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Electronic Supporting Information

A Microchip-Based Model Wound with Multiple Types of Cells

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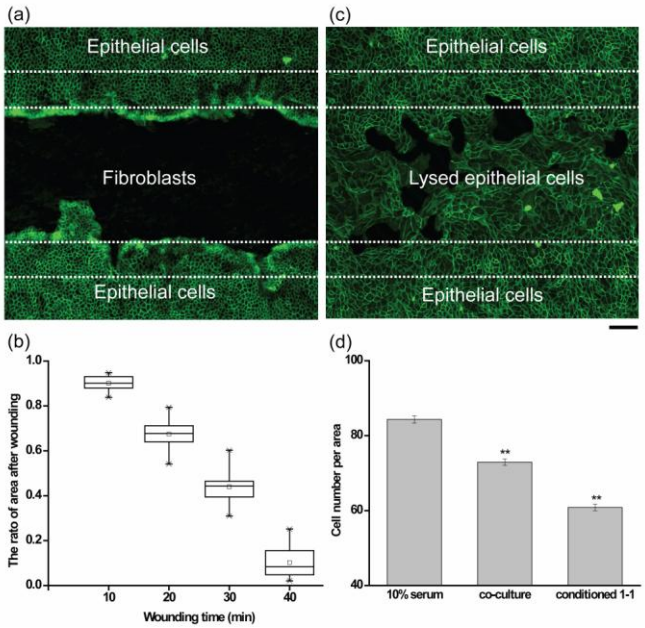
SI figures and text:
SI Figure 1. Character of co-culture and wounding chips.
SI Figure 2. Immunofluorescent images of E_cadherin junction under various conditions.
SI Figure 3. Quantitative analysis of epithelial collective migration for control experiments.
SI Figure 4. Quantitative analysis of the movement of individual epithelial cells at the edge of a group.
SI Figure 5. Progression of the leader for district fingers in 3 independent experiments.

SI methods

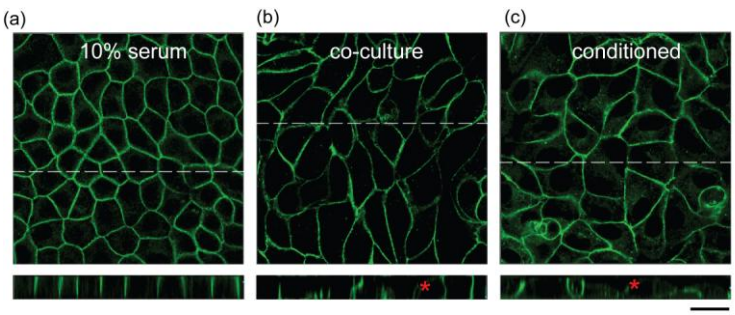
SI Movies

SI Movie 1. Time lapse movie of MDCK cells lysed in distilled water contained Annexin V/PI.
SI Movie 2. Time lapse movie of ROS formation in injured MDCK cells after lysis 3 min.
SI Movie 3. Time lapse movie of ROS formation in injured MDCK cells after lysis 10 min.
SI Movie 4. Time lapse movie of MDCK-MDCK-MDCK culture.
SI Movie 5. Time lapse movie of MDCK-NIH 3T3-MDCK culture.
SI Movie 6. Time lapse movie of MDCK-MDCK (lysed)-MDCK culture.

