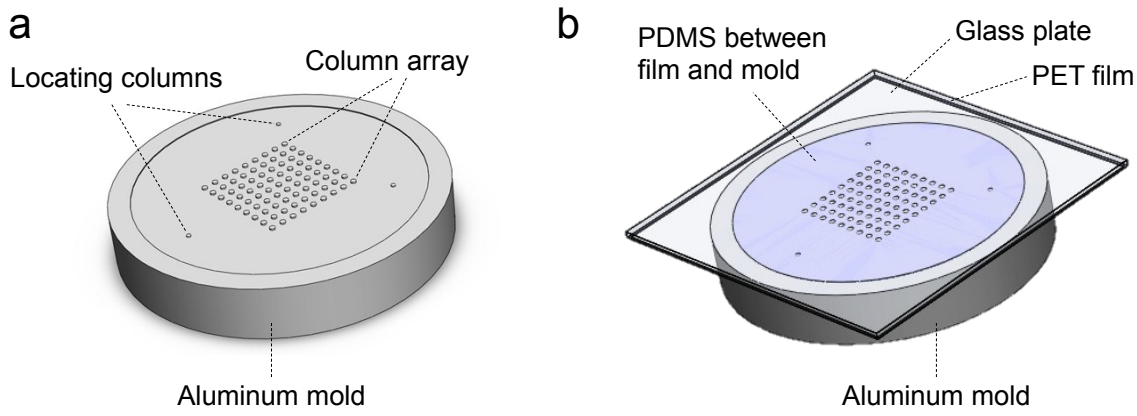

Figure S2. Schematic diagram of fabrication process for polydimethylsiloxane (PDMS) layer with through-hole array for pattern-I droplet chain array. (a) Aluminum mold for fabricating PDMS layer with column (300 μm height, 1000 μm diameter) array and three location columns (200 μm height, 800 μm diameter). (b) Casting fabrication for PDMS layer. First, the PDMS prepolymer with a mixing ratio of 10:1 (w/w) for PDMS base and curing agent is poured on the mold. A glass plate covered with a flat and smooth polyethylene terephthalate (PET) film is pressed on the mold by a 3-kg metal block, with the PET membrane closely contacting with the mold columns to ensure the PDMS thickness of 300 μm. After curing at 65 °C for 4 h, the PDMS layer is demoulded from the aluminum mold and PET film.

