

Microfluidic capture device for simple, cell surface marker-based quantification of senescent CD8+ T cells

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Resistance channels and corresponding flow rates, flow speeds, and shear stress

Resistance channel length [mm]	Flow rate [$\mu\text{l/min}$]	Mean flow speed in capture chamber [mm/s]	Shear stress [dyne/cm^2]
15	5.6	1.66	4.0
30	2.8	0.83	2.0
45	1.9	0.55	1.3
60	1.4	0.42	1.0

Table S1. Resistance channels and corresponding flow rates, flow speeds, and shear stress

With pressure driven flows, the fluidic resistance determines the flow rate and speed, and the corresponding shear stress in the capture chamber. Along with the resistance channel length, these values are summarized in Table S1. Fluidic resistances were calculated with the following formulas.

$$R \approx \frac{12}{1-0.63\left(\frac{h}{w}\right)} \eta L \frac{1}{h^3 w} \quad \cdots \text{Rectangular channel } (w > h).$$

$$R = 28.4 \eta L \frac{1}{w^4} \quad \cdots \text{Square channel } (w = h).$$

The values were calculated with the viscosity, $\mu = 1.00 \text{ mPa}\cdot\text{s}$, assuming the sample viscosity was similar to that of water. The resistance channel has a cross-sectional dimension of $25 \mu\text{m} \times 25 \mu\text{m}$, which is much smaller compared to that of the capture chamber. The total fluidic resistances of flow channels can be approximated as the fluidic resistance of the resistance channels. For the flow rate calculation, a pressure difference of 1 bar was assumed.

Shear stress was calculated with the following formula based on the calculated flow rate.

$$\tau = \frac{6\mu Q}{h^2 w}$$

Determination of PEG/biotin-PEG ratio

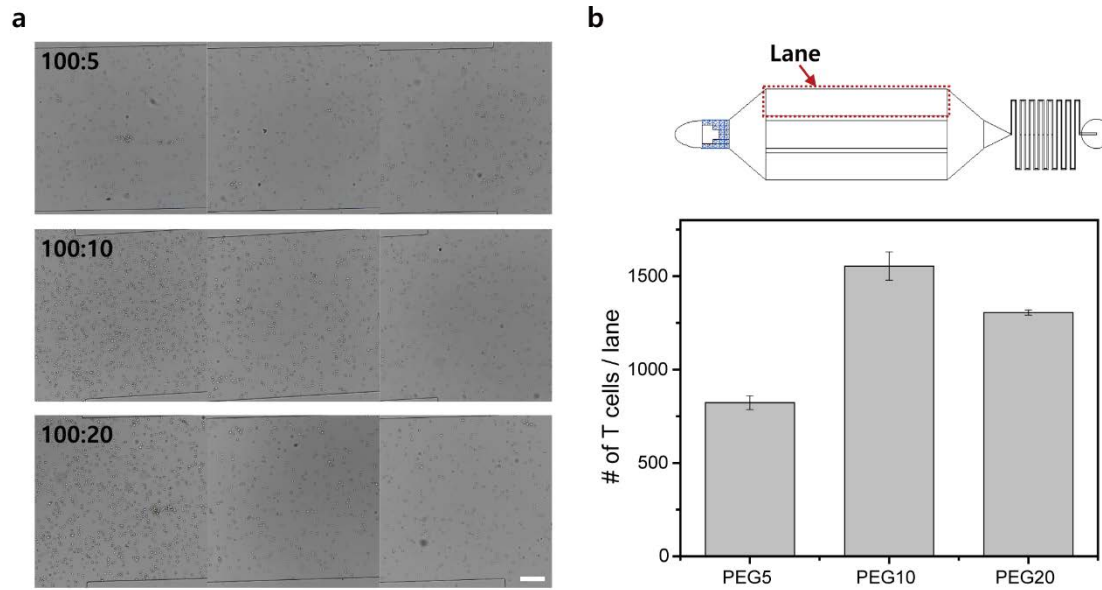


Figure S1. Determination of PEG/biotin-PEG ratio. (a)T cells capture on substrates with varying PEG:biotin-PEG ratio. The scale bar is 100 μ m. **(b)** Number of captured T cells per lane. N=3

