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TECHNICAL NOTE

# Facile immunostaining and labeling of nonadherent cells using a microfluidic device to entrap the cells

Hironori Yamazoe,[1,*] Yukihiro Sugiyama,[2] Abdelfatteh El Omri,[2] Yoshihisa Hagihara,[1] and Takao Okada[2]

*Stress Signal Research Group, Health Research Institute, National Institute of Advanced Industrial Science and Technology (AIST), 1-8-31 Midorigaoka, Ikeda, Osaka 563-8577, Japan*[1] *and Research Institute of Biomolecule Metrology (RIBM), 807-133 Enokido, Tsukuba, Ibaraki 305-0853, Japan*[2]

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**We fabricated a microfluidic device for the entrapment of nonadherent cells. Solution exchange was easily performed by introducing the solution into the cell-trapping microchannel. Immunostaining and labeling of the cell membrane of THP-1 cells were demonstrated using this device, which does not require cumbersome repetition of centrifugation and resuspension steps.**



[**Key words:** Microfluidic device; Albumin film; Microbeads; Immunostaining; Cell membrane labeling; Solution exchange]

Immunostaining of cells is a fundamental technique for determining the existence of specific proteins and their location on the cell surface or inside the cell and for investigating changes in protein expression level accompanying various cellular events, such as cell proliferation, differentiation, and apoptosis. Labeling the cell membrane of live cells with fluorescent molecules is also a useful technique for *in vitro* and *in vivo* cell-tracking applications (1,2). However, immunostaining of nonadherent cells or labeling the cell membrane of living cells requires repeated performance of tedious and time-consuming steps. For example, in each step of the procedure, cells must be centrifuged in conical tubes, the supernatant must be aspirated, and the cells must be resuspended in the next solution called for in the protocol. Several techniques have been developed that reduce the burden of these solution-exchange procedures during immunostaining, such as electrostatic attachment of nonadherent cells onto glass plates coated with a positively charged polymer, or attachment of nonadherent cells onto glass plates using a device such as a cytospin (3,4). However, these methods have several drawbacks. The native shape of nonadherent cells may change after attachment onto the substrate, and these methods cannot be used for the labeling of the whole cell membrane of living cells. The use of microfluidic devices capable of trapping cells inside a microchannel would reduce the burden of solution-exchange procedures. A microfluidic device composed of a U-shaped array structure was proposed for cell entrapment; however, the design did not enable sufficient entrapment of cells (5,6).

In this study, we fabricated a microfluidic device composed of microchannels with a dam-like structure, microbeads, and cross-linked albumin film. All cells introduced into the device are trapped in the microchannels and are retained there without loss during solution exchange. Exposure of cells to different solutions is easily performed by passing each solution from the inlet to the outlet of the cell-trapping microchannels. Because the trapped cells are cultured in a free-floating state in the microchannel, native cell shape is retained after immunostaining, and the entire cell membrane can be uniformly labeled with dye. Here, we demonstrate the use of this device through immunostaining of the cytoskeletal protein actin in nonadherent cells and through labeling the cell membrane of living cells with a fluorescent dye.

A microfluidic device possessing a 10-μm gap was constructed by thermocompression bonding of two (upper and lower) custom-made cyclo-olefin polymer (COP) plates (Fig. 1a; Sumitomo Bakelite Co., Ltd., Tokyo, Japan). Both COP plates were fabricated using injection molding. A top view and cross-sectional view of the microfluidic device and an indication of its size (μm) are shown in Fig. 1b. Various types of liquid solutions, bead suspensions, or cell suspensions can be introduced through the inlet and are then drawn through the outlet using a syringe. Although microfluidic devices with small gaps can be fabricated with various designs (7), the dam-like structure described here is a suitable design for packing microbeads. Cells are trapped in the region of the dam (in front of the 10-μm gap), which is called the “cell culture chamber.” Fig. 1c shows the structure of the 10-μm gap region in detail. The upper plate is supported with the nine pillars of the lower plate to prevent bending of the upper plate during thermocompression bonding, thereby ensuring that the gap size remains uniformly 10 μm at all positions.

We tested the device for its ability to entrap nonadherent cells in the cell culture chamber, using human monocytic THP-1 cells. THP-1 cells (RCB1189, Riken Cell Bank, Ibaraki, Japan) were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum

* Corresponding author at: National Institute of Advanced Industrial Science and Technology (AIST), 1-8-31 Midorigaoka, Ikeda, Osaka 563-8577, Japan. Tel.: +81 29 861 6264; fax: +81 72 751 9517.
*E-mail address:* hironori-yamazoe@aist.go.jp (H. Yamazoe).

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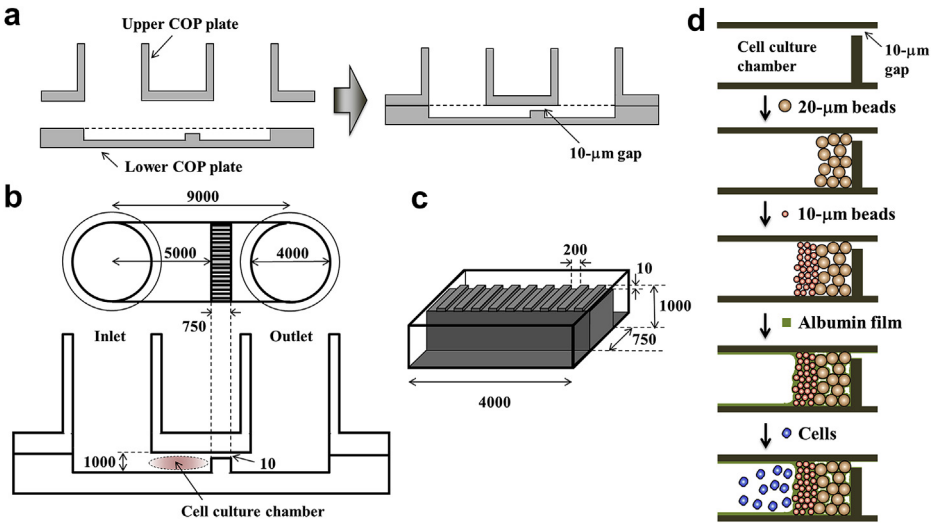


