Analysis of pressure-driven air bubble elimination in a microfluidic device

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Table of Contents:

S1. Analytical model of the bubble elimination method

An analysis model for the bubble removal is based on the gas permeation equation.[1] In the scheme described in Fig. 1, the permeability of a polymer to a permeating gas is defined by

$$P = \frac{Nb}{(p_2 - p_1)} \qquad (1)$$

where P is the permeability, N is the flux of the penetrant under steady-state condition, b is the penetrating thickness, and p_1, p_2 are the permeate and feed pressure, respectively. When the pressure difference is formed in the steady state across the gas permeable membrane (PDMS), the gas flux penetrating the polymer membrane is given by

$$N_A = -\frac{dV}{dt} = \frac{PA(p_2 - p_1)}{b} \frac{T}{273} \frac{76}{P_{atm}} \qquad (2)$$

where T is the absolute temperature in Kelvin, P_{atm} is the atmospheric pressure in cmHg, A is the penetration area, and dV/dt is the volumetric flow rate driven by pressure gradient across the membrane. Eqn (2) can be adapted for an analysis of the trapped-bubble removal in the microfluidic channels as shown in Fig. S1. The volume of a bubble can be roughly defined by the product of height of the microchannel, h, and the area observed by a microscope, A with the assumption that the bubble forms a column of gas which permeates only through the ceiling of PDMS neglecting the penetrant across the bottom slide glass. Here, we did not consider the dissolution of the gas into the aqueous solution because the gas flux through the PDMS wall is dominant compared to that into the surrounding aqueous solution. Through eqn (3), we have reached a first-order decay expression in eqn (4) by which we can predict a bubble removal rate in accordance with time under certain

experimental condition.

$$-\frac{dA}{dt}h = \frac{PA(p_2 - p_1)}{b}\frac{T}{273}\frac{76}{P_{atm}} \tag{3}$$

$$A(t) = c_1 \exp\left(\frac{aP(p_1 - p_2)}{hb}\frac{T76}{273P_{atm}}t\right) + c_2 = c_1 e^{kt} + c_2 \tag{4}$$

Through this equation we can generally predict the bubble removal rate according to the exponential decay model. However, it becomes difficult to estimate the removal rate if we use aqueous solution containing volatile organic components such as acetone, toluene and methanol which can be absorbed by PDMS substrates consequently affecting the gas transport parameters. In this study, we used water-based aqueous solution, where the vapor pressure of water molecules was negligible due to the low temperature around 25˚C.
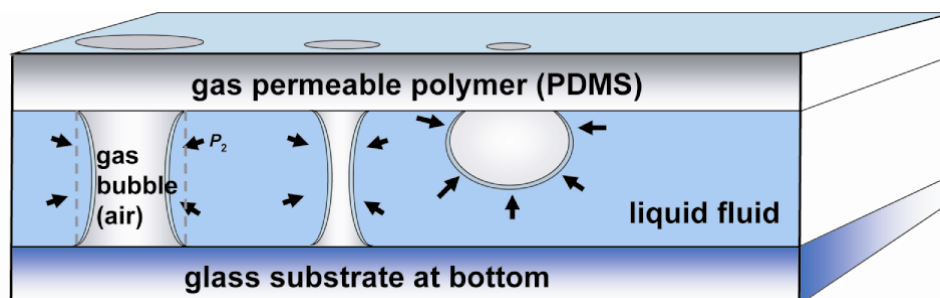

Fig. S1 Scheme of disappearance of the bubbles trapped in a microfluidic channel. As we pressurize the microchannel, the trapped air vanishes through the PDMS ceiling. At the last moment of the bubble removal, the hanging bubble clings to the ceiling of the PDMS microchannel because the ceiling surface is hydrophobic while the bottom slide glass is hydrophilic. Therefore, we do not have the isolated bubbles surrounded by liquid solution and the bottom glass substrate.