The ratio of PEG and biotin-PEG determines the density of the binding sites for the neutravidin, therefore, it influences the cell capture efficiency. Surfaces with three different PEG:biotin-PEG ratios (100:5, 100:10, and 100:20) were prepared to determine a sufficient biotin-PEG density. Isolated WBCs were injected in the capture chambers for 5 minutes and incubated for 15 minutes after immobilizing anti-CD8 antibodies (Fig. 1c step ④). After the capture and washing step (Fig. 1c step ⑤), the number of T cells was counted for the substrates with varying PEG:biotin-PEG ratio (Fig. SI 1a and 1b). The number of captured T cells was almost twice as larger in the case of 100:10 ratio compared to 100:5 ratio, while the number decreased slightly when the ratio was increased to 100:20. The results indicate that the PEG:biotin-PEG ratio of 100:10 is sufficient for the T cell capture. For all the subsequent experimental conditions, the PEG:biotin-PEG ratio of 100:10 was used.

Neutravidin and biotin-FITC binding kinetics

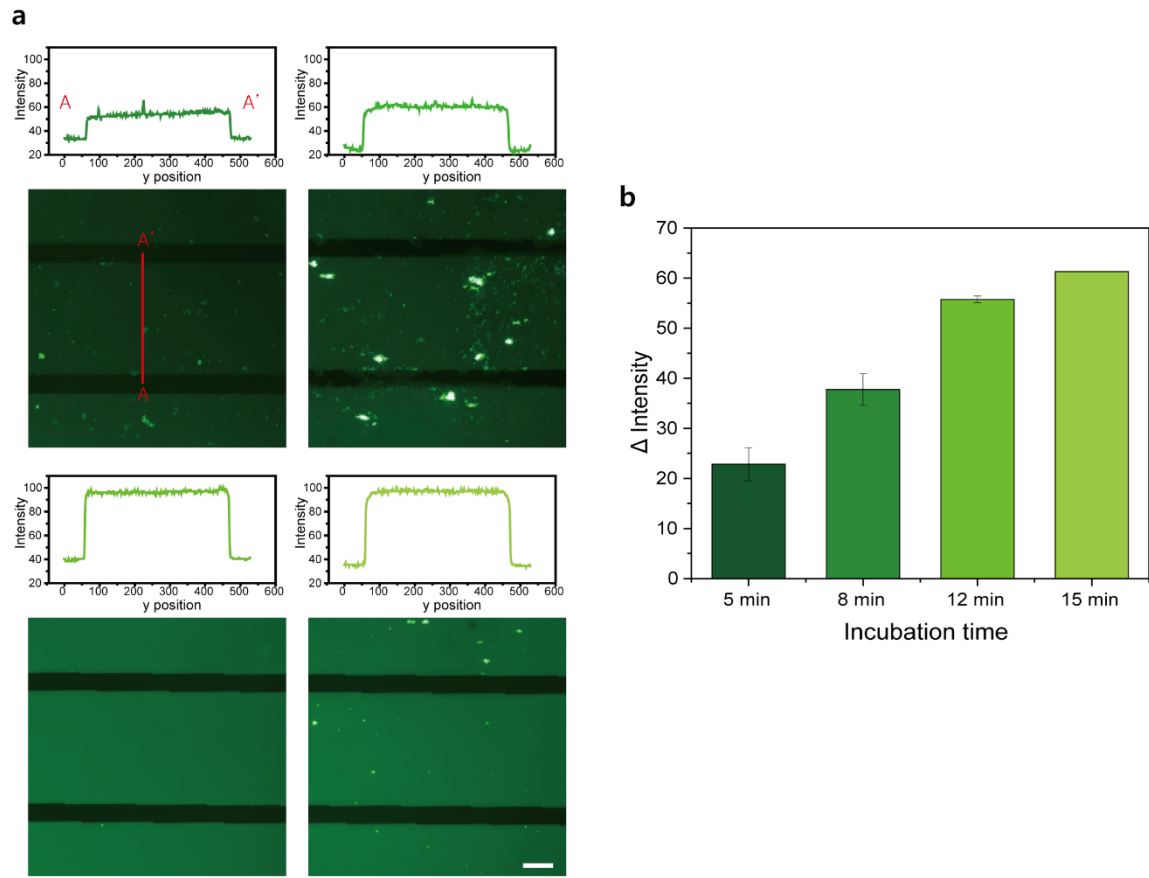


Figure S2. Binding biotin-FITC with varying incubation time. (a) FITC intensity profile depending on the incubation time. The scale bar is 200 μ m. (b) FITC intensity in the chamber depending on the incubation time.

