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

Supplementary Information

A microscale, full-thickness, human skin on a chip assay simulating neutrophil responses to skin infection and antibiotic treatment

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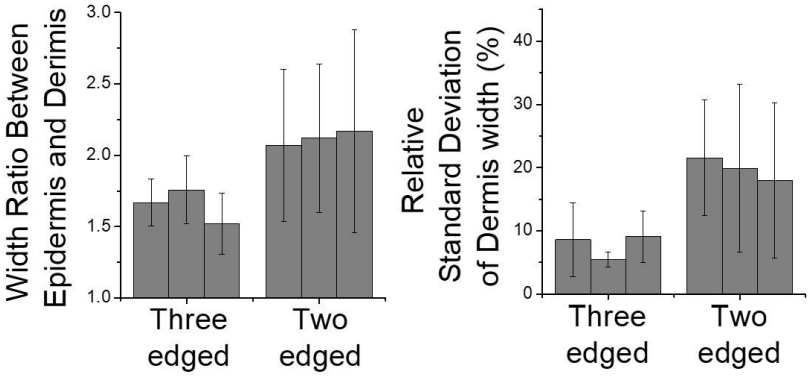

Fig. S1 Geometric parameters of MSTCs depending on the number of micro-biopsy needle edge. Each bar represents the result obtained with an individual needle. n = 3 needles, n = 6 MSTCs per needle.

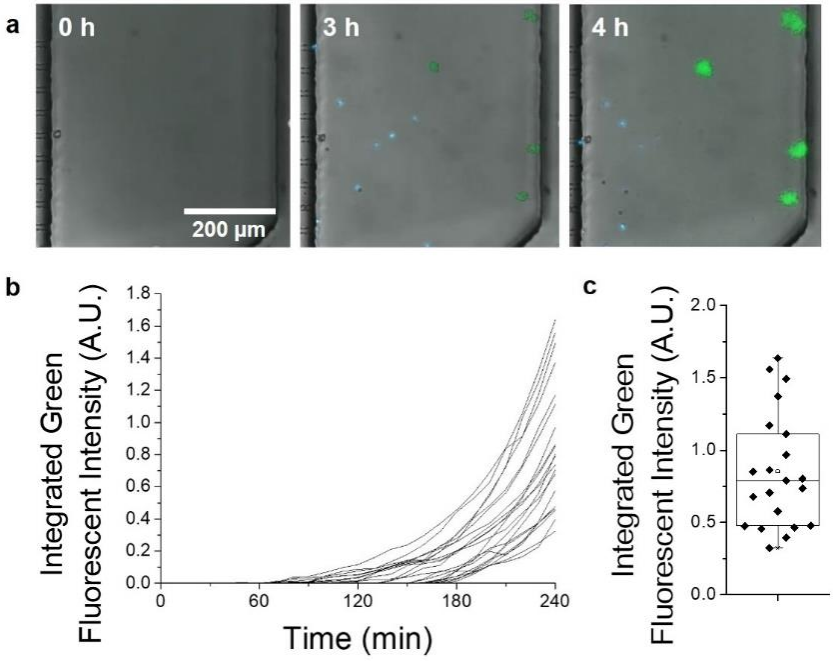

Fig. S2 *S. aureus* growth inside the device. (a) Time-lapse image of *S. aureus* growth. When bacteria inoculated MSTCs are loaded, a few bacteria become detached and grow inside SOC over the course of the experiment. (b) Integrated FITC intensity of each *S. aureus* colony in SOC over time. We only analyzed the colonies which did not come into contact with neutrophils during the experiment. (c) Integrated FITC Intensity of a single *S. aureus* colony at 4 hours. n = 7 experiments, n = 12 devices, n = 21 colonies.

