Investigations on T Cell Transmigration in a Human Skin-on-Chip (SoC) Model

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Electronic Supplementary Information (ESI)

Figure S1

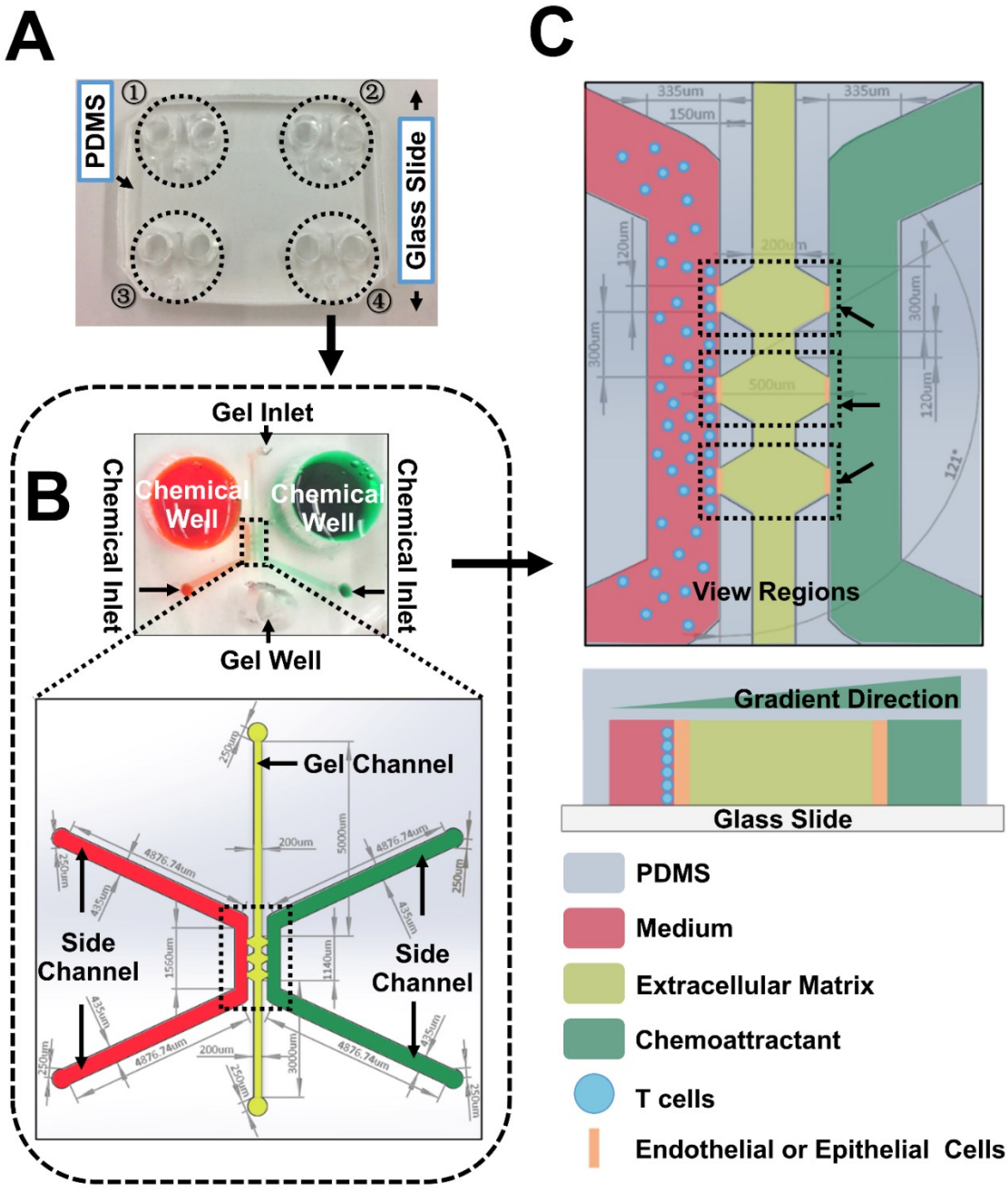

Figure S1. Illustration of the micropillar device and skin-on-chip (SoC) model. (A) A representative image of the dimensions of the micropillar device. The top transparent part is PDMS and the bottom part is a glass slide. The numbered areas circled with black dashed lines show the four independent units on a single device. **(B)** The magnified view of one selected unit from Figure A shows the major structures in the real device (top image) and schematic diagram (bottom image). **(C)** Schematic illustration of the selected area from Figure B (dashed line) to show the magnified view of the SoC model from a top- and side-view, respectively.