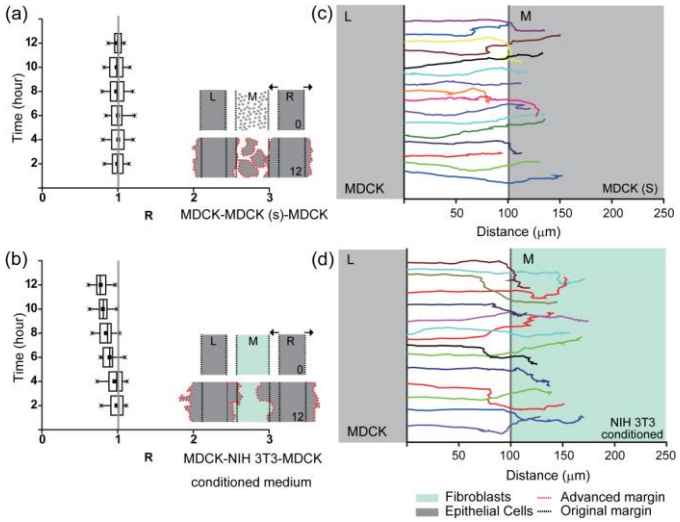
SI figures and text



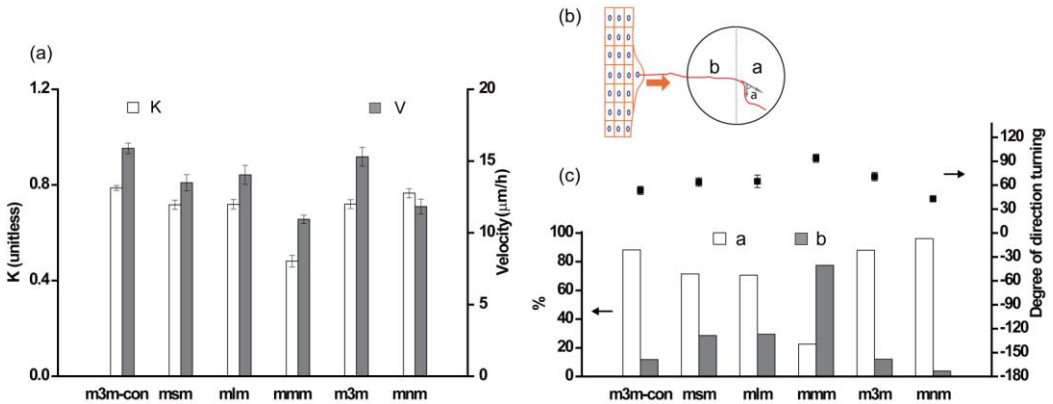
SI Figure 1 Character of co-culture and wounding chips. Images of E_cadherin staining and the statistics of the density of nuclei after culturing for 12 h in (a) co-culture chip and (c) wounding chip. Scale bar, 100 μm; (b) we calculated the area of residual cells after various wounding time to character the size of wound. The area of cells before lysis is (669.51× 500 μm). At least 12 areas from 3 independent experiments were measured at each lysis time; (d) we counted the number of cells per area in MDCK-alone, co-culture chip, and MDCK- alone cultured in conditioned medium (1:1). We stained cells with Hoechst 33342 and measured the mean value by selecting 27 areas (150 × 150 μm) at random from 3 independent experiments. **P < 0.001. Error bar, s.e.m.



SI Figure 2 Immunofluorescent images of E_cadherin junctions under various conditions. (a) MDCK- alone cultured in DMEM contained 10 %FBS, (b) in co-culture chip, (c) MDCK- alone cultured in conditioned medium (1:1). Confocal images were taken every 0.6 μm per slide, and the images were reconstructed in 3D using Image Pro plus 5.0. Bottom is the enlarged view of confocal XZ section. Dotted lines show the position of XZ section. From these images, we can observe that cell-cell junctions in co-culture chip (b) and conditioned medium (c) were loose and weak (indicated by red asterisks), while more extensive and tight in control (a). Scale bar, 20 μm.



