Regular-Sized Cell Creation in Microchannel Emulsification by Visual Microprocessing Method

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ABSTRACT: A novel emulsification method was developed for making monodispersed regular-sized cells. Both oil in water (O/W) and water in oil (W/O) emulsion cells were generated by permeating an internal phase into a continuous phase through a silicon microchannel, which was designed and prepared by using semiconductor technology. The microprocessing of O/W (or W/O) emulsion cells was monitored and controlled with a microscope video system. Regular-sized O/W cells were made by a normal hydrophilic microchannel and a glass plate with use of an appropriate surfactant. On the other hand, W/O emulsion cells were made by a hydrophobic microchannel and a glass plate modified with a silane coupler reagent. Regular-sized W/O cells were also obtained; therefore, a suitable combination of organic phase, surfactant, and electrolyte should be carefully selected. There is a possibility for creating artificial biological cells with this method. In the water/triolein and lecithin system, when the amount of oil was decreased on the permeate side, polygon or fiber cell types were created, and each cell contacted its neighbors across a thin oil layer like a biological tissue.

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Scientific and technological efforts on mechanical emulsification in mixers, colloid mills, homogenizers, and sonicators have focused on making more homogeneous and stable emulsions (1). Membrane filtration can be applied to obtain greater homogeneity. Olson *et al.* (2) used several polycarbonate membranes to remove large emulsion cells. Suzuki (3) mentioned that the size distribution of emulsion cells was narrow after the emulsion was filtered repeatedly. Membrane emulsification was first reported by Nakashima *et al.* (4,5), who created an emulsion by filtrating an internal phase into a continuous phase. The significant point of their method is that a (porous glass) membrane was used for cell creation and not separation. Because the emulsion droplet size can be controlled by membrane pore size, the method is advantageous for making a monodispersed emulsion in comparison with conventional methods. The method is now commercially used to produce low-fat margarine (6).

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Kikuchi *et al.* (7,8) developed a system in which a microscope was attached to a video recorder (microscope video system) for viewing optically accessible microchannels formed in a single crystal silicon substrate, which was manufactured with semiconductor technology. The microscope video system was used for diagnosing blood cell deformability by observing permeability through the silicon microchannel. The large advantage of this method is real-time optical observation of the behavior of micro-sized materials.

In this paper, a novel emulsification method for making and observing monodispersed regular-sized cells is proposed in which the silicon microchannel and the microscope video system are used. Visual images are recorded during the creation of O/W (oil in water) and W/O (water in oil) emulsion cells. The possibility for making artificial biological cells is suggested with lecithin as a cell wall material.

EXPERIMENTAL PROCEDURES

Materials. High-oleic sunflower oil (triolein, >90% purity) was obtained from Nippon Lever B.V. (Tokyo, Japan). Food-grade sorbitan monolaurate, L10 (HLB: 8.6), and sorbitan monooleate, O10 (HLB: 4.3), were obtained from Kao Chemicals (Wakayama, Japan). Special reagent-grade oleic acid, sodium dodecyl sulfate (SDS, HLB: 40), KCl, NaCl, and reagent-grade soybean lecithin were purchased from Wako Pure Chemical Ind. (Osaka, Japan). Kerosene was purchased from Japan Energy Corporation (Tokyo, Japan). The silane coupler reagent, octyltriethoxysilane, LS-5580, was obtained from Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan) and applied for surface modification of the silicon microchannel.

Visual microprocessing system. Figure 1 shows the microscope video system, the silicon microchannel module, and the silicon microchannel plate for microchannel emulsification. An inverted metallographic microscope (TS–V; Chuo Precision Industrial Co., Ltd., Tokyo, Japan) was used to observe the microchannels, and images were recorded through an eyepiece by a 8-mm video camera (CCD-TR900; Sony Corporation, Tokyo, Japan). Optical magnifications of the objective lens and eyepiece were both 10 ×. The zoom lens of the video camera (usually applied at a focal length of 62.4 mm) gave additional magnification. Therefore, the final magnification was about 1,000 ×. The silicon microchannel plate measured 15 mm × 15 mm. The average channel width, channel wall height, and the ter-