FIG. 1. Preparation of the microfluidic device, consisting of a microchannel with a dam-like structure, microbeads, and cross-linked albumin film. (a) Construction of the device with a 10-μm gap by thermocompression bonding of upper and lower COP plates. (b) Top view and cross-section of the device along with the size in each dimension (μm). The area for the entrapment of cells is referred to as the cell culture chamber. (c) Three-dimensional image of the 10-μm gap region along with the size in each dimension (μm). (d) Schematic illustrations of the albumin film-coated microfluidic device filled with 20- and 10-μm beads.

(FBS), 100 U/ml of penicillin, and 100 μg/ml of streptomycin. Following culture, the cells were collected by centrifugation and resuspended in fresh culture medium. A 50-μl aliquot of the cell suspension ($6 \times 10^5$ cells/ml) was introduced into the device inlet using a pipet. Although the 10-μm gap is smaller than the diameter of a THP-1 cell (approximately 20 μm), we found that the cells passed through the gap without being trapped in the culture chamber (Table 1, condition no. 1). Cells were able to pass through the gap due to deformation of their shape as a consequence of their softness and capacity to change conformation. The passage of cells through spaces smaller than the size of the cell has been reported elsewhere (8).

Microbeads were employed to prevent passage of cells through the culture chamber gap. A 5-μl volume of 20-μm-diameter carboxylate-modified polystyrene beads (Micromod Partikeltechnologie, Rostock, Germany) suspended in water at a concentration of $5.8 \times 10^6$ beads/ml was placed into the device inlet, and the bead suspension was then drawn through the outlet. The rigid structure of the 20-μm beads prevented them from passing through the 10-μm gap, causing the beads to collect at the dam region (Table 1, device no. 2; Fig. 1d) (9). The narrow crevices between the beads prevented the passage of some, but not all, cells through the 10-μm gap. To overcome this problem, a 5-μl volume of a suspension of 10-μm carboxylate-modified polystyrene beads at a concentration of $9.3 \times 10^7$ beads/ml was added to the device. The smaller beads collected in front of the 20-μm beads and completely inhibited the passage of cells out of the culture chamber (Table 1, device no. 3; Fig. 1d). However, after several exchanges of solution, the crevices between the beads became filled with trapped cells, blocking the flow of solution from the inlet to the outlet. The amount of beads used (a 5-μl volume of bead suspension at the concentration indicated above) was determined by experience based on the results of a pilot study, which indicated that when a small amount of beads (1 or 3 μl) was used, the dam region did not become completely packed with beads, leading to failure of cell entrapment.

**TABLE 1.** Entrapment of nonadherent cells while ensuring the flow of solution.

| Device no. | Device conditions[a] | | | | Entrapment of cells[b] | Flow of solution[c] |
|---|---|---|---|---|---|---|
| | 10-μm gap | 20-μm beads | 10-μm beads | Albumin film | | |
| 1 | + | − | − | − | Not trapped | Not blocked |
| 2 | + | + | − | − | Not trapped | Not blocked |
| 3 | + | + | + | − | Trapped | Blocked |
| 4 | + | + | + | + | Trapped | Not blocked |

[a] Device with (+) or without (−) indicated component.
[b] Cells were trapped or not trapped in cell culture chamber.
[c] Flow of solution was blocked or not blocked after repeating the solution exchange.

We previously prepared a water-insoluble, cross-linked albumin film that possesses various properties of native albumin, such as the ability to bind drugs and resistance to cell adhesion (10). The surfaces of plastic and PDMS microchannel walls were coated with the albumin film using a simple process involving infusion of the microchannels with a solution of cross-linked albumin and then allowing them to dry (11,12). The cross-linked albumin film was utilized to prevent the crevices between the beads from becoming filled with cells. The solution of cross-linked albumin was prepared as previously described (12). Briefly, bovine serum albumin (BSA; Sigma, St. Louis, MO, USA) was dissolved in phosphate-buffered saline (PBS, pH 7.4) to yield a 3% solution, which was then reacted with 215 mM ethylene glycol diglycidyl ether (EGDE; Wako, Osaka, Japan) with vigorous stirring for 24 h at 25°C. The reaction mixture was dialyzed for 3 days at room temperature against Milli-Q water using cellulose tubing (molecular weight cutoff = 12 kDa; Nihon Medical Science, Gunma, Japan) to remove unreacted EGDE. Next, the reaction mixture was adjusted to a final concentration of 2% by the addition of PBS. A 40-μl volume of the cross-linked albumin solution was infused into the microfluidic device packed with 20- and 10-μm beads as described above, leading to the adsorption of cross-linked albumin molecules onto the surfaces of the microbeads and microchannel. The device was washed once by flushing with Milli-Q water, and then allowed to dry overnight at room temperature (Fig. 1d). Testing indicated that the device trapped cells in the culture chamber without the flow of solution from the inlet to the outlet becoming blocked after repeated solution exchange (Table 1, device no. 4; Fig. 2a). The crevices between the beads are thought to be filled by coating the surface of the beads with the albumin film, which prevents the cells from entering and becoming trapped in the crevices. Furthermore, nonspecific adsorption of cells onto the microchannel surface was suppressed by coating the walls with albumin film. No cell damage or change in