Electronic Supplementary Material (ESI) for Analyst.

Microfluidic compartments with sensing microbeads for dynamic monitoring of cytokine and exosome release from single cells

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Supporting information

Reconfigurable microfluidic device containing picolitere-compartment arrays

In this paper, we utilized reconfigurable microfluidic devices containing control layer as well as microstructured flow layer to create subpicolitere-compartments isolating single cells with sensing microbeads for detecting cell secreting molecules with enhanced sensitivity and reduced analysis time. Using this reconfigurable microfluidic device, we patterned the APTES-GA treated substrates with cell capture moieties (anti-CD4 Abs for T cell capture and collagen I for HepG2 cell capture) to confine the site for cell attachment (FIG. S-1(a)). Cell capture moieties were covalently immobilized on the surface through reactions with APTES and subsequent activation with glutaraldehyde, with the same pattern as that of chambers on the reversible PDMS membrane. The rest of surface was blocked with 1% BSA to prevent nonspecific binding of cells or proteins. The presence of cell capture moieties patterns on surface was verified using PE-labelled secondary antibodies (Alexa 546 donkey anti-mouse IgG, Life

Technologies, Inc.). FIG. S-1(b) illustrates that the pattern of anti-CD4 Ab surface is almost same as that of compartments shown in FIG. 2.

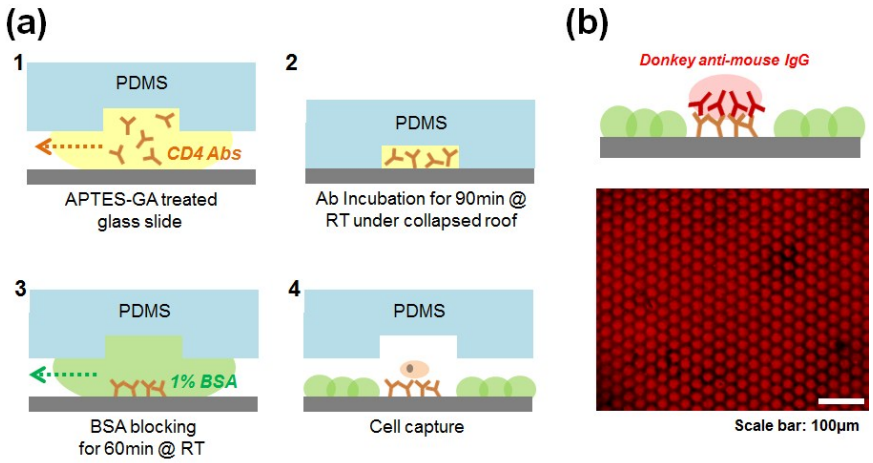

FIG. S-1. Surface patterning with cell capture moieties using micropatterned reconfigurable microfluidic devices. (a) Scheme of preparing anti-CD4 Ab patterned substrates. Anti-CD4 Abs were coated on APTES-GA treated glass slide, showing the same pattern as that of chamber arrays on the reversible membrane. (b) Confirmation of the presence of anti-CD4 Ab on surface using PE-labelled secondary antibodies. Drawing and fluorescent microscopic image showing PE-labelled secondary antibodies bound to patterned anti-CD4 antibodies on surface.

Viability/Cytotoxicity test

The viability of T cells encapsulated with sensing microbeads and detection antibodies inside the chamber was determined using LIVE/DEAD assay (Molecular Probes, Eugene, OR). FIG. S-2 demonstrates that most cells were alive during measurement, and cell viability ranges from 98.8 ± 0.7% (t = 0 hr) to 93.8 ± 2.1% at the end of the experiment (t = 6 h). This suggests that heterogeneity in IFNγ secretion may due to intrinsic differences in cellular functionality, not due to cell death.

