Electronic Supplementary Material (ESI) for Integrative Biology.

A dual-docking microfluidic cell migration assay (D^2-Chip) for testing neutrophil chemotaxis and the memory effect

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Supplementary Information S1

Mathematical modeling

The model considers the motion of cells as a biased random walk [1]. The bias of cell motion toward the ligand gradient is proportional to the ligand-receptor occupancy difference (*LROD*) across the cell body in the gradient direction, which is determined in the cell's local ligand field at each time step. Ligand-receptor kinetic reactions are evaluated to determine the *LROD*.

$$P_g = a \times LROD + b \quad (1)$$

$$LROD = LRO_{front} - LRO_{back} \quad (2)$$

$$LRO = \frac{c}{c + k_d} \quad (3)$$

where p_g is the biased probability of a cell to move toward the ligand gradient; C is the local ligand concentration; K_d is the equilibrium dissociation constant for ligand-receptor binding. a and b are the linear fitting coefficient. a is determined based on the nearly perfect chemotaxis ($p_g > 0.8$) at the starting position of the docked cell in the 100 nM fMLP gradient. b is set at 0.2 as the basal bias at low *LROD* (*LROD* ~ 0) considering total 5 possible cell migration directions (up, down, left, right or staying at the same spot in the ligand gradient). The 10 nM and 100 nM fMLP gradient profile in the middle microfluidic channel is shown in **Fig. S1A**. The calculated corresponding p_g in a 10 nM fMLP gradient or a 100 nM fMLP gradient is shown in **Fig. S1B**.

The remaining possibility of cell motion toward other possible directions is uniformly distributed. Fixed cell speed is estimated from the experiments for the 10 nM fMLP gradient or the 100 nM fMLP gradient. Then the model is used to simulate cell migration in comparison to the experimental results.

Computer simulations

Cell migration in 2D is simulated using MATLAB based on the above described biased random walk model and the D^2-Chip. The parameters used in the simulation are summarized in **Table S1**. Total 100 cells are simulated for each ligand gradient condition. The cells are initially aligned along the

side of the middle gradient channel by the sink channel (**Fig. S2**). Representative simulation results are shown in **Fig. 5B, Fig. S2** and **SI Movie 3**.

Figure S1

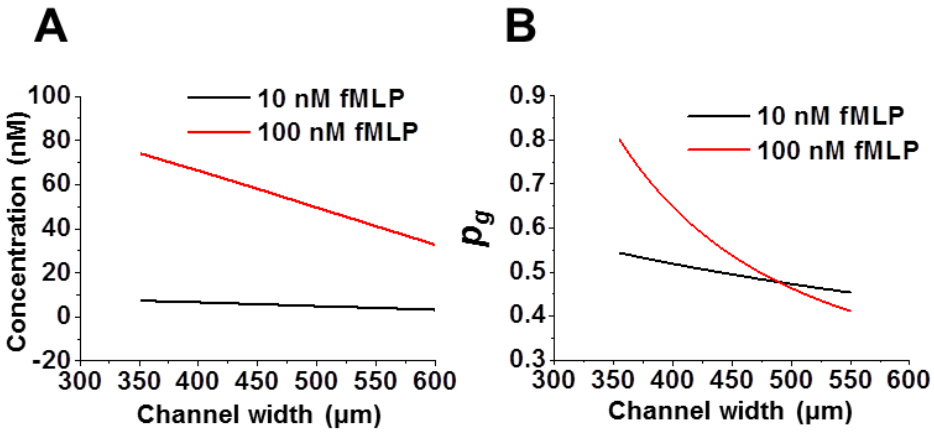

Fig. S1. fMLP gradients (A) and the probability bias (B) in fMLP gradients in the middle channel of the D^2-Chip.