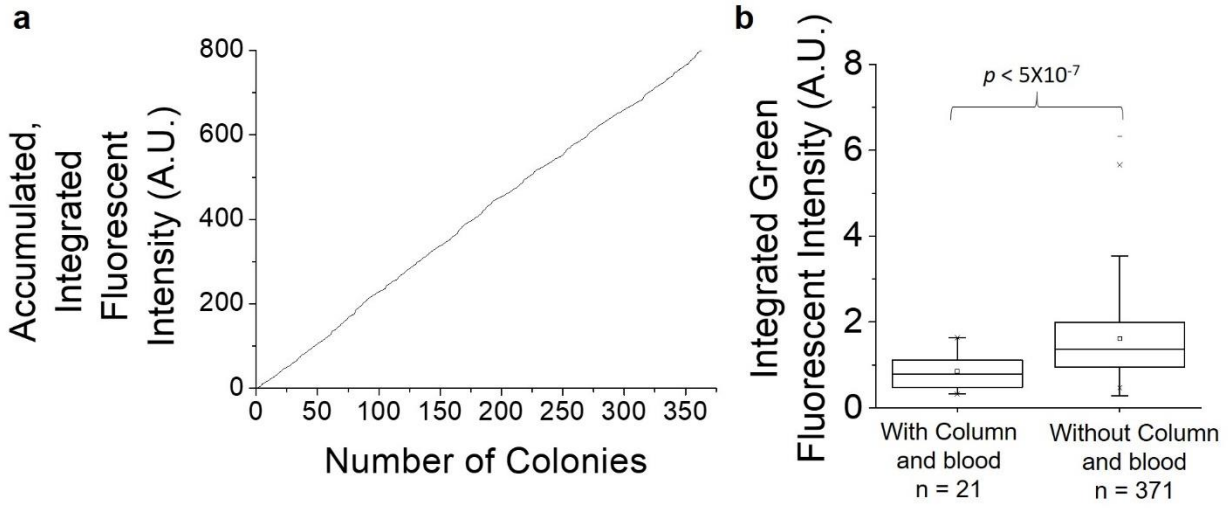

Fig. S3 The relationship between integrated fluorescent intensity and the number of colonies. (a) Accumulated, integrated fluorescent intensity linearly increases with the number of colonies. Subcultured *S. aureus* suspension is purified to remove the tetracycline and diluted by IMDM with 10% FBS. The column loading channel is filled with this bacterial suspension. After 4 hour-incubation at 37°C, we quantify the integrated fluorescent intensity of each colony and plot the accumulated, integrated fluorescent intensity compared to the number of colonies. (b) The fluorescent intensity of the single colony varies depending on the culture condition. Integrated fluorescent intensity of the single colony after 4 hour-incubation shows a significant difference depending on whether the skin column and diluted whole blood are loaded into the channel together. To quantify the number of bacteria on the column, we use the value while they are loaded with the column and blood (Fig. S2c).

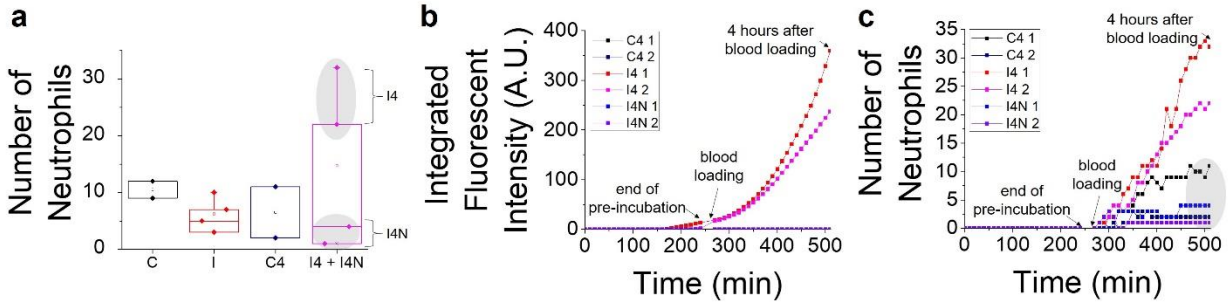

Fig. S4 Some MSTCs have no *S. aureus* after soaking in the bacterial suspension at extremely low concentrations. (a) Number of migrated neutrophils 4-hour after blood loading. n = 12 MSTCs. (b) Integrated green fluorescent intensity over time. (c) Number of migrated neutrophils over time. Among four pre-incubated, bacterial-suspension-soaked columns, only two columns (*I4*) show bacteria growth, and high neutrophil migration. Another two samples (*I4N*) show no bacteria growth even after 12 hours (data is not shown), resulting in a no difference in neutrophil migration from its control (shaded area in Fig. S4c). Thus, for the purpose of the study, two *I4N* are excluded from the data analysis, shown in Fig. 5. Such columns were observed only in the amplified condition of the second trial.