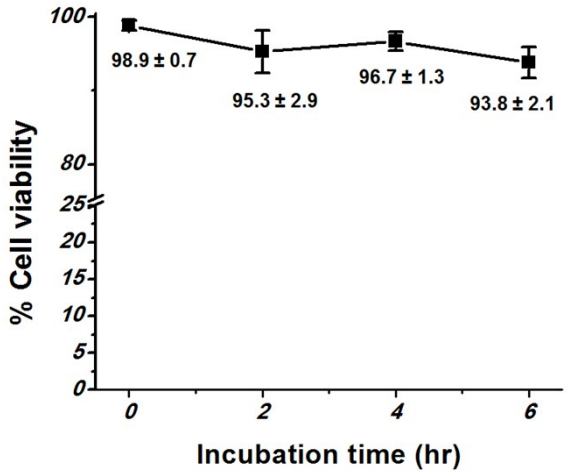

FIG. S-2. Cell viability during measurement.

Binding capacity of antibody-microbeads

The binding capacity of antibody-micobeads was determined by ELISA method and subsequently confirmed using fluorescence based sandwich immunoassay. For the ELISA experiment, we prepared two types of antibody-conjugated microbeads, IFN-γ sensing microbead and TNF-α sensing microbead as a negative control (concentration = 8.5×10^3 beads mL^{-1}) as illustrated in FIG. S-3(a). TNF-α sensing microbeads were prepared by conjugating streptavidin-microbeads (0.1 mg) with biotinylated anti-TNFα antibodies (0.05 mg mL^{-1} in PBS, R&D systems, Minneapolis, MN) for 2 h at RT or overnight at 4°C. Both IFN-γ sensing beads and TNF-α sensing beads were blocked with 1% BSA for 30 min at RT. And then, both sensing beads were challenged with 1 ng mL^{-1} of recombinant human IFN-γ for 3 h at 37°C. The supernatants from both samples were collected and analyzed for IFN-γ level using ELISA (R&D systems, Minneapolis, MN). The IFN-γ level of IFN-γ sensing bead sample was determined to be 861.6 ± 38.5 pg mL^{-1} and that of TNF-α sensing bead sample was 1014.9 ± 41.5 pg mL^{-1}.

Therefore, based on this ELISA data, we could determine the binding capacity of IFN-γ sensing beads to be $\frac{(1014.9 - 861)}{8.5 \times 10^3} =$ 0.0175 pg per bead.

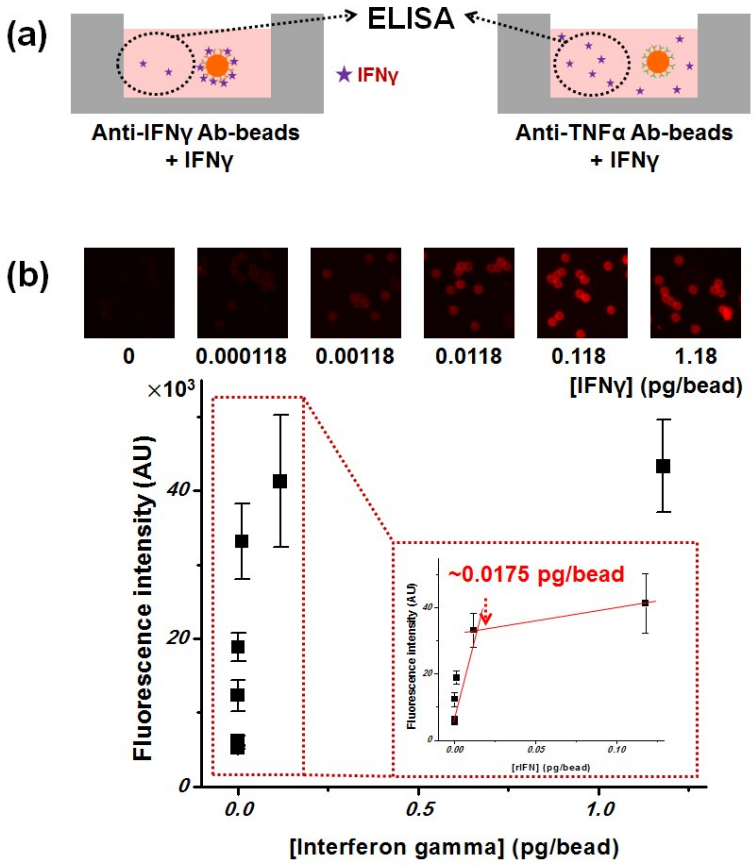

FIG. S-3. Binding capacity of antibody-microbeads determined by ELISA and sandwich assay. (a) Drawing showing ELISA method. (b) Fluorescence intensities of anti-IFN-γ cAb-beads after incubating with different concentrations of recombinant human IFN-γ (expressed as the amount of IFN-γ divided by number of beads) and PE-labelled anti-IFN-γ dAb (conc. = 5 μg mL^{-1}) for 60 min.