Figure S2

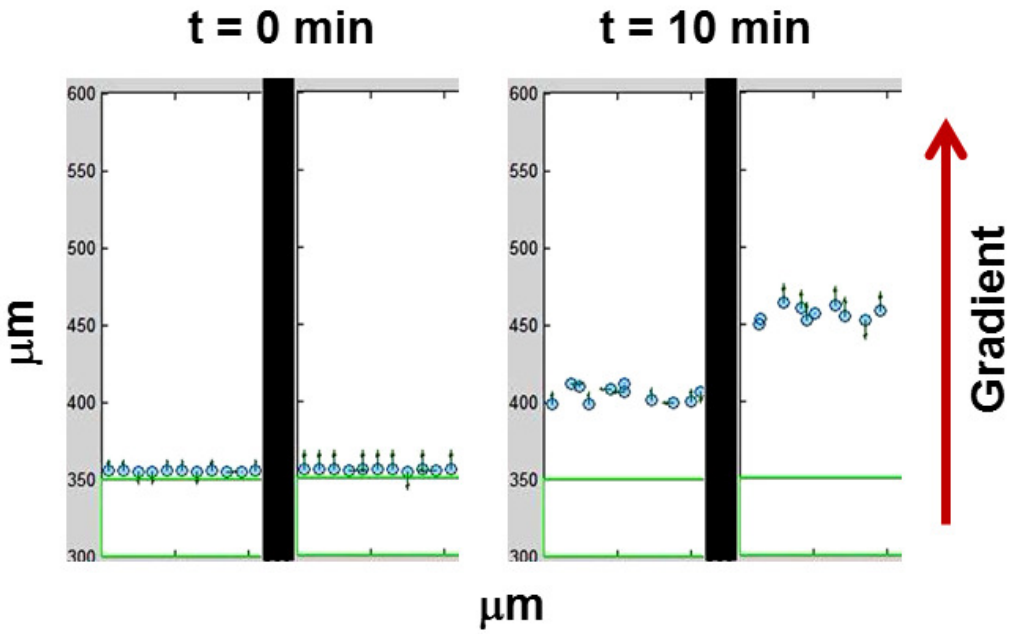

Fig. S2. Cell migration simulation in a fMLP gradient in the middle channel of the D^2-Chip. The left channel is the 10 nM fMLP gradient and the right channel is the 100 nM fMLP gradient.

Table S1.

Parameter	Description	Value
C	ligand concentration	Variable based on the linear fMLP gradient in the D^2-Chip
K_d	equilibrium dissociation constant for ligand-receptor binding	15 nM [2]
p_g	probability bias of migration toward the gradient	Variable based on equation 1
LRO	ligand-receptor occupancy	Variable based on equation 3
LRO_{front}	ligand-receptor occupancy at the front of the cell facing the ligand gradient	Variable based on equation 3
LRO_{back}	ligand-receptor occupancy at the back of the cell	Variable based on equation 3
$LROD$	ligand-receptor occupancy difference between the front and back of the cell	Variable based on equation 2
a	linear fitting parameter in equation 1	48.4
b	linear fitting parameter in equation 1	0.2
d	cell length	10 μm
t	time step	1 step = 2.5 s
T	total simulation time	600 steps = 25 min
v	migration speed	0.8 μm/step for the 100 nM fMLP gradient 0.5 μm/step for the 10 nM fMLP gradient

Transendothelial migration experiment

Human Umbilical Vein Endothelial Cells (HUVEC) was cultured in DME/H medium supplemented with 10% FBS and 1% penicillin/streptomycin at 37ºC in a humidified incubator containing 5% CO_2. For the neutrophil transendothelial migration experiments, HUVEC were first loaded into the source channel of the device and the pressure difference between the source channel and middle gradient channel was adjusted to align HUVEC along the docking barrier in the source channel. After HUVEC are settled in source channel, medium in all the ports were emptied and neutrophils were loaded into the middle gradient channel. Then the fMLP gradient was applied and time-laps microscopy of cell migration experiment was performed. Preliminary results were shown in **Fig. S3A**.

Cell turning number analysis

We performed the cell turning number analysis for the memory effect experiment. Briefly, we measured the total times that a cell makes turns or switches migration direction toward or away from the gradient (**Fig. S3B-C**).