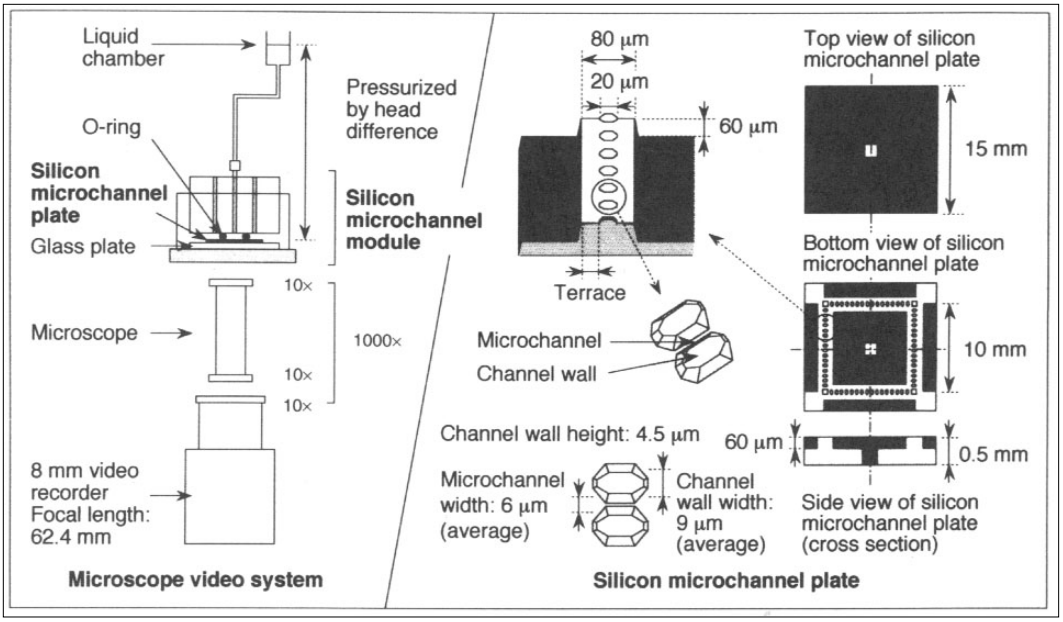

FIG. 1. Microscope video system, silicon microchannel module, and silicon microchannel plate for microchannel emulsification.

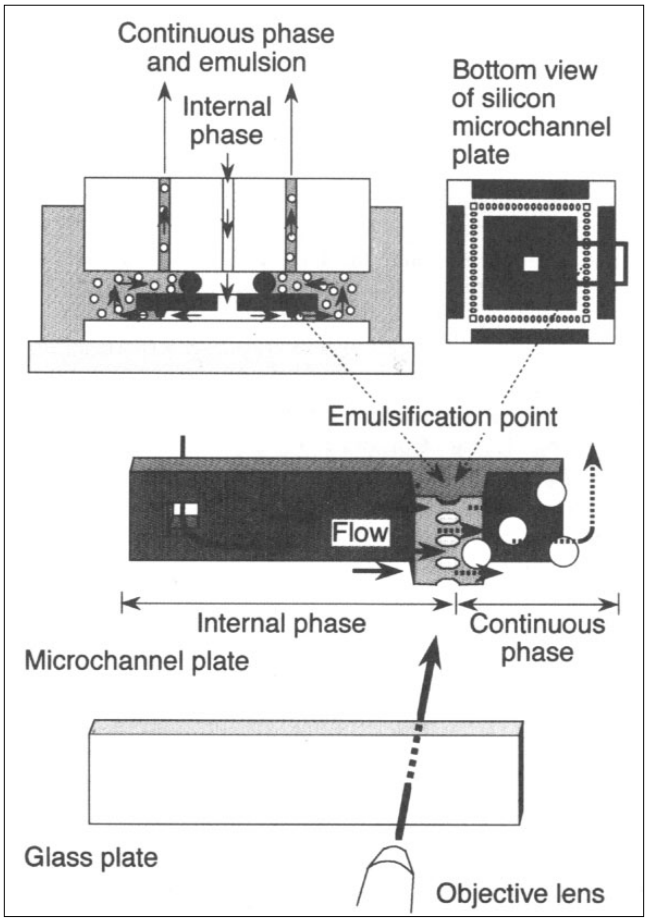

FIG. 2. Schematic flow mechanism through the silicon microchannel.

race height were 6 μm, 4.5 μm, and 60 μm, respectively. The silicon material was originally hydrophobic; however, after the processes of photolithography and orientation-dependent etching, the silicon plate became hydrophilic (7,9).