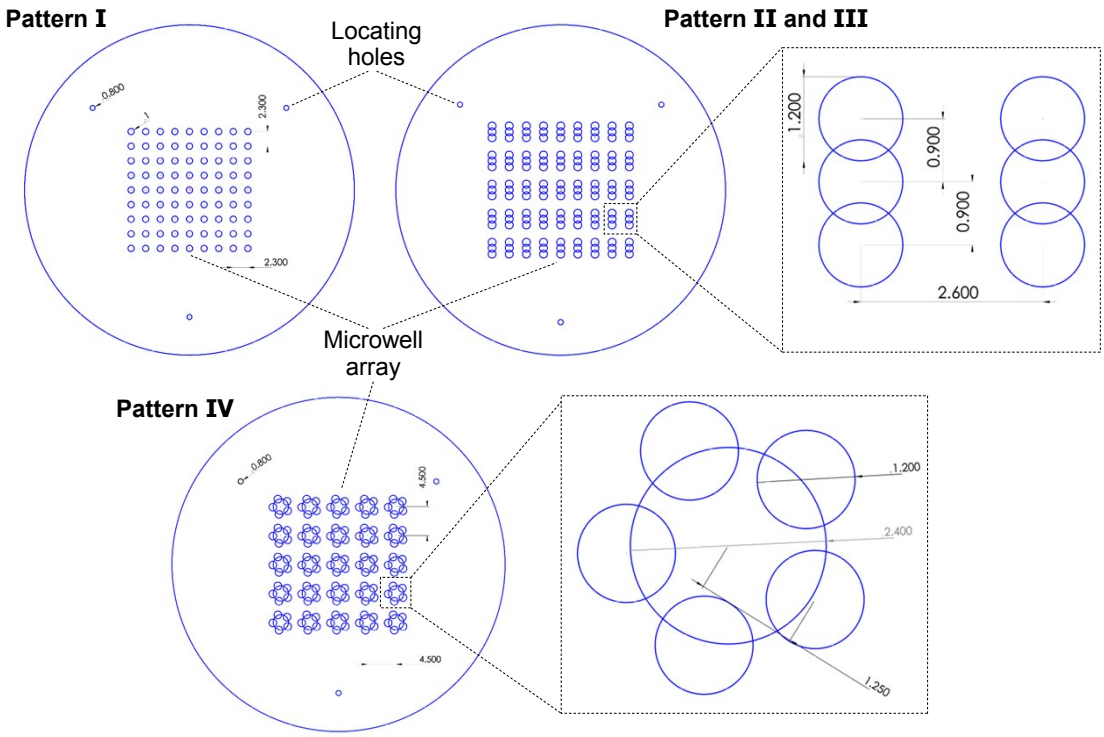

Figure S3. Schematic diagrams of the configuration design for microchips with droplet chain arrays of pattern I, II, III and IV.