Figure S2

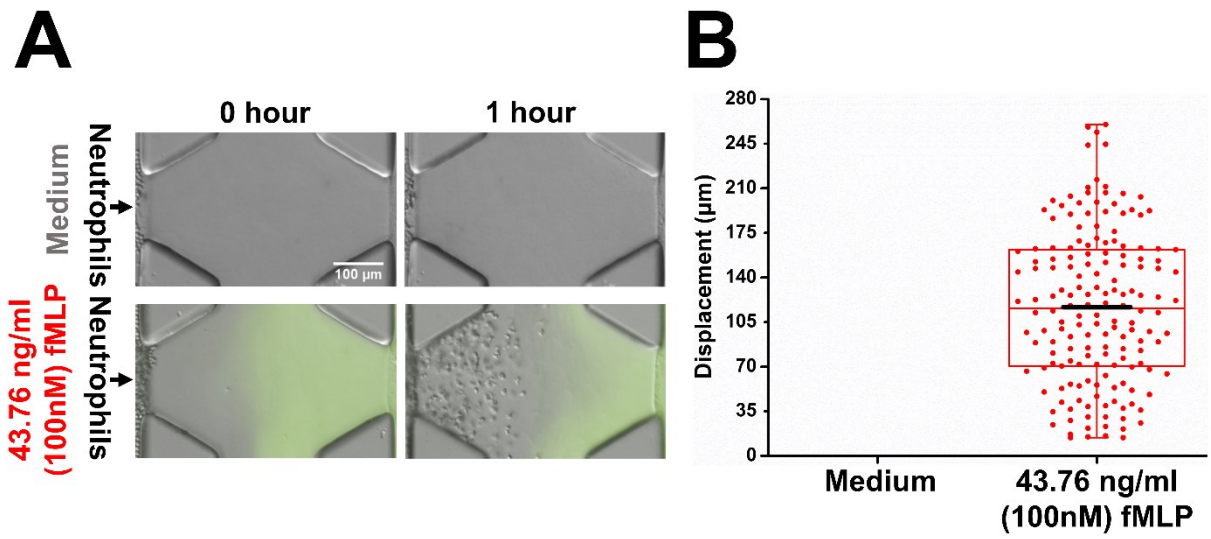

Figure S2. Neutrophil transmigration in the SoC model. Human blood neutrophils were isolated from the peripheral blood samples of healthy donors using a magnetic negative selection kit (EasySep Direct Human Neutrophil Isolation Kit, STEMCELL). The isolated neutrophils were cultured in complete RPMI-1640 culture medium (RPMI-1640 with 1% penicillin-streptomycin and 10% FBS) in an incubator (37 °C; 5% CO_2) prior to cell migration experiments within 8 hours (h) of isolation. Neutrophils were re-suspended in the same culture medium and loaded into the device. Two different experimental groups, fMLP (N-formylmethionyl-leucyl-phenylalanine; 100nM) in complete RPMI-1640 culture medium and medium control, were tested in parallel on each micropillar device following the same experimental protocol and data analysis method for T cells. Each experiment was independently replicated at least three times using separate devices. **(A)** Representative images of neutrophil migration through collagen gel in the medium and fMLP groups in the micropillar device at 0 h and 1 h, respectively (scale bar: 100 µm). The green color in the images indicates the gradient profile and most concentrated area of the fMLP gradient. **(B)** The displacement analysis of neutrophils in the different experimental groups at 1 h from Figure A. The colored box chart shows the total displacement of each human neutrophil in the corresponding experimental groups in Figure A. The top and bottom whiskers show the maximum and minimum values. The box includes the migrated cells within the range from 25% - 75% of total neutrophil cells based on the ranked displacement value. The bold black line in each box indicates the mean displacement value. Note that no human neutrophils migrated through collagen gel in the "Medium" group.