Microchannel emulsification. Figure 2 schematically shows the flow mechanism in the module and through the microchannel. The silicon microchannel plate was tightly covered with a flat glass plate, and the channel image was obtained through the glass plate. The silicon microchannel module was initially filled with the continuous-phase liquid of the desired emulsion, and the internal-phase liquid was pressed into the module by lifting the liquid chamber filled with the internal phase liquid. During the microprocessing of emulsion cells, the operating pressure and the flow rate were regulated by changing the height of the chamber with real-time optical observation.

Surface modification for making hydrophobic microchannels. To make a stable hydrophobic microchannel plate and bottom glass plate, the silane coupler reagent, octyltriethoxysilane, was applied for surface modification. The microchannel plate and glass plate were washed with 0.1 M nitric acid for 30 min and with water for 30 min. After drying at 80°C, they were dipped in 5 wt% octyltriethoxysilane solution in toluene and heat-treated at 110°C for 1 h (temperature gradient was 1°C/min). Finally, they were ultrasonicated several times in toluene, and the unreacted materials were washed out.

RESULTS AND DISCUSSION

Creation of regular-sized O/W emulsion cell. Triolein was used as the internal oil phase with 0.3 wt% sorbitan monolaurate. SDS was also used for making O/W emulsion cells and

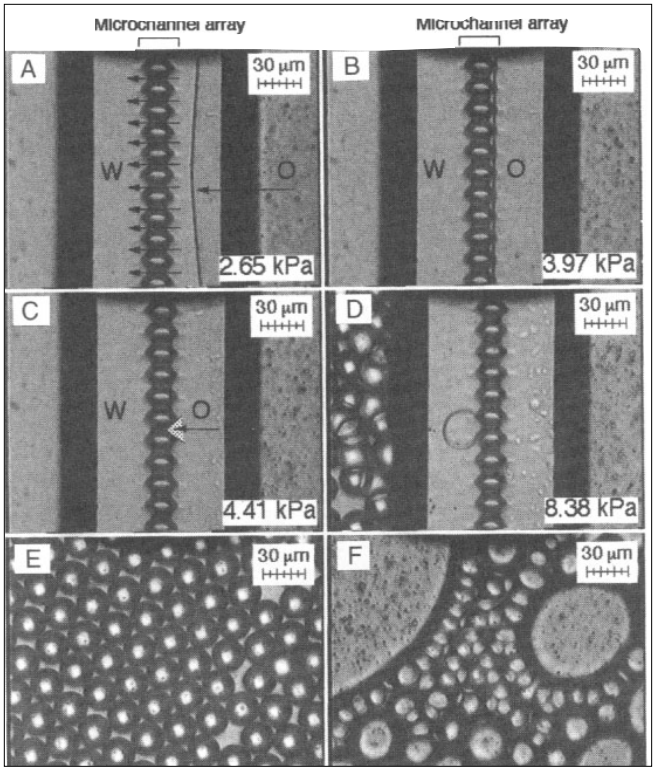

FIG. 3. The process of microchannel emulsification for making oil in water (O/W) cells in the system: triolein with 0.3 wt% sorbitan monolaurate/water. (A) Intrusion into the terrace of the microchannel; (B) contact to the entrance of the microchannel; (C) full contact to the entrance of the microchannel; (D) breakthrough the microchannel and cell creation; (E) created regular-sized cells of 22.5 μm; (F) irregular-sized cells created under reduced water conditions in the permeate side.

was dissolved in the water phase, in contrast to sorbitan monolaurate, which was dissolved in oil.