S2. Microfabrication details

We fabricated two types of PDMS microfluidic devices for an analysis of the bubble elimination and large-area cell culture. For the measurement of the bubble removal rate, the straight closed-end microfluidic channels having 50 µm of height, 100 µm of width and about 18 mm of length were fabricated as shown in Fig. S2(a). This simple feature of the microchannel was employed for reliable and repeatable measurement of the bubble removal because complicated or large-area microchannels make irregular air bubbles trapped in the channel, which causes analytical variation of the correction factor. In order to demonstrate a large-area microfluidic cell culture device working for long period, we also fabricated a PDMS microchannel of 1.7 cm^2 of area and 8.5 µL of volume as presented in Fig. S2(b). PDMS substrates for microfluidic channels were prepared by conventional PDMS (Sylgard 184, Dow Corning) molding process using SU-8 2025 photoresist, and the peeled-off PDMS substrates were bonded with a clear slide glass using air plasma treatment.
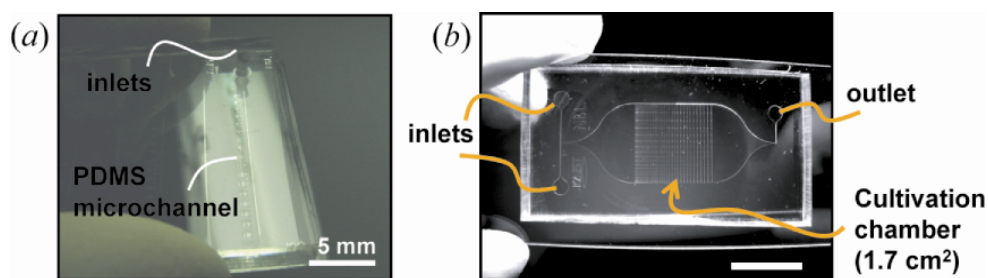

Fig. S2 Pictures of the fabricated PDMS devices. (a) The device for an analysis of the trapped air bubble removal (see Fig. S5(a)). (b) Microfluidic large-area cell culture device (area; 1.7 cm^2 and volume; 8.5 µL). Scale bar is 1.0 cm. The PDMS micropillar arrays are patterned in the chamber for a demonstration of the complicated geometry of the device.

S3. Experimental setup and cell culture procedures

Experimental setup

Fig. S3 presents experimental setups for simply removing the trapped bubbles in the PDMS microchannels. There are two kinds of injection methods by an air compressor or syringe pump. For precise pressure control to obtain reliable experimental data, we used the method Fig. S3(a) while we carried out a continuous perfusion cell culture using the method Fig. S3(b) for convenient injection of cell culture medium. The area of air bubble was observed by an inverted microscope and images were captured by CCD every 1 s, and the observed bubble area was analyzed by a commercial image processing program, i-Solution (Image and Microscope Technology, Korea). We also considered the correlation between the applied pressure in the PDMS microchannels and the measured pressure by a pressure sensor in Fig. S3. Through the Boyle's law ($PV = P'V'$), we evaluated the locally applied pressure inside the channel calculating the reduced bubble volume (V') at P'. This result reveals that the error between the measured pressure of the sensor and the local pressure in microchannels are less than 1.5%, which can be caused by pressure resistance throughout the pressure control manifold.
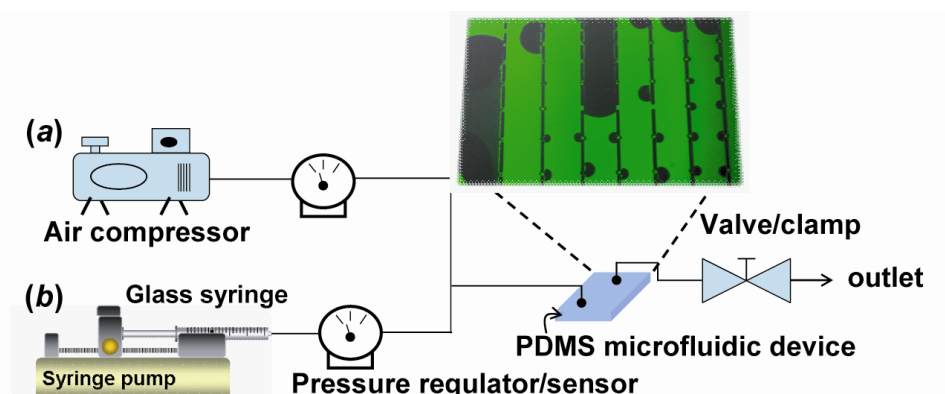
Fig. S3 Experimental setups for simply eliminating air bubbles in a PDMS device. While liquid samples are injected to the device, closing valve or clamping off tubes at the outlet ports makes pressure in PDMS chamber accumulated and consequently the trapped gas removed. The inset picture is the fluorescence microscopic image of the air bubbles trapped in the microfluidic channel when fluorescence solution was injected into the microchannel. The PDMS micropillar arrays caused the air bubbles trapped in the microfluidic device.