Figure S3

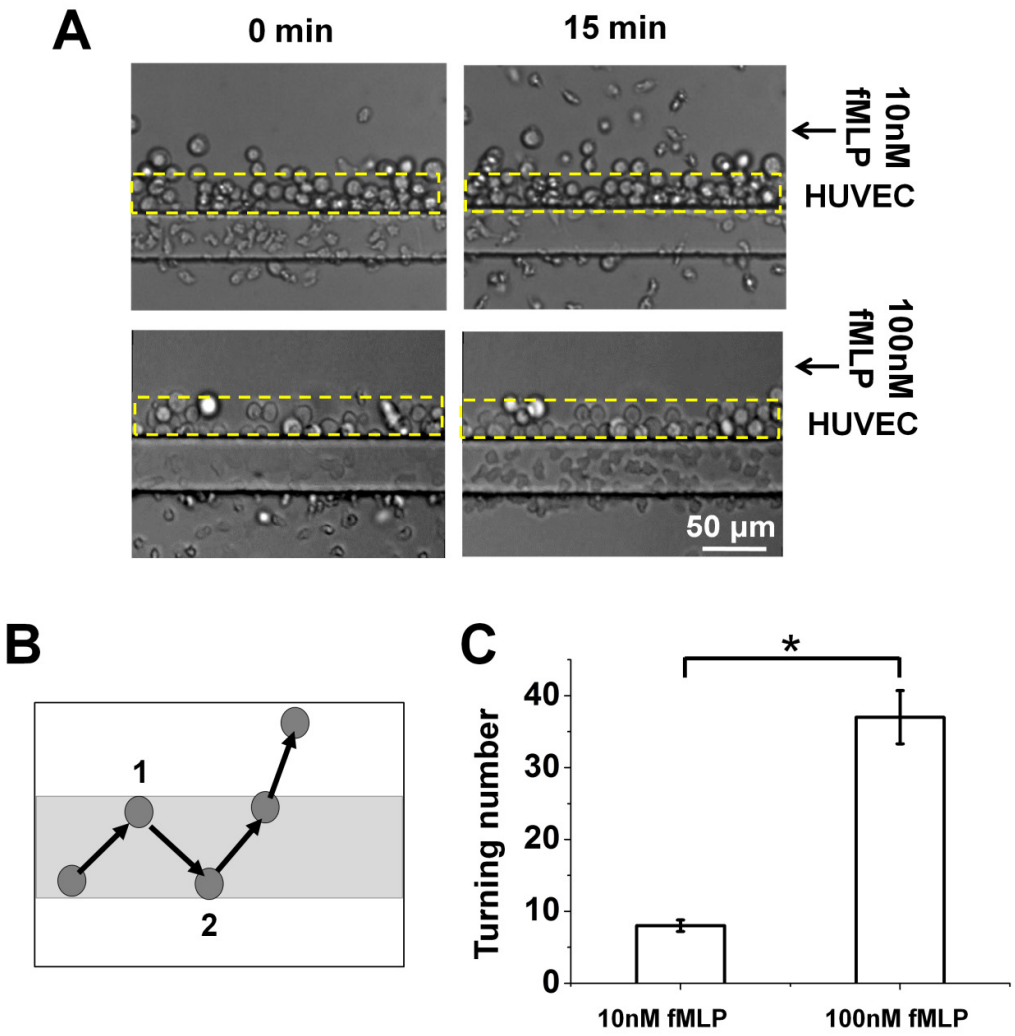

Fig. S3. Transendothelial migration experiment and cell turning number analysis for the cell memory effect using the D^2-Chip. (A) Representative images of neutrophil transmigration experiment through a HUVEC layer in response to 10 nM fMLP or 100 nM fMLP in the source channel at the 0^{th} min and the 15^{th} min; (B) Illustration of the cell turning number analysis; (C) Comparison of the cell turning number in response to 10 nM fMLP or 100 nM fMLP in the source channel.

SI Movie S1. Representative neutrophil chemotaxis in a 10 nM or a 100 nM fMLP gradient in the middle channel of the D^2-Chip.

SI Movie S2. Representative synchronized neutrophil chemotaxis in a 100 nM fMLP gradient but not a 10 nM fMLP gradient in the middle channel of the D^2-Chip.

SI Movie S3. Representative computer simulations of synchronized neutrophil chemotaxis in a 100 nM fMLP gradient but not a 10 nM fMLP gradient in the middle channel of the D^2-Chip based on the biased random walk model.

SI Movie S4. Representative experiment showing memory effect of neutrophil chemotaxis in response to 10 nM fMLP but not 100 nM fMLP in the source channel of the D^2-Chip.

References

1. Codling, E. A., Plank, M. J., Benhamou, S. (2008) Random walk models in biology. Journal of the Royal Society Interface 5, 813-834.
2. Herzmark, P., Campbell, K., Wang, F., Wong, K., El-Samad, H., Groisman, A., Bourne, H. R. (2007) Bound attractant at the leading vs. the trailing edge determines chemotactic prowess. Proceedings of the National Academy of Sciences 104, 13349-13354.