Figure S3

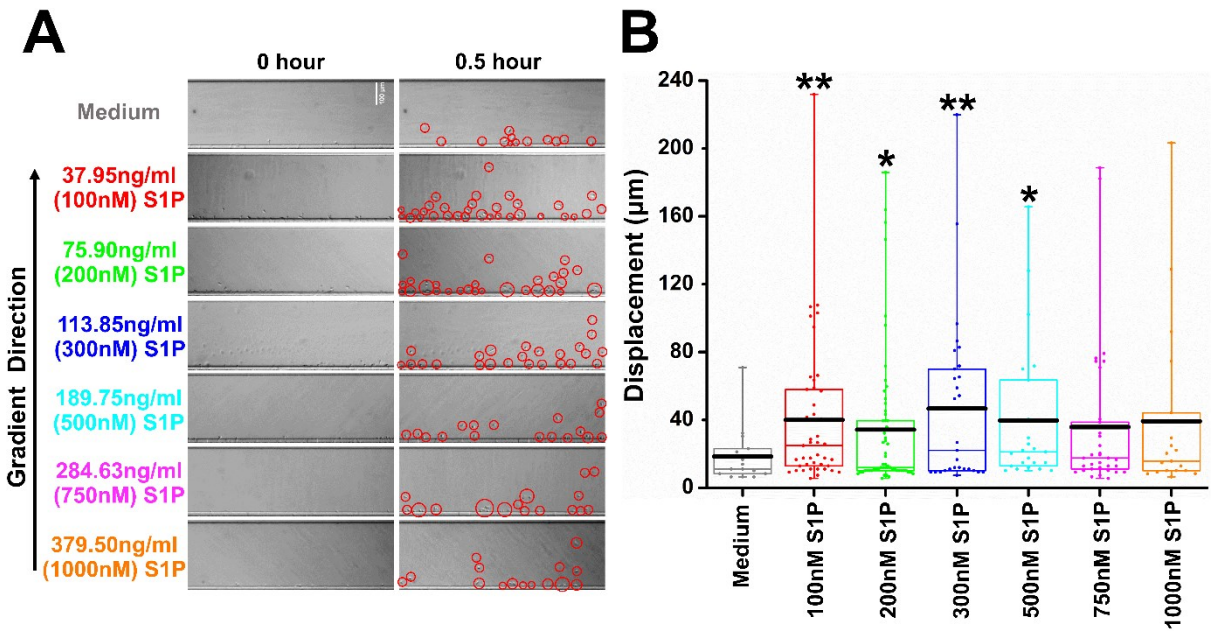

Figure S3. Human T cell migration to S1P gradients in the 2D radial microfluidic device. The activated human peripheral blood T cells (ahPBTs) were prepared as described in the manuscript, and T cells were re-suspended in RPMI-1640 medium (serum/antibiotics-free) when loading into a previously published radial microfluidic device.[1] Seven different experimental groups (i.e., Medium: RPMI-1640 medium and S1P: Sphingosine-1-phosphate (S1P) prepared in the same medium at final concentrations of 100 nM, 200 nM, 300 nM, 500 nM, 750 nM, 1000 nM) were performed in parallel on the same radial microfluidic device, and each experiment was independently replicated at least three times using separate devices. **(A)** Representative images of T cell migration in the medium and the different S1P groups in the radial microfluidic device at 0 h and 0.5 h, respectively (scale bar: 100 μm). The black arrow besides the images indicates the gradient direction of S1P in all the groups except for the medium control. The red circles in the images label all the migrated human T cells in the different groups. **(B)** The displacement analysis of T cells in the different experimental groups at 0.5h from Figure A. The colored box chart shows the total displacement of each cell in the corresponding experimental groups in Figure A. The interpretation of box chart is the same as previously described. The data in different groups were compared using the two sample Student's t-test available in OriginPro, significant difference compared to the "Medium" group was indicated using *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Figure S4