Because the silicon microchannel is hydrophilic, it is more easily wetted with water than oil. Therefore, for oil (triolein) injection into the microchannel plate module and permeation through the microchannel, it was necessary to apply pressure by increasing the height of the liquid chamber. Figure 3 (A–D) shows the process of microchannel emulsification for making O/W cells with 0.3 wt% sorbitan monolaurate in triolein (internal oil phase). At 2.65 kPa (head difference: 30 cm), the oil phase intruded into the terrace of the microchannel (Fig. 3A). The boundary line between water and oil phases gradually moved to the entrance of the microchannel, and at 3.97 kPa (45 cm), the line contacted the entrance (Fig. 3B). At 4.41 kPa (50 cm), it had fully contacted the entrance (Fig. 3C). The operating pressure was slowly increased with simultaneous observation of the boundary position, and when the pressure reached 8.38 kPa (95 cm), the oil phase broke through the channel, and the creation of regular-sized cells had started (Fig. 3D). The breakthrough site appeared to be random. The speed of cell creation at 8.38 kPa was high (about 2 cells per second for each creation site) and gradually decreased as the operating pressure was reduced. Cell creation continued until the operating pressure reached 4.85 kPa (55 cm). The cells were regular with a size of 22.5 μm, which is 3.75 times larger than the average microchannel width (6 μm) (Fig. 3E). This is in good agreement with the membrane emulsification results obtained by Nakashima and Shimizu (4). They empirically found that the cell size was 3.26 times larger than the membrane pore size. The cell size was independent of the operating pressure in the range examined. During emulsification, generated cells gradually accumulated, and the amount of water was relatively reduced in the permeate side. When the water became insufficient to cover the surface of the O/W cells, irregular ones began to appear (Fig. 3F).

As a substitute for sorbitan monolaurate, SDS was dissolved in the continuous water phase. Emulsion cells were also created with this system. Table 1 shows the effect of pressure (head difference) on microchannel processing of O/W emulsion cells. The pressure difference needed to make O/W cells decreased as the SDS concentration increased because the interfacial tension between water and triolein became lower.

Creation of regular-sized W/O emulsion cells. Because both the microchannel silicon plate and the bottom glass plate were hydrophilic, the microchannel was always wetted by water without pressurization, and a W/O emulsion could not be created. When the water for the internal phase was pressurized, a

TABLE 1
Effect of Pressure (head difference) on Microchannel Processing for Creation of Regular-Sized O/W and W/O Cells[a]

Cell type: internal phase/continuous phase (surfactant or electrolyte)	Intrusion into the terrace of the microchannel: pressure (kPa) [head diff. (cm)]	Full contact to the entrance of the microchannel: pressure (kPa) [head diff. (cm)]	Breakthrough the microchannel and cell creation: pressure (kPa) [head diff. (cm)]	Lower limit for cell creation after breakthrough: pressure (kPa) [head diff. (cm)]
Triolein (0.3 wt% L10)/water	2.65	4.41	8.38	4.85
	(30)	(50)	(95)	(55)
Triolein/water (0.1 wt% SDS)	3.70	4.06	7.94–9.08	4.76–5.91
	(42)	(46)	(90–103)	(54–67)
Triolein/water (0.2 wt% SDS)	1.85	1.94	2.21–2.65	1.50–1.59
	(21)	(22)	(25–30)	(17–18)
Water (0.75 wt% KCl)/kerosene	1.03	1.13	1.62	1.37
(3 wt% L10)	(10.5)	(11.5)	(16.5)	(14)

[a]O/W, Oil in water; W/O, water in oil; L10, Kao Chemicals (Wakayana, Japan).