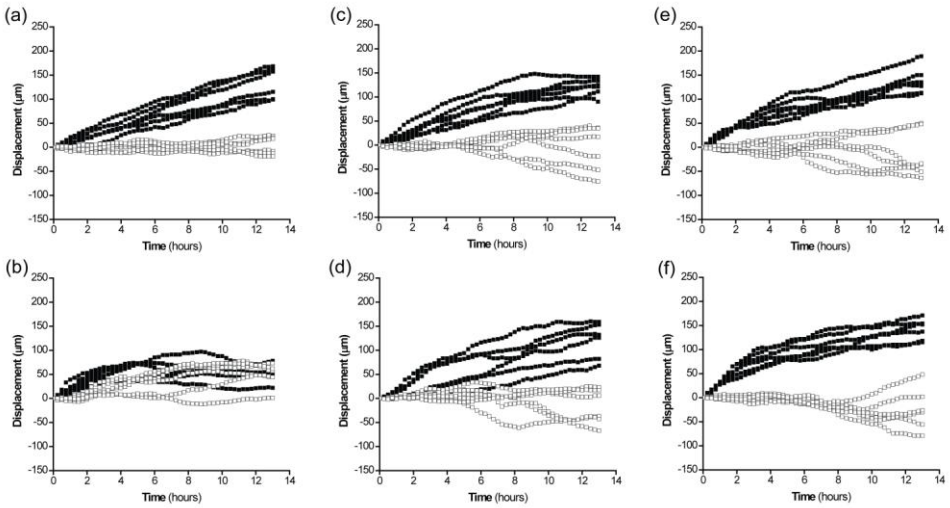
SI Figure 3 Quantitative analysis of collective epithelial migration for control experiments. Plot of the ratio ($R_{c/s}$) between the epithelial sheet migration rate toward cells and that toward free space (a and b) and cell trajectory (c and d) in MDCK-alone (a and c, MDCK (S) means: patterned stripe M with reduced density of normal MDCK to replace lysed MDCK, a: 16 samples, n=3; c: 16 cells, n=3) and co-cultured chip (NIH 3T3 in stripe M, in conditioned medium (1:1), b and d, b: 16 samples, n=3; d: 15 cells, n=3). Error bar, s.e.m.



SI Figure 4 Quantitative analysis of the movement of individual epithelial cells at the edge of a group. (a) Statistical summary of the average velocity of cells (v) and the extent of random turning (k) in various conditions used in this study. We measured the total distance ($\sum\Delta d$) that the cell traveled, and the average velocity of cells v was equal to $\sum\Delta d /nt$ (t is the time interval of each step, and n is the number of steps). We used the ratio $k = \Delta r / \sum\Delta d$ to identify how often the cell tended to turn (the displacement Δr, the distance between the initial and final positions). Cells that frequently make turns will yield a k value close to 0; whereas cells that persistently move along one direction will yield a k value close to 1(see reference 1) Error bar, s.e.m. (b) The scheme of re-direction of an observed trace. Only the leader cells originally located at the open edge were included in the analysis. In each trajectory, we measured only the changes in moving direction that happened after the first contact with cells from stripe M. We classified each trace into one of the following two categories by a circle divided into two regions (the circle was centered on the centroid of the cell just before re-direction; the dotted line represents a diameter paralleled to the initial margin of stripe M): The region "a" and "b" represents a final location that cells moved into. (c) Statistical analysis of the distributions of final locations and the degree of re-direction in various conditions used in this study. m3m-con represents co-culture chip with NIH 3T3 in stripe M incubated with conditioned medium; msm represents MDCK-alone with reduced density of normal MDCK in stripe M; mlm represents MDCK-alone with lysed MDCK in

stripe M; mmm represents MDCK-alone with normal MDCK in stripe M; m3m represents co-cultured chip with NIH 3T3 in stripe M; mnm represents MDCK-alone without cells in stripe M.

SI Figure 4c summarizes the statistical analysis of the directions of cell motility in all of conditions we used in this work. In the condition of mnm, the degree of direction turning was replaced by the largest direction turning in the whole process for its deficiency of contacting with cells from another stripe. m3m-con (34 cells, n=3); msm (21 cells, n=3); mlm (19 cells, n=3); mmm (40 cells, n=3); m3m (25 cells, n=3); mnm (26 cells, n=3). If epithelial cells moved into region a in a total observing time, which represents a forward motility; if cells moved into region b, which implies a reverse direction.



SI Figure 5 Progression of the leader for district fingers in 3 independent experiments. White squares denote the progression parallel to the initial edge; black squares denote the progression perpendicular to initial edge. In MNM, the leader cells persis in the direction perpendicular to the initial edge. In MNM (a), cells sustain a movement perpendicular to the initial edge at a constant speed; In MMM (b), cells took the direction perpendicular to the initial edge first, and followed an increasing reversed motility in this direction; in M3M, MLM, MSM, and M3M-con (c-f), the leaders possessed a highly directional motility perpendicular to the initial edge. An increasing motility parallel to the initial edge happened after about 8 hours, which is consistent with the time cells began to have a contact with cells from the opposite side.

