Electronic Supplementary Information

Analysis of the paracrine loop between cancer cells and fibroblasts using a microfluidic chip

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1. Concentration distributions in the culture chambers of round and square shapes

We used COMSOL Multiphysics (COMSOL, Burlington, MA) to calculate the concentration distributions of the medium flowed from the other chamber into the culture chambers of round and square shapes. The volume of the round shape chamber is 64.2 μl, the same as that of the horn-shape chamber; while the volume of the square chamber is 64 μl. Other parameters are the same as those used for the simulation of the horn-shape chamber. The results are shown in Fig. S1. After 60

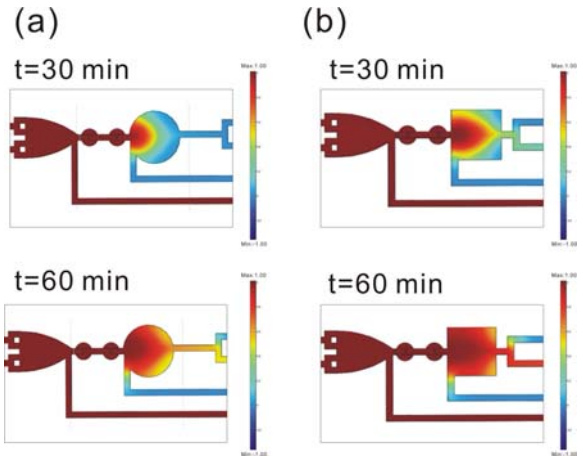

Fig. S1 Simulation results of the concentration distribution in (a) the round shape of culture chamber and (b) the square shape of culture chamber after the medium flowing from the left chamber for 30 and 60 minutes.

minutes of flow from the left chamber, the concentration distributions in the round and square shapes of chambers are not as uniform as that in the horn-shape chamber. Therefore, using the horn-shape chamber provides us the largest area of observation on cell behaviours.

2. Effects of flow fields on cell activities

It is essential to verify that the PDMS chip and the medium flows do not influence the cell activities without the cytokines. We first cultured fibroblasts in one chamber with the other chamber empty, and carried out the same experimental procedures. The results are shown in Fig. S2: the minimal aspect ratios of fibroblasts are not increased when the cells are cultured in the PDMS chamber with only the flows of the culture medium. The cell numbers show a steady increase. We also carried out the similar experiment on cancer cells, and the results show that the migration speeds of cancer cells are not affected by the medium flows. The number of cancer cells is increased constantly with a higher rate than that of the fibroblasts.

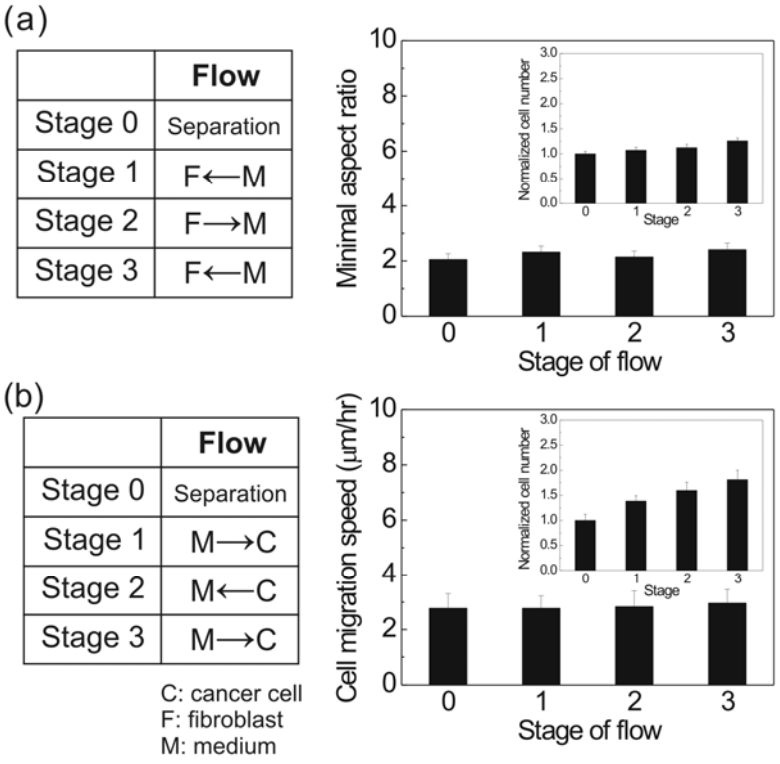

Fig. S2 Cell responses to the medium flows. (a) Fibroblast morphology. (b) Cancer cell migration speeds. The inserts are the cell numbers at each stage of flow. The data are from 3 independent experiments, and the cell number counted in each experiment is 100. Error bars, standard error of the mean.