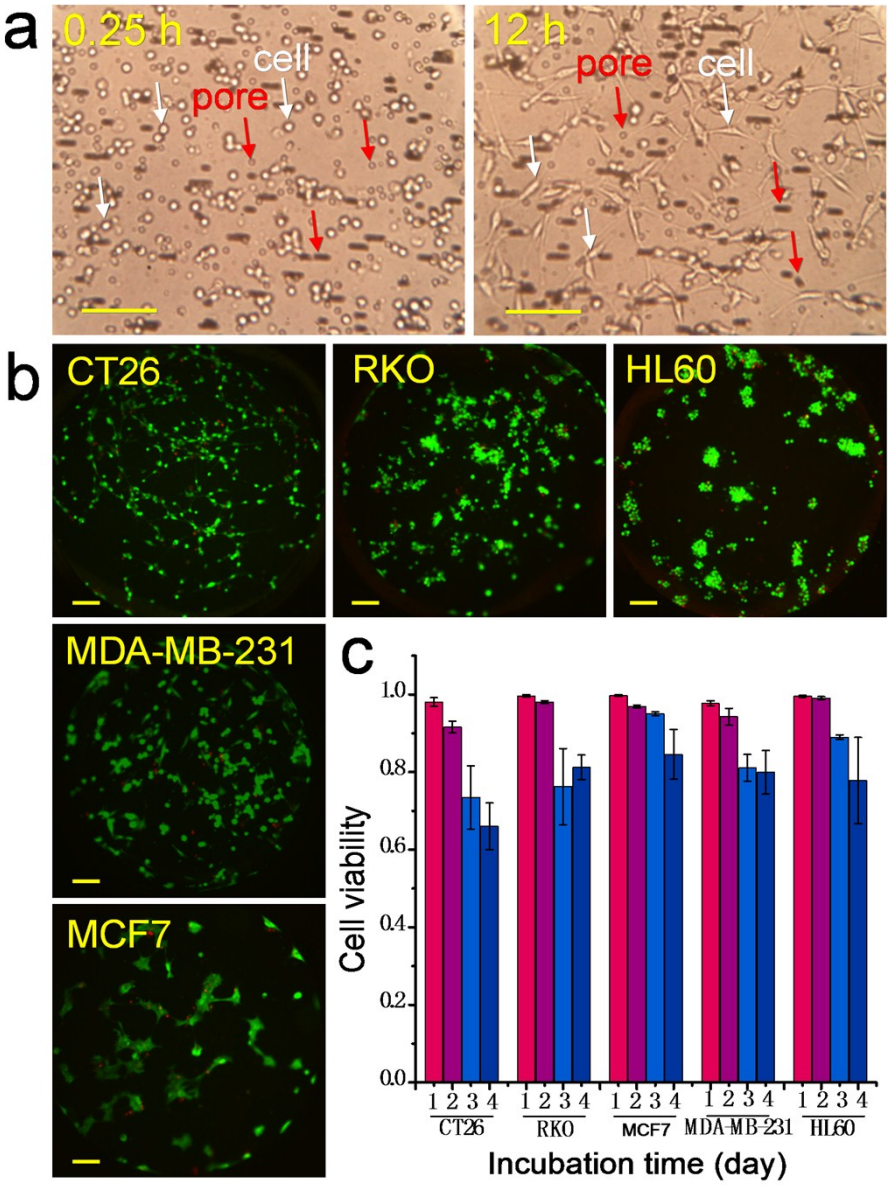

Figure S4. Test of device biocompatibility. (a) Typical microscope images of in-droplet CT26 cells after 0.25 h (left) and 12 h (right) culture. Red arrows show the pores on the PC membrane, and white arrows the CT26 cells. After 12 h culture, CT26 cells adhere well to the membrane. (b) Typical fluorescent images (live/dead stain) of CT26, RKO, MCF7, MDA-MB-231, and HL60 cells after 1 days' culture on the microchip, showing that most of the cells are alive. (c) Histogram of CT26, RKO, MCF7, MDA-MB-231, and HL60 cell viability in droplets after different incubation days in the microchip device. Cell viabilities show higher than 90% after two days' culture.

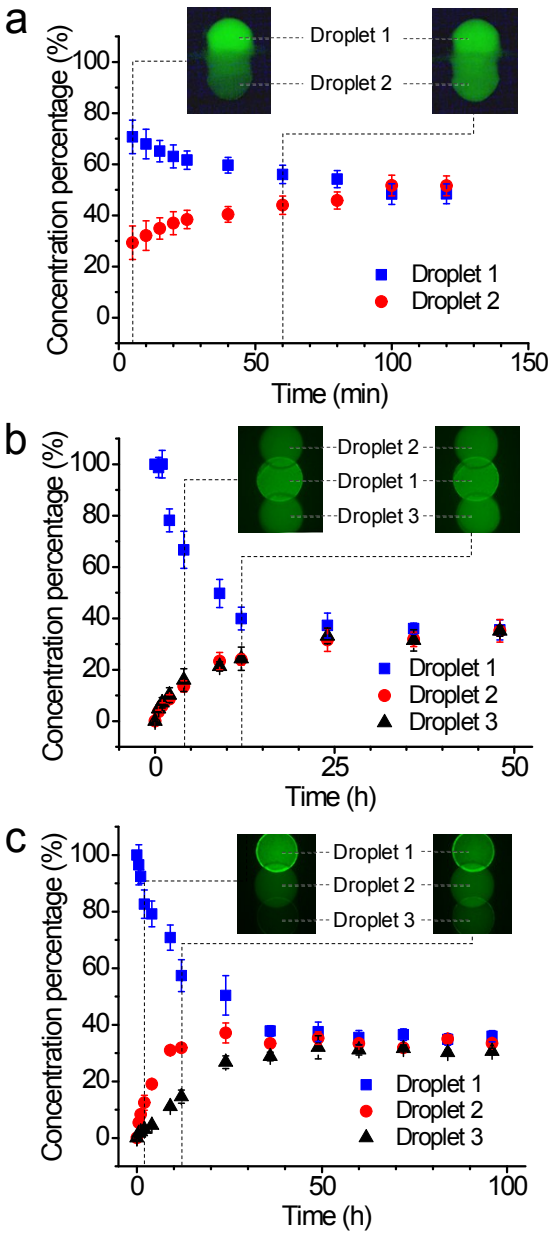

Figure S5: Characterization of chemical concentration gradients in droplet chains. (a) Variation of FITC-dextran concentration gradient in a droplet chain (pattern I) during 2 h. (b) Variation of FITC-dextran concentration gradient in a pattern II droplet chain during 2 days. (c) Variation of FITC-dextran concentration gradient in a pattern III droplet chain during 4 days. Conditions: droplet 1 contains 0.5 mM model sample of FITC-dextran (10 KDa) in culture medium and cancer cells with a density of 5×10^5 cells/mL. The underside droplets are culture medium with matrigel (1.1 mg/mL). The inserted figures show typical fluorescence images of droplet chains at corresponding times. Shown are representative results of average of three independent experiments.