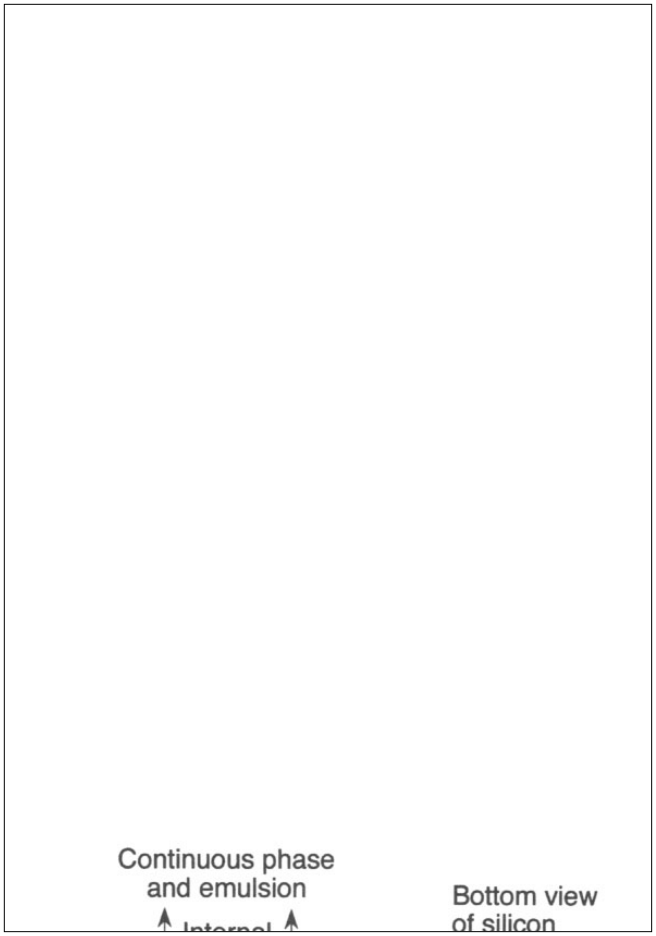

FIG. 4. Microchannel emulsification for making water in oil (W/O) cells: (A) water permeation through the hydrophilic channel; (B,C) water permeation through the channel, partially wetted by water, in the system: water/triolein with 0.3 wt% sorbitan monooleate; (D) water permeation through the channel, wetted by water, in water/triolein with 0.1 wt% sorbitan monooleate system; (E) breakthrough the microchannel and cell creation in the system: water with 0.75 wt% KCl/kerosene with 3 wt% sorbitan monolaurate; (F) regular-sized W/O cells of 21 μm packed in the closest conformation; (G) the W/O cells observed at a different focus point; (H) W/O cells in the system: water with 0.86 wt% NaCl/oleic acid with 3 wt% sorbitan monolaurate.

large amount of water permeated into the continuous oil phase, as shown in Figure 4A. We concluded that, for making a W/O emulsion, the microchannel and the bottom glass plate should be hydrophobic, and therefore, modified with a silane coupler reagent, octyltriethoxysilane. After silane coupler surface modification, the contact angle of water to the silicon microchannel plate changed from 36 to 86°, and to the bottom glass plate from 32 to 80°. The contact angle values were constant, and the hydrophobicity remained for several months during this study. In the following studies, the hydrophobic microchannel and bottom glass plate were used.

For W/O emulsion, the surfactant HLB value should be from 3 to 6 (1). However, this is only a general consideration, and there is little information regarding vegetable oils. Triolein was used as the continuous oil phase with the food-grade surfactant sorbitan monooleate (HLB: 4.3). Figure 4B shows the W/O emulsification process by using water with the sorbitan monooleate/triolein system. The surfactant concentration was 0.3 wt% in the triolein phase. The permeate side of the microchannel and terrace was partially wetted by water like a stain. We considered that the surfactant adsorbed on the channel and terrace so strongly that they became hydrophilic. With 0.1 wt% of sorbitan monooleate, the stain decreased. However, a water droplet at the edge of the channel appeared to grow continuously on the permeate side (Fig. 4C). Probably, at the low surfactant concentration, the interfacial tension between water and triolein was still high, and the water droplet expanded strongly against surrounding oil. As referred by the membrane emulsification method (4,5), application of additional shear around the droplet is required for effective release from the edge and to make W/O cells successfully and continuously.

For making a W/O emulsion with a water/kerosene system, the appropriate surfactant HLB value is from 6 to 9, and for a water/oleic acid system it should be from 7 to 11 (10). Therefore, sorbitan monolaurate (HLB: 8.6), the same surfactant used for making the O/W cells, was selected and dissolved in the oil phase. Figure 4D shows an image captured in the creation process of W/O cells in the water/kerosene system. The concentration of sorbitan monolaurate was 3 wt% in kerosene, and that of KCl was 0.75 wt% (0.1 M) in water. Electrolyte, such as KCl, is generally added to the internal water phase as a stabilizer for W/O emulsion cells (11,12). The process observed was similar to that in the reversed system for creating O/W cells. At the breakthrough pressure, the speed of cell creation was about 2 cells per second, and the obtained W/O emulsion cells had a regular size of 21 μm (Fig. 4E). Although the W/O emulsion cells were slightly distorted by contact with the glass plate, Figure 4 (F,G) shows that they were regular-sized and packed in the closest conformation. In the water/oleic acid system, W/O cells were also created continuously. However, cell stability was poor, and they fused easily (Fig. 4H). Concentration of sorbitan monolaurate was 3 wt% in oleic acid, and that of NaCl was 0.86 wt% in water. The effects of operating conditions, water and oil phase components, and the structure and surface modification of the silicon microchannel should be investigated further. However, it is promising that the microchannel emulsification method allows possibilities to create regular-sized O/W and W/O cell types.