Cell culture in a microfluidic device

We demonstrated a continuous perfusion culture of MCF-7 human breast cancer cells (mammary gland epithelial cell) in a large-area microfluidic device (see Fig. S7). The pressure influence of MCF-7 has been recently investigated,[2] and we successfully cultivated without the pressure influence on the cell viability (see Fig. S8). Cell culture medium, RPMI 1640 was supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units mL^{-1} penicillin, and 100 μg mL^{-1} streptomycin. When we injected medium solution into the microchannel, we frequently met the air bubbles trapped in the PDMS channel, which disturb the microfluidic device and are rarely removed even by attentive

handling of the fluidic system. Even though the high pressure-driven injection can remove the trapped bubbles,[3] some bubbles around outlet channels did not disappear (see Fig. S4) because the pressure accumulation about the outlet ports is significantly reduced due to the pressure drop. Therefore, by closing the outlet ports with a clamp we applied the uniform pressure over the microfluidic network. If the bubbles were unfortunately trapped in the channel during the cell seeding and media exchange, we measured the bubble area and estimated the required time to absolutely remove them under the certain pressure that is safe for the cells. Once the required time exceeded the critical time limit for the cell viability, we should discard the microfluidic culture while we can simply predict how long the bubble removal will take if it is within the critical time. Microfluidic cell cultures were maintained at 37 °C and passaged by trypsinizing the proliferated cell layer with 0.25% trypsin/EDTA•4Na. Resuspending the cells by tapping the PDMS microchannels resulted in dissociation of confluent cell layers into the individual cells. Resuspension of the confluent cells is critical to drain out a portion of the cells for subculture. Irregular micropatterns in a PDMS microchannel locally generate a shear flow that makes clumps of MCF-7 cells into individual cells, where the shear force is negligible compared to the resuspending procedure in a conventional cell passage. Cell viability was determined using calcein AM (calcein acetoxy-methyl ester; green dye; live cells) of the LIVE/DEAD® Viability/Cytotoxicity Kit for mammalian cells (Molecular Probes, Inc.). The calcein AM solution (2 μM) was injected into the microfluidic channels in which MCF-7 cells were cultured, and we stopped the flow for 30 min for incubation.

8

References

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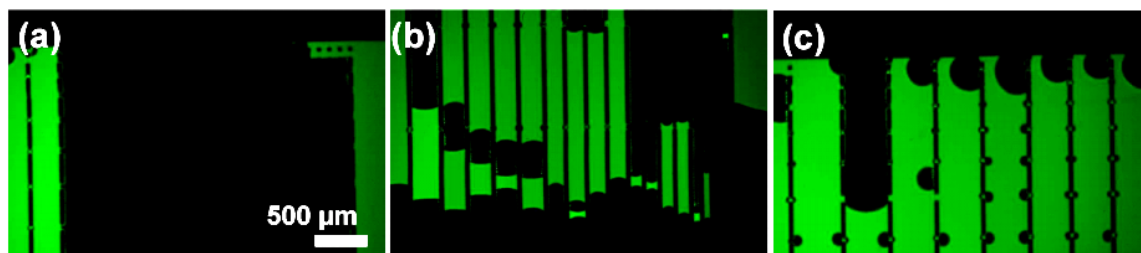3   L. Kim, M. D. Vahey, H.-Y. Lee and J. Voldman, *Lab Chip*, 2006, **6**, 394-406.