The response of IFN-γ sensing beads to varying IFN-γ concentrations was measured using fluorescence-based sandwich assay as shown in FIG. S-3(b). The concentration of IFN-γ is

expressed as the amount of IFN-γ divided by number of beads. For instance, the concentration would be 1.18 pg per bead if 8.5×10^3 beads mL^{-1} of microbeads were challenged with 10 ng mL^{-1} of IFN-γ. The fluorescent signal reached the point of saturation when the IFN-γ concentration was between 0.0118 and 0.118 pg per bead, which is comparable to the value of 0.0175 pg per bead determined with ELISA experiment.

Relationship between signal and number of microbeads

The number of microbeads inside each sensing compartment follows the Poisson distribution, in which the probability $P(X = x)$ of having x microbeads per compartment is given as follows:

$$P(X = x) = \frac{e^{-\lambda}\lambda^x}{x!}$$

where λ is the mean number of beads in the volume of each compartment. In our system, we have 0.56 beads per 20-pL compartment (λ = 0.56) on average, since the concentration of beads we prepared for sensing is 2.8×10^7 particles mL^{-1}. Consequently, we can estimate the probability of co-encapsulation of single cells and x microbeads per compartment on the assumption that the capturing of cells and beads occurs independently. The probability of finding single cell and x beads per compartment is

$$P(single\ cell, X = x) = P(single\ cell) \times \frac{e^{-\lambda}\lambda^x}{x!}$$

where P(single cell) is the probability of having single cell inside each compartment, which is experimentally determined to be 0.45 in this system. Therefore, co-encapsulation efficiency is 0.45 × 0.3199 = 0.144, which means that only 14.4% of chambers will contain single bead and

single cell. On the other hand, the probability of having single cell and multibeads (at least one bead) inside a comparment can be obtained as follows:

$$P(single\ cell, multibeads) = P(single\ cell) \times (1 - e^{-\lambda})$$

As a result, co-encapsulation efficiency is 0.45 × 0.4288 = 0.193, indicating that 19.3% of chambers will have single cell and at least one bead. It implies that we can get information additionally from 356 chambers (7,280 chambers × (0.193-0.144)) for individual measurement if the correlation between signal and number of encapsulated beads is elucidated. To identify the relationship between signal and number of beads during cytokine detection, we used the numerical model we developed to achieve concentration profiles of cytokines secreted from single cells. Using the numerical model described in the current paper, we calculated how many cytokine molecules would or would not be captured by one bead when single cells are incubated with one bead, two beads, or three beads. As shown in FIG. S-4(a), the differences between amounts of cytokine captured on each bead from single beads and those from multi-beads were negligible. It indicates that the number of encapsulated beads may have its least impact on signals, since signals closely correlate with amounts of cytokines on beads. Also, it is of note that there would be about 3 times more free-floating cytokine molecules (which would not be captured by microbeads, dAb or cAb) inside the chamber having single cell and single bead, compared to the chamber having single cell and three beads (FIG. S-4(b)).