As a relatively low-concentration biotinylated antibody was used, a sufficiently long incubation time was needed to maximize the antibody binding to the neutravidin layer. To assess the optimal incubation time, the fluorescence intensity was measured using FITC-biotin (53608, Sigma-Aldrich) (Fig. S2). FITC-biotin solution was injected and kept in the chamber for 5, 8, 12, and 15 min for biotin-neutravidin binding. The intensity of FITC was measured after washing the chamber with DPBS.

CD8⁺CD57⁺/CD8⁺ ratio of T cells captured with various shear stress conditions

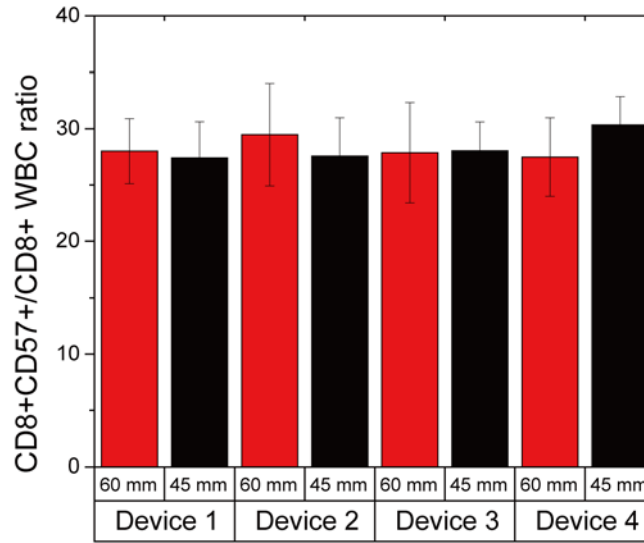


Figure S3. CD8⁺CD57⁺/CD8⁺ ratio of T cells captured with various shear stress conditions.

The capture condition should not affect the measurements of the CD8⁺CD57⁺/CD8⁺ ratio. Since the cell capture is based on the CD8 binding to its antibody, the amount of CD8 on the cell surface can influence the chance of capture. To check whether this is true in the capture condition we used, CD8⁺CD57⁺/CD8⁺ ratios were measured in capture chambers with 60 mm and 45 mm resistance channels. The samples used for the experiments in the SI Fig 3 were collected from the same donor corresponding to the black dots in Fig. 3B, where the CD8⁺CD57⁺/CD8⁺ ratio is ~30%. All measurements give similar values regardless of the shear stress.