Fig. S4 Sequential photographs during filling the large-area microfluidic channel (1.7 cm^2) with FITC solution (3.0 mM) using high flow rate (10.0 µL min^{-1}). Even at the high flow injection rate, the trapped bubbles nearby a large outlet port did not partially removed yet because the pressure and the shear stress on the bubbles are reduced near the opened outlet. Therefore, to apply the uniform pressure over the large-area microfluidic channel, we employed the method presented in Fig. S3.
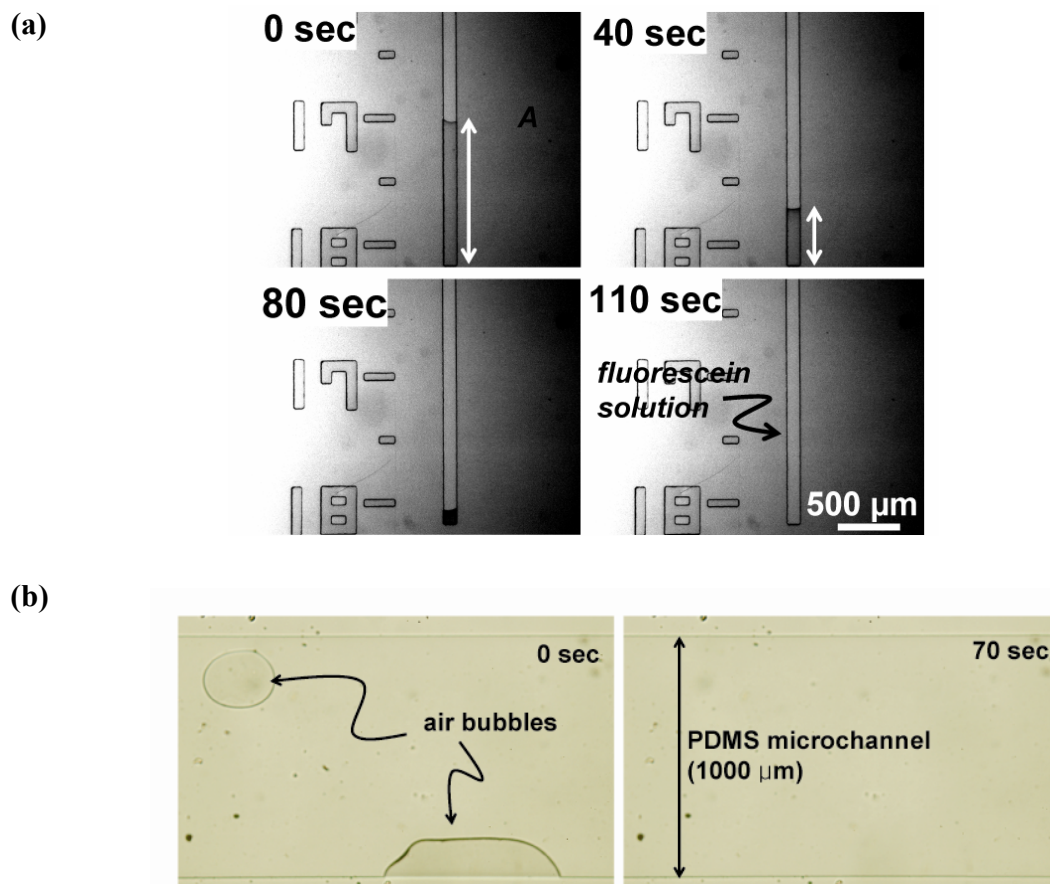
(a)

(b)