Approach for creating artificial biological cells. There have been many research publications that describe artificial biological cells. Although actual biological cell walls have complex structures with many components, the liposome has been studied as one type of artificial cell. Liposome cell walls are usually made of phospholipid, such as phosphatidylcholine. A large liposome cell (large unilamellar vesicle, LUV) was prepared by Oku *et al.* (13) with a vesicle size between 10–100 μm. Szoka *et al.* (14) made intermediate-sized unilamellar vesicles of around 0.1 μm by the reverse-phase evaporation vesicle (REV) method. With our microchannel

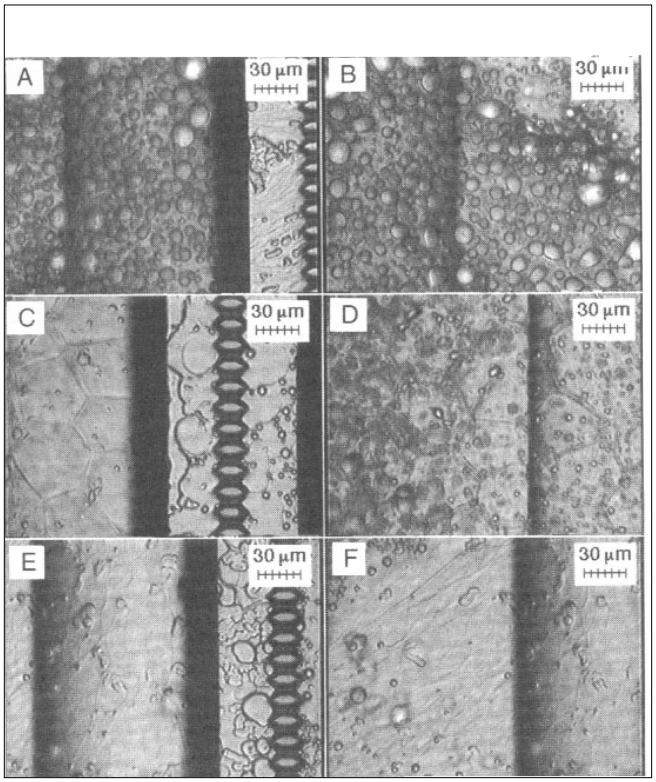

FIG. 5. Microchannel emulsification for making W/O artificial biological cells in the system: water with 0.86 wt% NaCl/triolein with 25 wt% lecithin; (A) microchannel processing under triolein-rich conditions on the permeate side and creation of a normal cell; (B) normal cells; (C) microchannel processing under triolein-depleted conditions in the permeate side and the creation of a polygonal cell; (D) polygonal cells; (E) microchannel processing under virtually depleted triolein conditions in the microchannel and terrace, and creation of a fibriform cell; (F) fibriform cells.

emulsification method, large reverse-phase vesicles (W/O cells) were created. Therefore, it is possible to create large artificial cells that are covered with a thin continuous oil layer. Triolein, containing 25% lecithin, was used for the oil phase, and 0.86% NaCl was dissolved in the water phase. Triolein oil was added to give fluidity in the lecithin phase, which should be altered in the future because cell walls generally do not contain oil. Figure 5 (A–F) shows the images during the creation of an artificial biological cell by using water/triolein with the lecithin system. Microchannel processing and normal W/O cells, created under triolein-rich conditions, are shown in Figure 5 (A,B). The microchannel was wetted by water, and the cell size was not regular. It is interesting that the cell structure changed drastically when the amount of triolein decreased in the permeate side, as shown in Figure 5 (C–F). Figure 5 (C,D) shows the W/O cell covered with a thin oil layer. Most of the triolein that initially filled the permeate side was replaced by water. Triolein remaining in the microchannel and terrace was supplied little by little with water flow toward the permeate side. Each cell became polygonal and contacted neighboring cells like a biological tissue. Figure 5 (E,F) displays the images obtained in the late stage of the microchannel emulsification when only little triolein remained in the microchannel and terrace. Triolein remaining in the permeate side seemed to be more than that of the polygonal cell. Triolein and water were mixed slowly because the water flow rate was low. Each cell had a fibriform shape, like a muscle fiber cell. Because these types of cells were obtained unexpectedly, further investigation is necessary to control cell type and stability.

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