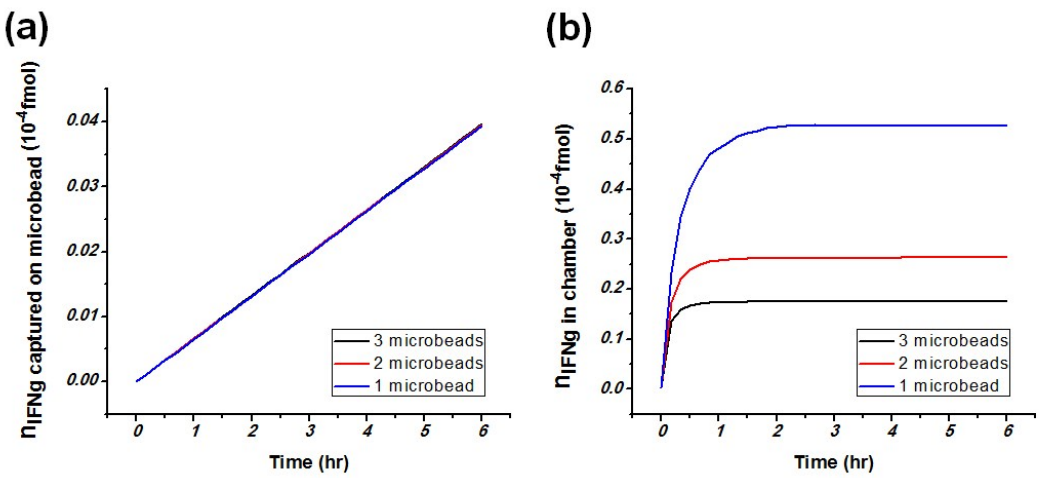

FIG. S-4. Computational modeling on response of IFN-γ sensing beads in relation to the number of beads. (a) Comparison of the simulated amount of IFNg (= IFN-γ) captured on bead when there would be 1 bead (blue), 2 beads (red), or 3 beads (black) encapsulated with single cells inside the chamber. (b) Simulation results showing change in the amount of floating IFNg molecules in chamber, not captured by bead(s) or cAb/dAb, over time.

Flow cytometry for detecting IFN-γ secreted from CD4$^+$ T cells

We performed the flow cytometry for detecting IFN-γ secretion from single CD4$^+$ T cells using commercially available IFN-γ secretion assay (Miltenyi Biotec, Bergisch-Gladbach, Germany). Briefly, 80 μL of T cells (1×10^6 cells for both mitogenically activated cells and quiescent cells) were incubated with 20 μL of IFN-γ catch reagent for 10 min on ice. After adding 1 mL of warm X-vivo medium, cells were incubated for 6 h at 37 °C in a humidified 5% CO_2 atmosphere for IFN-γ secretion, followed by washing with cold X-vivo medium. Subsequently, cells were centrifuged at 300 g for 10 min and supernatant was removed completely. Cells resuspended in 80 μL of cold medium were incubated with 20 μL of PE-labeled IFN-γ detection antibody for 30 min on ice. After washing with medium, cells were fixed

with 2% paraformaldehyde fixative for 15 min, and their IFN-γ secretion was confirmed using flow cytometry. FIG. S-5 shows that activated single T cell secretes IFN-γ in a heterogeneous manner: 17.8% of activated single T cells are 'high-secretor' while 82.6% of them are 'low-secretor'. On the other hand, only a few quiescent T cells (0.23%) are considered as 'high-secretor'. The classification of 'high-' and 'low-secretor' was arbitrarily determined based on the fluorescence intensities.

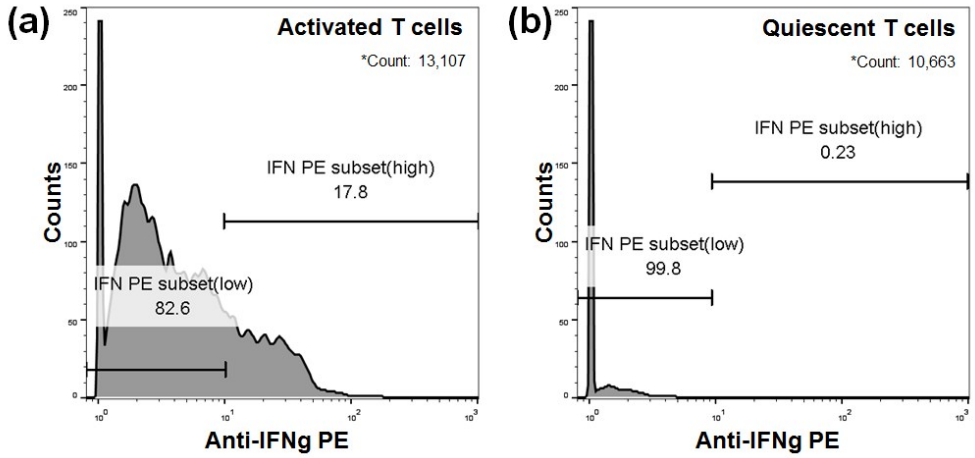

FIG. S-5. Flow cytometry result for IFN-γ secreted from (a) activated $CD4^+$ T cells and (b) quiescent $CD4^+$ T cells.