Supplementary Methods

Device Fabrication The microchip for co-culture and wounding comprise 2- to 3-mm-thick PDMS (Sylgard, Dow Corning) microfluidic system and a polystyrene culture dish. The PDMS microfluidic system has 3 parallel channels with the width of 500 μm, the depth of 100 μm, and separated by 100 μm. The cross-linked PDMS was treated by high pressure processing (125^{o}C, 25 min) and dried at a temperature of 80 ^{o}C for 2 hours. Then we placed the PDMS channels on the surface of a culture dish to form enclosed microchannels. The microchannels were incubated with fibronectin (20 μg ml^{-1}, BD) at 37^{o}C for 2 hours, and then blocked with 3% bovine serum albumin (BSA, Sigma) in PBS at 37^{o}C for 1 hour.

Cell Culture All the reagents were purchased from Invitrogen, except addition statements. NIH 3T3 mouse embryonic fibroblast cells (ATCC) and MDCK (Madin-Darby canine kidney, Peking Union Medical College, Tsinghua University) were cultured in high glucose Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2mM L-glutamin and 1% penicillin/streptomycin. Cells were passed by washing in phosphate buffer solution (PBS) and trypsin/EDTA. After centrifugation at 1000 rpm for 2 min, the cell pellet was

re-suspended in DMEM with 10% FBS with suitable cell densities (MDCK: 2×10^7/ml to 3×10^7/ml; NIH 3T3 1×10^6/ml to 2×10^6/ml). Different types of cells were introduced into the channels and cultured in an incubator (Thermo) with 5% CO_2, 50–75% humidity, and at 37 ^oC. After cell attachment, we add 3 ml DMEM with 10% FBS into the culture dish to cover the microchip, then peeled off the PDMS channels with forceps, and then washed with fresh medium gently. The procedure of removing was operated in the medium. Time lapse were performed in a chamber (PeCon) with 5% CO_2 at 37 ^oC, mounted on an inverted microscope (DMI 6000B, Leica Microsystems). Conditioned medium was obtained by isolating medium cultured with NIH 3T3 fibroblasts overnight and mixed with fresh medium in different proportions.

Wounding After cell attachment, cell wounding can be selectively performed in one of the channels before peeling off. We filled cold distilled water into the channel to lyse MDCK cells. The suitable treating time was tested from 10 to 40 minutes. After treating 10 minutes, cells became rounded and lose cell-cell contact. Before removing the PDMS channel, the distilled water in the lysis channel were replaced by DMEM with 10% FBS. Then we added 3 ml DMEM with 10% FBS into the culture dish immediately to cover the whole microchip and peeled the PDMS channel off with forceps gently. Finally, we washed the chip with fresh DMEM with 10% FBS gently to remove detached cells. Most of the injured cells will recover from the damage and re-spread, and less than 5% -10% cells will died and detach from the substrate after washing. After treating 20, 30, and 40 minutes, about 30%, 50%, and 80% of the cells will died in the manipulation of peeling off and washing, and the remnant cells will detach in the process of incubation at random (data not shown). We choose 10 min as the suitable wounding time for its ability to recover and maintain relatively constant cell amount after washing. The procedure of removing was operated in the medium and wounding will not influent cells in the adjacent channel according to the morphology of cells.

Microscopy and Image Live cell images were taken on an inverted Leica DMI 6000B microscope with a photometric CCD camera (Leica DFC350 FX) under the control of LAS AF. Time-lapse images were acquired at 15 min intervals for at least 12 h using 10 × magnifications. E_Cadherin images were acquired using an Olympus FV1000 scanning laser confocal microscope with an X100/ 1.4 NA oil objective. Imaged were processed with Image-Pro Plus.