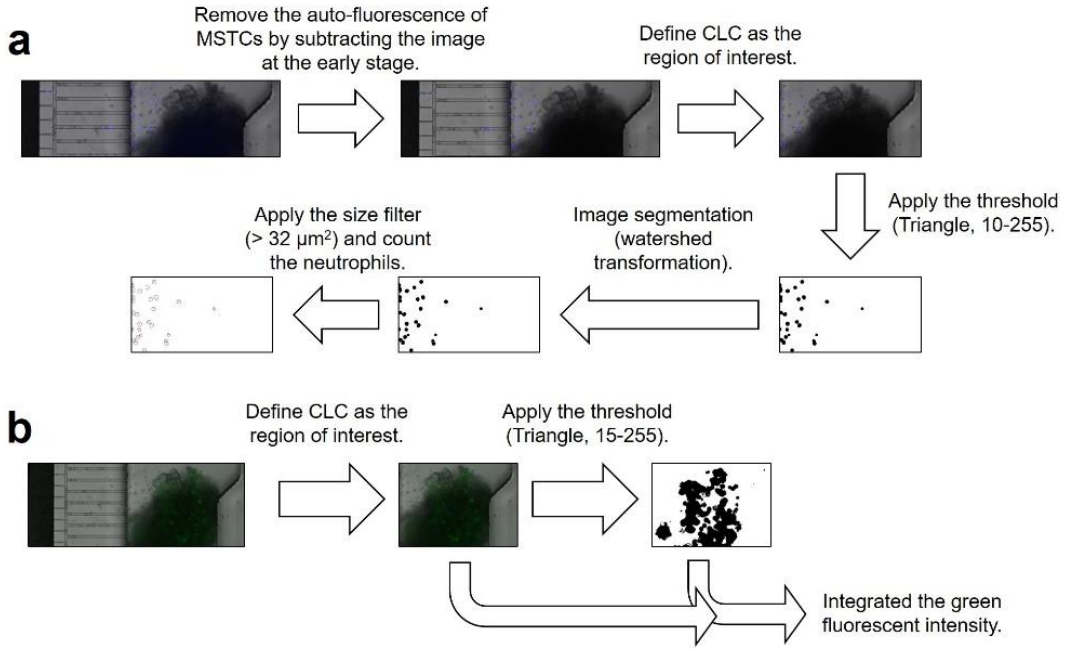

Fig. S5 Quantitative analysis using an ImageJ. (a) Neutrophil counting. (b) Integration of the green fluorescent intensity. Bright field image is overlapped for a better understanding of processes.

Effect of bacteria loading method on neutrophil migration

We conducted the following experiment to test the effect of bacterial distribution. For this experiment, we harvested MSTCs from the fresh skin sample. We inoculated skin columns with *S. aureus* using two different methods: the soaking method described in the method section; and an injection method which allows for a spatial distribution. For the injection method, we injected ~ 1 ml of bacterial suspension into the skin using a 27-gauge needle and syringe. The needle was inserted into the skin tangentially to generate a bacteria distribution in epidermis and dermis. After injection, skin columns were collected in the infiltrated region where the bacterial suspension was injected. Collected columns were separately stored in 1 ml normal saline and washed by BHI and IMDM with 10 % FBS as described in the method section.

We found that neutrophils showed similar migratory behavior regardless of infection mimicking methods (Fig. S6). Thus, we utilized the soaking methods throughout the study.

In a parallel experiment, we filled the column loading channel with diluted bacterial suspension in the absence of a skin column. The bacterial suspension was loaded using hydrostatic pressure. Compared to the bacterial inoculated skin columns, fewer bacteria are required to induce the same extent of immune response when *S. aureus* was loaded without the skin column (See the dotted line and arrow in Fig. S6). We have two hypotheses to explain this result. First is the location of bacteria. When we only load the bacteria, bacteria settle down and grow at the bottom of the channel where the migration channel locates. On the other hand, when bacteria inoculated skin column is loaded, bacteria are distributed over the height of the channel and not confined to the bottom of the channel. When all bacteria are located at the

bottom of the channel, they could generate a chemoattractant gradient more rapidly. Note that there is a higher chance that neutrophils encounter the bacteria right after passing through the migration channel in this case. The second hypothesis is the role of the skin tissue. The skin tissue might suppress the neutrophil's migration as it interacts with the bacteria.