Monitoring exosome production of single HepG2 cells

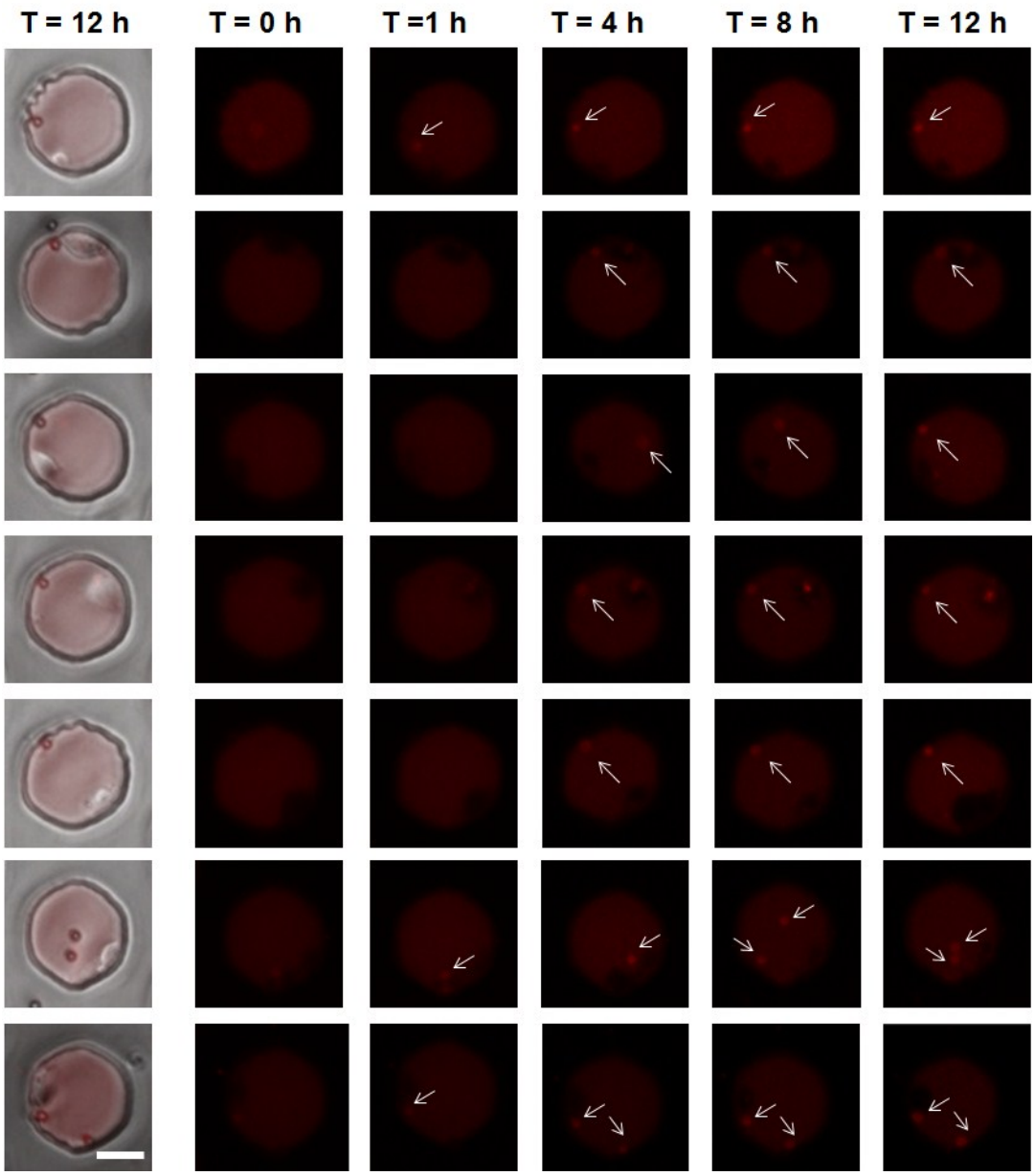

FIG. S-6. Fluorescence images of anti-CD63 cAb-bead encapsulated with single HepG2 cell and anti-CD63 dAbs over time. Scale bar = 20 μm.