Fig. S5 (a) Sequential photographs prove that the air bubbles were successfully removed in the PDMS microchannels at an applied pressure of 11.0 psi. The bubble removal rate was measured in the device shown in Fig. S2(a), where the figures patterned on the PDMS substrates in the pictures are the reference scale for measuring the bubble area (A). (b) In another PDMS microchannel with different geometry, the trapped bubbles are completely removed at 8.0 psi in 70.0 s. Comparing the theoretical removal time (65.0 s, where b is 0.40 cm, h is 20 μm and the other constants are same with that of Fig. S5 (a)), we can conclude that this method is applicable to the different geometric devices.
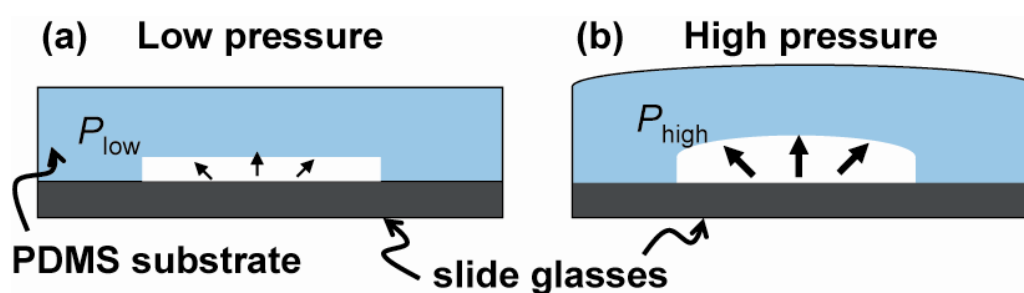
Fig. S6 The Schematic showing pressure-driven deformation of the PDMS microchannels.
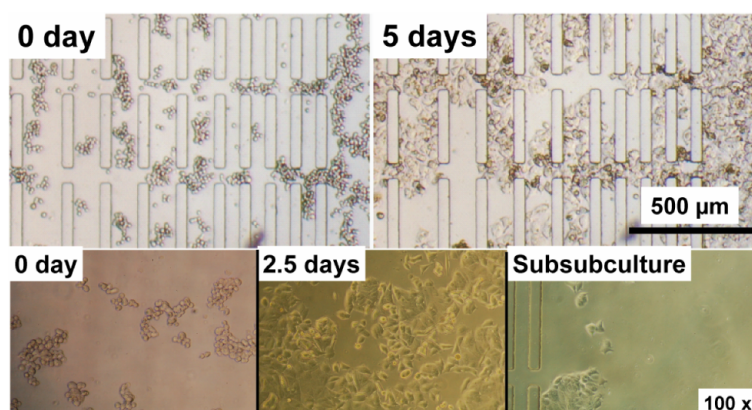
Figure (a) and (b) are not to scale.

Fig. S7 Large area (1.7 cm^2) human breast cancer cell (MCF-7) culture for 5 days and subculture of the third round. At the initial state of cell seeding (0 day in Fig. S7), cells started to settle down and attached on hydrophilic bottom slide glass showing the change of a cellular morphology from the round to the polygon-like. The proliferated cells increase the surface density, forming the confluent cell layers (2.5 days in Fig. S7), and the following trypsinization makes the detached cell clump discarded, leaving the partial group of cells on the microchannels. Although the cell density is reduced as much as that at the initial state after trypsinization, cellular morphology is quite different from that at the starting point.
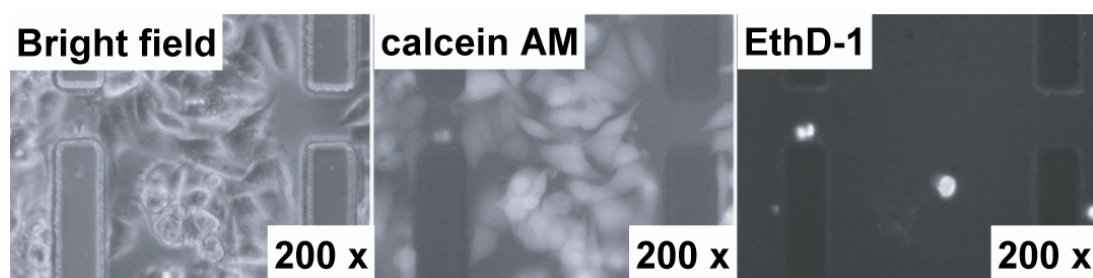
Fig. S8 Viability test of cultivated MCF-7 in the microfluidic device for 3 days using calcein AM (live cells)/ ethidium homodimer-1 (dead cells). Images were captured at the same position by switching fluorescence filters.