Capture efficiency using whole blood depending on the dilution factor

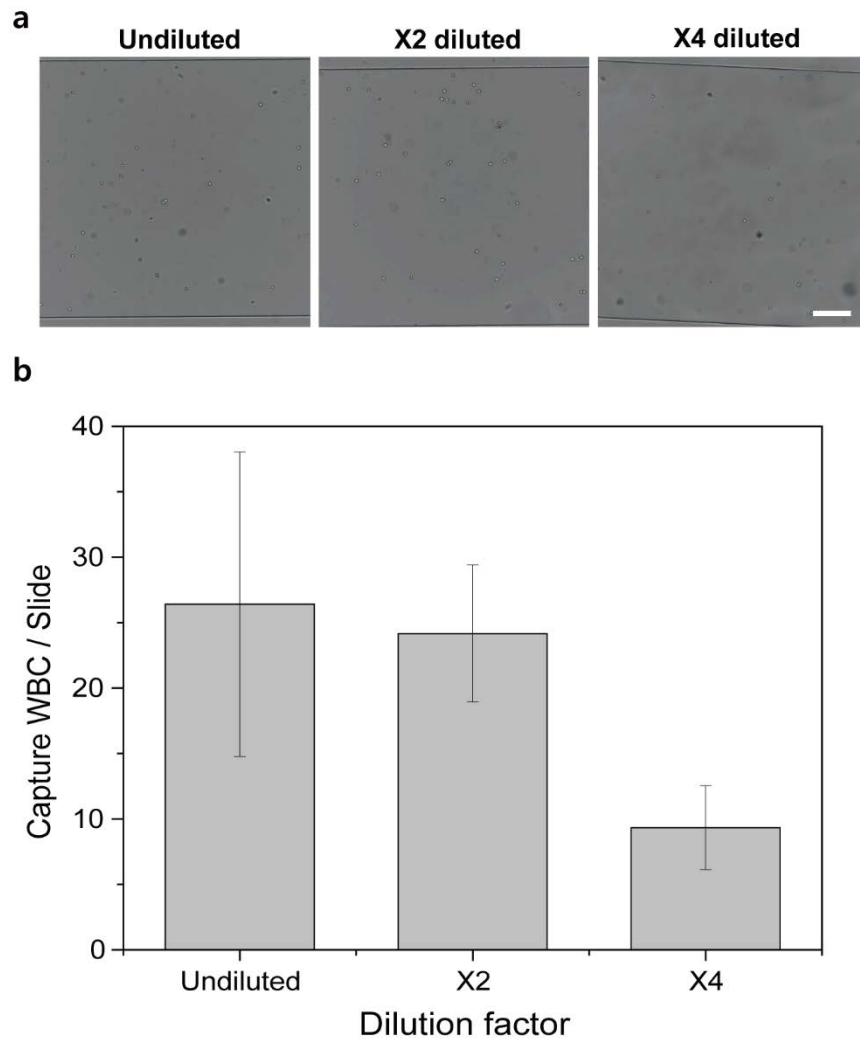


Figure S4. Capture efficiency depending on the dilution of the blood sample. (a) Images of captured T cells. The scale bar is 100 μm . (b) Number of captured T cells depending on the dilution factor.

Whole blood was diluted with DPBS and compared the number of WBCs captured with undiluted blood. Fluid flow conditions were the same as the condition for capture with isolated WBCs (flowing 5 min + incubation 15 min). The capture yield was significantly reduced compared to the capture with isolated WBC.