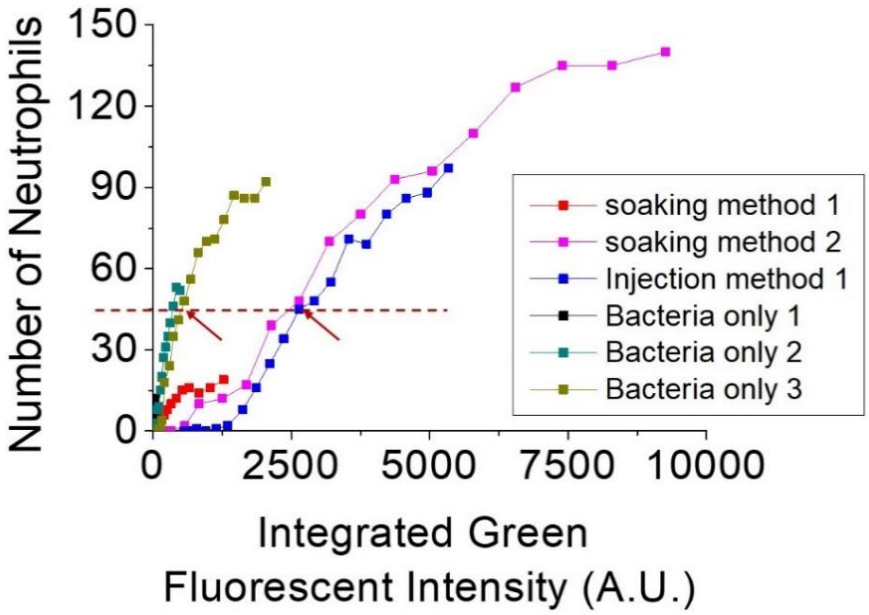

Fig. S6 Neutrophil migratory behavior with various bacteria loading methods. The plot shows the number of migrated neutrophils as a function of integrated green fluorescent intensity, which represents the number of bacteria in the channel. The plot shows the data over 3 hour-observation.

Figure	Data sets	Null hypothesis	Alternative hypothesis
Fig. 5e	C and I	C = I	C < I
	C4 and I4	C4 = C4	C4 < I4
Fig. 6e, h	C and I	C = I	C < I
	C and CP	C = CP	C < CP, or C > CP
	I and IP	I = IP	I > IP
	C and IP	I = IP	C < IP
	CP and IP	CP = IP	C < IP
	CP and I	CP = I	CP < I

Table S1. The null and alternative hypothesis for statistical analysis (*t*-test). Equal variation is not assumed.

T cell experiment

In order to validate that T cells do not migrate through the RBC filter in our setting, we isolated T cells by immunomagnetic negative selection. A blood sample was collected in the heparin-coated vacuum tube, and T cells were isolated using EasySep™ Direct Human T Cell Isolation Kit (STEMCELL Technology, Canada) by following the protocol provided by the vendor. Isolated T cells were centrifuged, re-suspended in IMDM with 10 % FBS at 2.55 X 10^6/ml, and stained with Hoechst 33342 at 33 μM. T cells were stained in the same way as the whole blood case. Isolated T cell concentration is higher than the T cell concentration of diluted whole blood to ensure that there are enough T cells near the migration channel. Bacteria inoculated MSTC that were harvested from the frozen/thawed skin sample were loaded into the SOC, and then the T cell suspension was loaded into the blood loading channel using hydrostatic pressure. T cell suspension flowed until we observed the flowing cells through the outlet of the blood loading channel. Video S1 shows that none of the isolated T cells passes through the RBC filter to migrate towards bacteria growing in the column loading channel.

Video S1. The effectiveness of RBC filter to block the T cell migration.