3. Cell numbers at each stage of flow

In order to verify that the cells proliferate regularly in the chip, we measured the cell numbers in the final image taken at each stage. The means of cell numbers were obtained in 3 individual experiments, and 15 fields of view were observed in each experiment. For each individual experiment, we normalized the cell numbers in Stages 1, 2 and 3 to that in Stage 0. Fig. S3 shows the normalized cell numbers at each stage in the EXP-A and EXP-B. The increase in cell numbers is within 50% for fibroblasts and 100% for cancer cells, respectively.

4. Measurement of the cancer cell migration speed

For measuring the migration speeds of cancer cells, we used MetaMorph

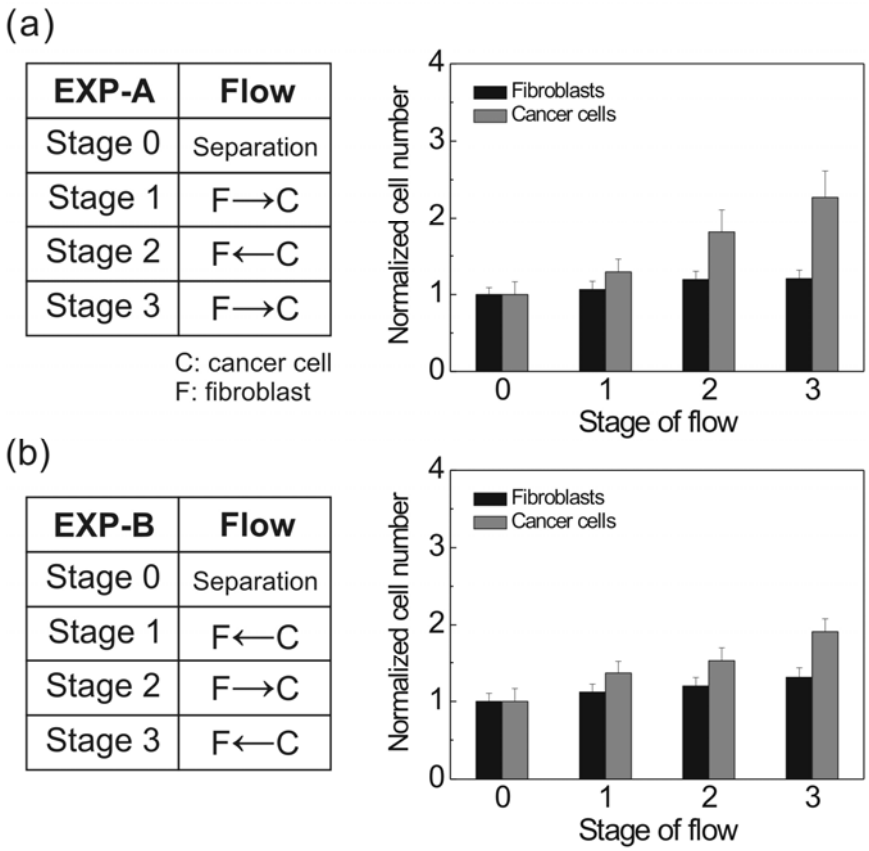

Fig. S3 The cell numbers at each stage of flow in (a) EXP-A and (b) EXP-B in the cell culture chips. The normalized cell numbers are obtained from 3 independent experiments and 15 fields of view are used for cell counting in every experiment. Error bars, standard error of the mean.

image analysis software (Molecular Devices, Sunnyvale, CA) to trace the trajectory of total migration distance of each single cell and obtain the average migration speeds of 100 cells from 15 fields of view. The images were acquired at

an interval of 30 minutes. We selected only the separate cells to measure migration speeds. Please see the time-lapse video of cancer-cell migration. Because the fields of view were switched during the experiments by a motorized translation stage, the time-lapse video look laterally shifted between each frame. The migration distance was therefore calculated relative to a fixed feature at the bottom of the culture chamber in each field of view.