Annexin V/PI staining We used the Alexa Fluor 488 annexin V/Dead Cell Apoptosis Kit with Alexa Fluor 488 annexin V and PI (Invitrogen) to character cell death using distilled water treatment. Apoptotic cells show green fluorescence, dead cells show red and green fluorescence, and live cells show little or no fluorescence. MDCK cells were lysed with distilled water in wounding chips at various treating time. After peeling off the microchannels, the wounding chips were incubated with Annexin V/PI diluted in DMEM contained 10% FBS for 30 min, and imaged after washing. In SI movie 1, Annexin V/PI diluted in distilled water was added into the microchannel and imaged at 3 min intervals.

Size of a wound After removing the PDMS microchannel, the wounding chip were incubated with DMEM with 10% FBS contained CellTrace calcein, AM (Invitrogen) for 30 min (37 ^oC). Fluorescent images were taken and analyzed using Image-Pro Plus to calculate the area of residual live cells. Each cell stripe (width of 500 μm) possess a fixed length (669.51 μm) were measured as an individual test point.

Visualization of ROS We used CM-H_2DCFDA (5-(and-6)-chloromethyl-2', 7'-dichlorodihydrofluorescein diacetate, acetyl ester, Invitrogen) to visualize the formation of ROS in injured cells. The dye was diluted according to the manufacturer and diluted to a working concentration of 5 μM in DMEM before used. CM-H_2DCFDA was added into the microchannel at different time after injury with distilled water. Time lapse images were acquired at 3 min intervals.

Immunofluoresence Cells were fixed using 3.7 % formaldehyde (37 ^oC, 30min) and treated with 0.1 % Triton X-100

in PBS (10min). After blocked with 10 % goat serum in PBS, cells were incubated with antibody to E_cadherin (1:50, BD) overnight at 4 ^{o}C. Finally Alexa Fluor 488 secondary antibody and Hoechst 33342 were added and incubated for 1 h. Cell adhesion junctions closed to the substrates were captured in this paper.

Quantification of the data To quantify the epithelial collective migration rate and proliferation, phase contrast and fluorescent imaging of a fixed area (3 ×669.51 × 896.55 μm) was taken at 0 and 12 h, and the area of the cell sheets that advanced into the space between two cell stripes and the number of cells was measured using Image-Pro Plus. In all figures, shown is the mean values ± s.e.m. from at least three independent experiments. Student's *t-test* was calculated using Origin. To calculate the ratio ($R_{c/s}$) between epithelial collective migration rate toward cells and that toward free space in various conditions, time-lapse imaging of the two edges of a cell stripe (R or L) with a fixed length (669.51 μm) were performed, and the area of advancement was measured every 15 minutes ($R_{c/s}>1$, represents MDCK move faster toward cells in stripe M; $R_{c/s}<1$, represents MDCK move faster toward free space). MDCK cells located at the edge of stripe L under various conditions were tracked using an Image-Pro Plus particle tracking function manually from at least three experiments. Only the leader cells originally located at the open edge were included in the analysis. In Fig. 4 and SI Fig. 3, the trace did not represent the absolute location; it is a relative position only for indicating the trajectory of cell. We offset the value of X_0 to 0, and subtract X_0 from Xn to obtain a Xn.' (X_0 and Y_0 represent the horizontal and vertical ordinate of the start point, Xn and Yn represent each tracking point, n=1, 2, 3…). We offset the value of Y_0 to a suitable value only for distinguishing each trace away.

Supplementary Movies

These movies were acquired with an inverted microscope.

Time lapse images were acquired at 3 min intervals, and played at 5 frames per second in movie 1 to 3, Magnification, 20x.

Supplementary Movie 1 were the overlay of phase contrast and fluorescence (red represented the PI staining; green represented Annexin V staining).

Supplementary Movie 2 and 3 were taken under phase contrast and fluorescence molds (green represented ROS staining)

Time lapse images were acquired at 15 min intervals, and played at 10 frames per second in movie 4 to 6, Magnification, 10x.

Supplementary Movie 4 and 5 were the overlay of phase contrast and fluorescence (blue represented the nuclei staining by Hoechst 33342).

Supplementary Movie 6 was taken under the phase contrast mold.

References

1. Xingyu Jiang & George M. Whitesides *Proc. Natl. Acad. Sci. USA* 102, 975–978 (2005).