CD3⁺ T cell capture

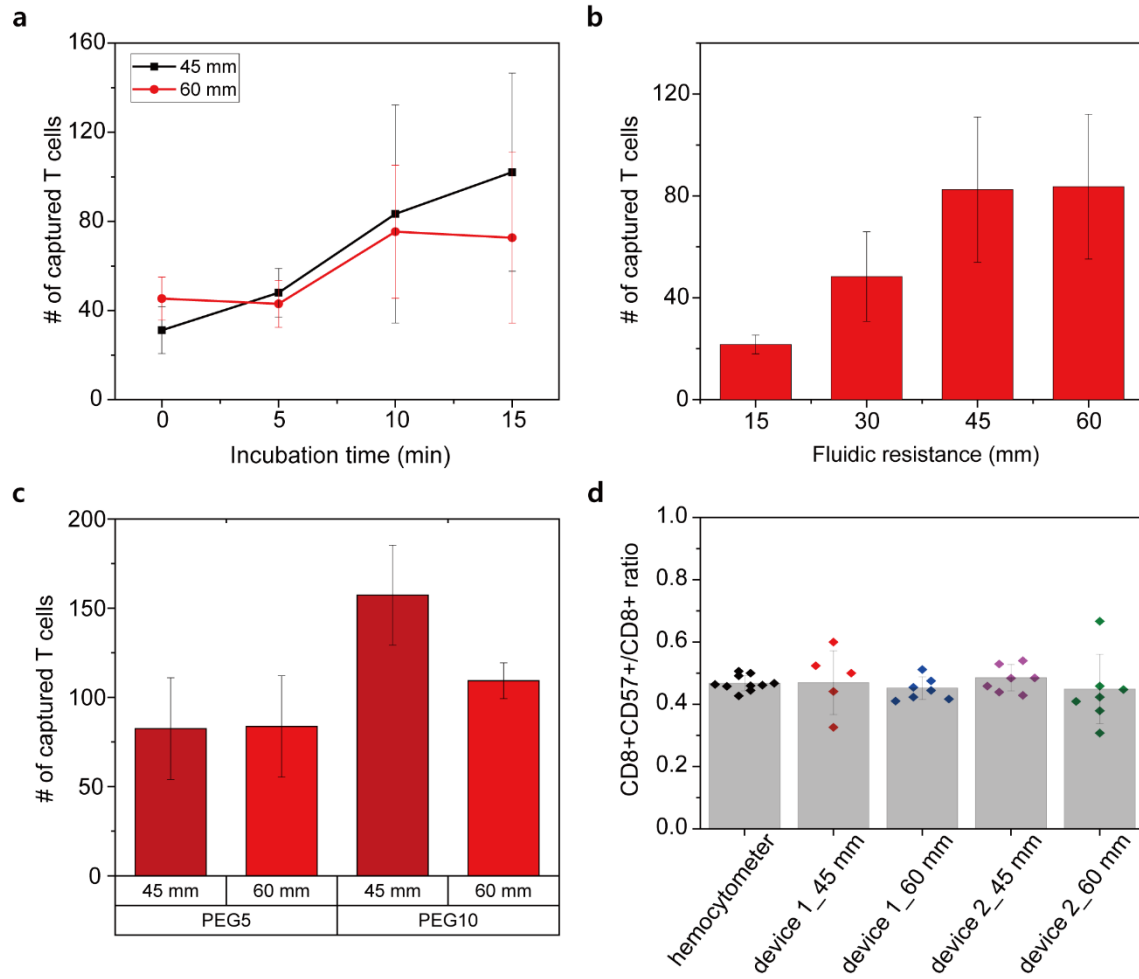


Figure S5. Optimization of CD3⁺ T cell capture. (a) Capture efficiency depending on incubation time. (b) Capture efficiency depending on fluidic resistance. (c) Capture efficiency depending on Biotin-PEG proportion and fluidic resistance. (d) CD8⁺CD57⁺/CD8⁺ ratio depending on capture conditions.

The capture efficiency using the CD3 conjugated antibody was confirmed. In general, the capture efficiency was not as good as the CD8-based capture. First, the incubation time required for capture was checked (Fig. S5a). As the incubation time increased, the amount of WBC captured increased, and it was confirmed that it increased significantly with >10 min. Using 10 min as incubation time, fluid resistance is varied for optimal capture (Fig. S5b). The fluidic resistances with 45 mm and 60 mm resistance channels resulted in good capture conditions. Finally, the biotin and biotin-PEG ratio was tested. (Fig. S5c). In addition, the

CD8⁺CD57⁺/CD8⁺ ratio was compared with the results from the hemocytometer (Fig. S5d). These results suggest that the capture using CD3⁺ also gives the same CD8⁺CD57⁺/CD8⁺ ratio that CD3 can also be a target for capturing T cells.