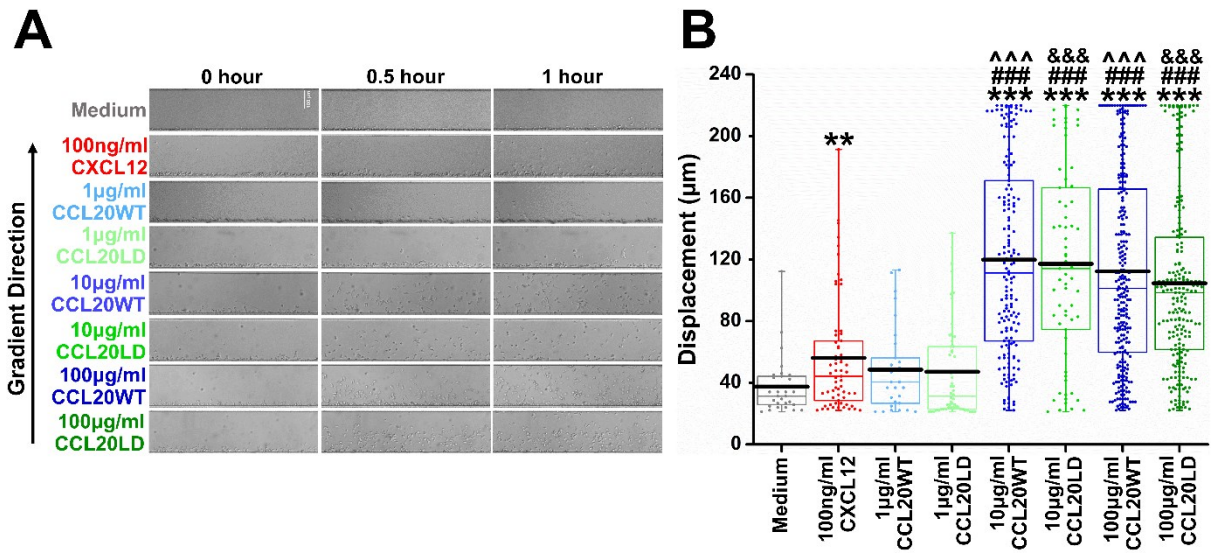

Figure S4. T cell migration to CXCL12, CCL20WT and CCL20LD gradients in the D^3-Chip. The ahPBTs were prepared as described in the manuscript, and T cells were re-suspended in complete RPMI-1640 medium (RPMI-1640 with 1% penicillin-streptomycin and 10% FBS) when loading into a previously designed D^3-Chip.[2, 3] Eight different experimental groups were tested and compared: RPMI-1640 medium (serum/antibiotics-free); C-X-C motif chemokine ligand 12 (CXCL12) in the same medium at 100 ng/mL; C-C motif ligand 20 wild-type (CCL20WT) in the same medium at 1 µg/mL, 10 µg/mL, and 100 µg/mL; CCL20 locked dimer (CCL20LD) prepared in the same medium at 1 µg/mL, 10 µg/mL, and 100 µg/mL. Each experiment was independently replicated at least three times using separate devices. **(A)** Representative images of T cell migration in the different groups in the D^3-Chip at 0 h, 0.5 h and 1 h, respectively (scale bar: 100 µm). The black arrow besides the images indicates the gradient direction in all the groups, with the exception of the medium control. **(B)** The displacement analysis of T cells in the different experimental groups at 1h from Figure A is shown. The colored box chart shows the total displacement of each cell in the corresponding experimental groups in Figure A. The interpretation of box chart is the same as previously described. The data in different groups were compared using the two sample Student's t-test available in OriginPro. Significant difference compared to the "Medium" group was indicated using $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$; Significant difference compared to the "100 ng/mL CXCL12" group was indicated using $^{\#}p < 0.05$, $^{\#\#}p < 0.01$, $^{\#\#\#}p < 0.001$; Significant difference compared to the "1 µg/mL CCL20WT" group was indicated using $^{\wedge}p < 0.05$, $^{\wedge\wedge}p < 0.01$, $^{\wedge\wedge\wedge}p < 0.001$; Significant difference compared to the "1 µg/mL CCL20LD" group was indicated using $^{\&}p < 0.05$, $^{\&\&}p < 0.01$, $^{\&\&\&}p < 0.001$.

Figure S5

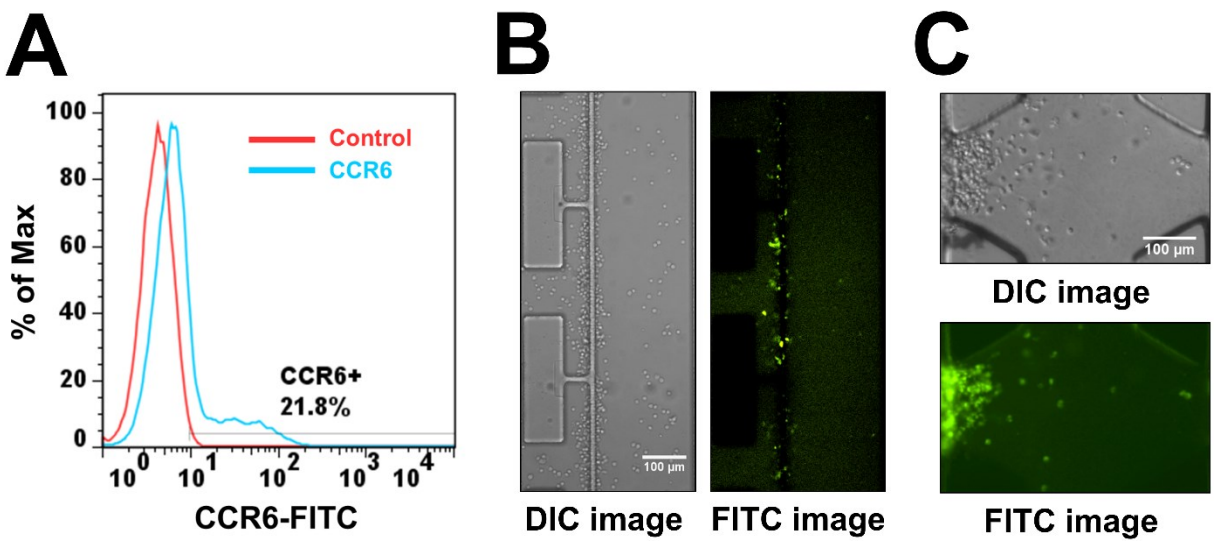

Figure S5. The CCR6 expression of ahPBTs by flow cytometry and on-chip staining in the D^3-Chip and the SoC model. For flow cytometry analysis, ahPBTs were collected and incubated with FITC anti-human CD196 (CCR6) antibody (BioLegend, Catalog# G034E3) at the final concentration of 2 μg/ml in PBS (1×) for 30 minutes at room temperature, then rinsed with PBS (1×) two times. Cells with or without antibody incubation were re-suspended in PBS and divided into different tubes for analysis in a flow cytometer (BD FACSCalibur, ON, Canada). For on-chip staining, chemical solutions were removed from the chip after cell migration experiments and rinsed once with PBS. Cells were fixed in 4% of paraformaldehyde (PFA) for 20 minutes at room temperature followed by another rinse with PBS. The D^3-Chip was incubated with the CCR6 antibody at the final concentration of 2 μg/ml in PBS for 30 minutes in an incubator (37 °C; 5% CO_2), while the micropillar device was incubated with the CCR6 antibody at the final concentration of 10 μg/ml in PBS for 1 hour in an incubator (37 °C; 5% CO_2). The devices were then rinsed once with PBS after incubation before taking images with the fluorescence microscope (Nikon Ti-U). **(A)** Representative data of CCR6 expression of ahPBTs in the flow cytometry analysis extracted from FlowJo software (FlowJo LLC., Ashland, Oregon, USA). **(B)** Representative images to show the CCR6 expression of ahPBTs at the end of 1 h migration experiment in the D^3-Chip. **(C)** Representative images to show the CCR6 expression of ahPBTs at the end of 2 h migration experiment in the micropillar device. DIC image: Differential interference contrast image; FITC image: Fluorescein isothiocyanate image; Scare bar: 100 μm.

References

1. J. Wu, A. Kumar-Kanojia, S. Hombach-Klonisch, T. Klonisch and F. Lin, *Lab on a Chip*, 2018, **18**, 3855-3864.
2. X. Ren, J. Wu, D. Levin, S. Santos, R. L. de Faria, M. Zhang and F. Lin, *Annals of the New York Academy of Sciences*, 2019, **1445**, 52-61.
3. X. Ren, A. Alamri, J. Hipolito, F. Lin and S. K. P. Kung, in *Methods in Enzymology*, Academic Press, 2019.