Electronic Supplementary Information (ESI) for Lab on a Chip
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A simple and reversible glass-glass bonding method to construct a microfluidic device and its application for cell recovery

Supplementary information

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Experimental

Channel fabrication

Glass plates were treated with piranha solution for 30 min and then washed with deionized water followed by blowing by nitrogen gas to make the surface clean and dry. Chromium layer was formed on the clean surface of the glass plates by operating an ion beam sputter device (EIS-220, Elionix, Tokyo, Japan) for 40 min. Gold layer was formed on the chromium layer by operating the ion beam sputtering device for 30 min. The glass plates with metal layers were treated with vacuum oxygen plasma at an intensity of 30 W and oxygen flow rate of 8 mL min^{-1} for 2 min in a chamber of a compact etcher (FA-1, Samco, Kyoto, Japan) right before applying photoresist. Positive photoresist (OFPR-800, Tokyo Ohka Kogyo, Kanagawa, Japan) was spin-coated on the gold layer at 2,000 rpm for 25 s with a spin-coater (MS-A150, Mikasa, Tokyo, Japan). The glass plates with photoresist were exposed to UV through a photomask to transcribe pattern of channels by UV exposure via operation of a mask aligner (MA-10, Mikasa). The exposed photoresist layer was developed with a developer (NMD-3, Tokyo Ohka Kogyo). Then the exposed gold layer was etched with an etchant (AURUM-302, Kanto Chemical, Tokyo, Japan) followed by etching the exposed chromium layer with an etchant (HICRETCH S-1, Fujifilm Wako Pure Chemical Corporation, Osaka, Japan). The glass plates were etched with 20% hydrofluoric acid (HF) containing 2% hydrochloric acid (HCl) and then washed with deionized water to terminate glass etching. After glass etching, the photoresist layer was removed with acetone and the metal layers were removed with the corresponding etchant.

Immobilization of hydrophobic pattern

Glass plates were polished with melamine resin and then washed with deionized water. The polished glass plates were coated with photoresist, ink, or adhesive tape to cover the areas that were not desired to obtain hydrophobic coating. In case of using photoresist, photoresist was spin-coated on the glass plates at 2,000 rpm for 25 s with the spin-coater. The pattern of hydrophobic areas was transcribed on the glass plates through a photomask of a desired pattern set to the mask aligner. The exposed photoresist layer was developed by the photoresist developer. In case of using ink, pattern was drawn with a pen on the glass plates. In case of using adhesive tape, pattern was formed by applying the tape. The glass plates coated with photoresist, ink, or adhesive tape were treated with vacuum oxygen plasma at an intensity of 30 W and oxygen flow rate of 8 mL min^{-1} for 2 min in the chamber of the compact etcher. Then the glass plates were placed in a vacuum desiccator to be exposed to the vapor produced by 20 μL of trichloro(1*H*,1*H*,2*H*,2*H*-heptadecafluorodecyl)silane (T2705, Tokyo Chemical Industry, Tokyo, Japan) while evacuating the desiccator for 1 h. The glass plates treated by the vapor were then heated at 70 °C for 1 h. After that, photoresist, ink, or adhesive tape were removed.

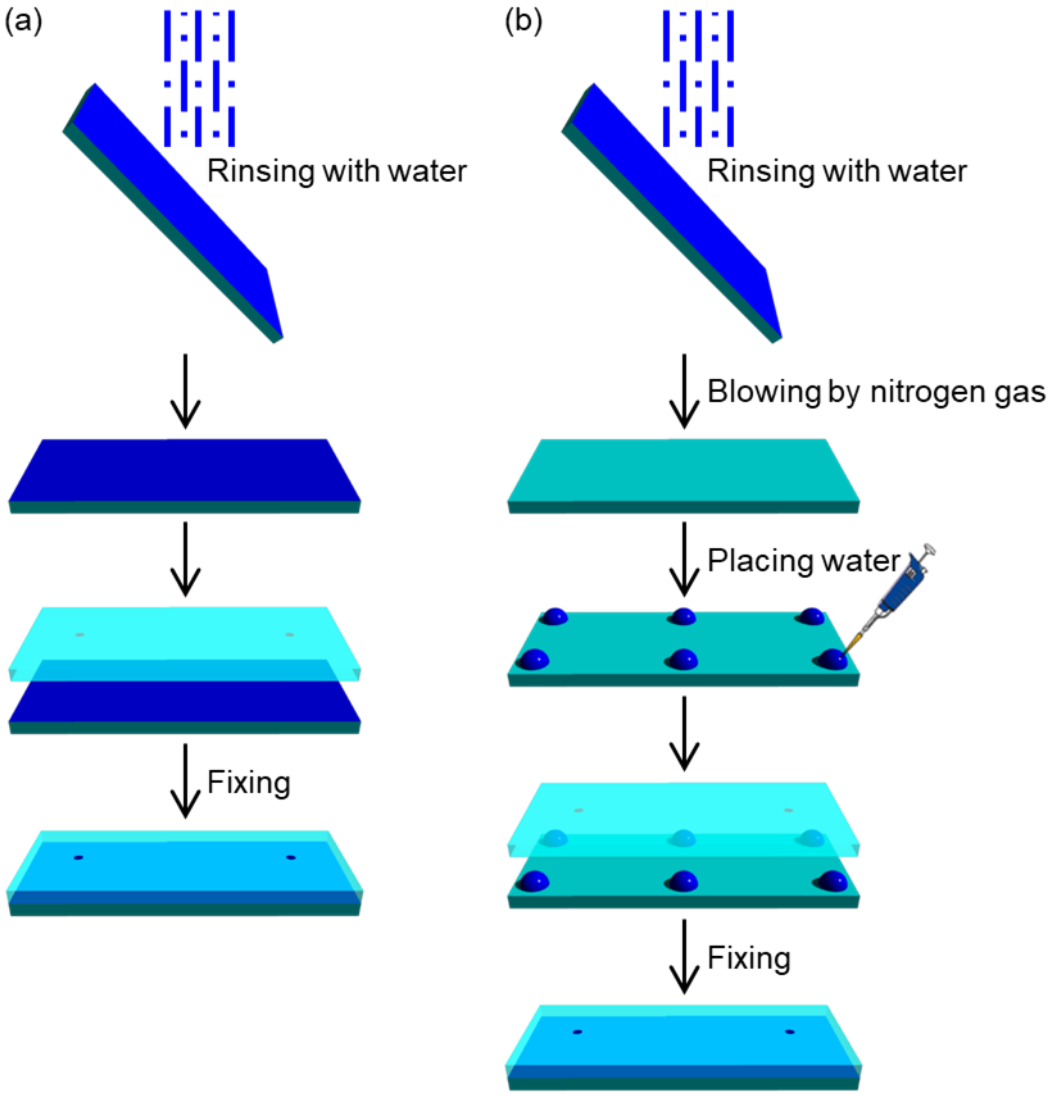

Fig. S1 Two procedures of the described wet glass-glass bonding method. (a) Whole-surface wet bonding procedure. (b) Water-droplet bonding procedure.

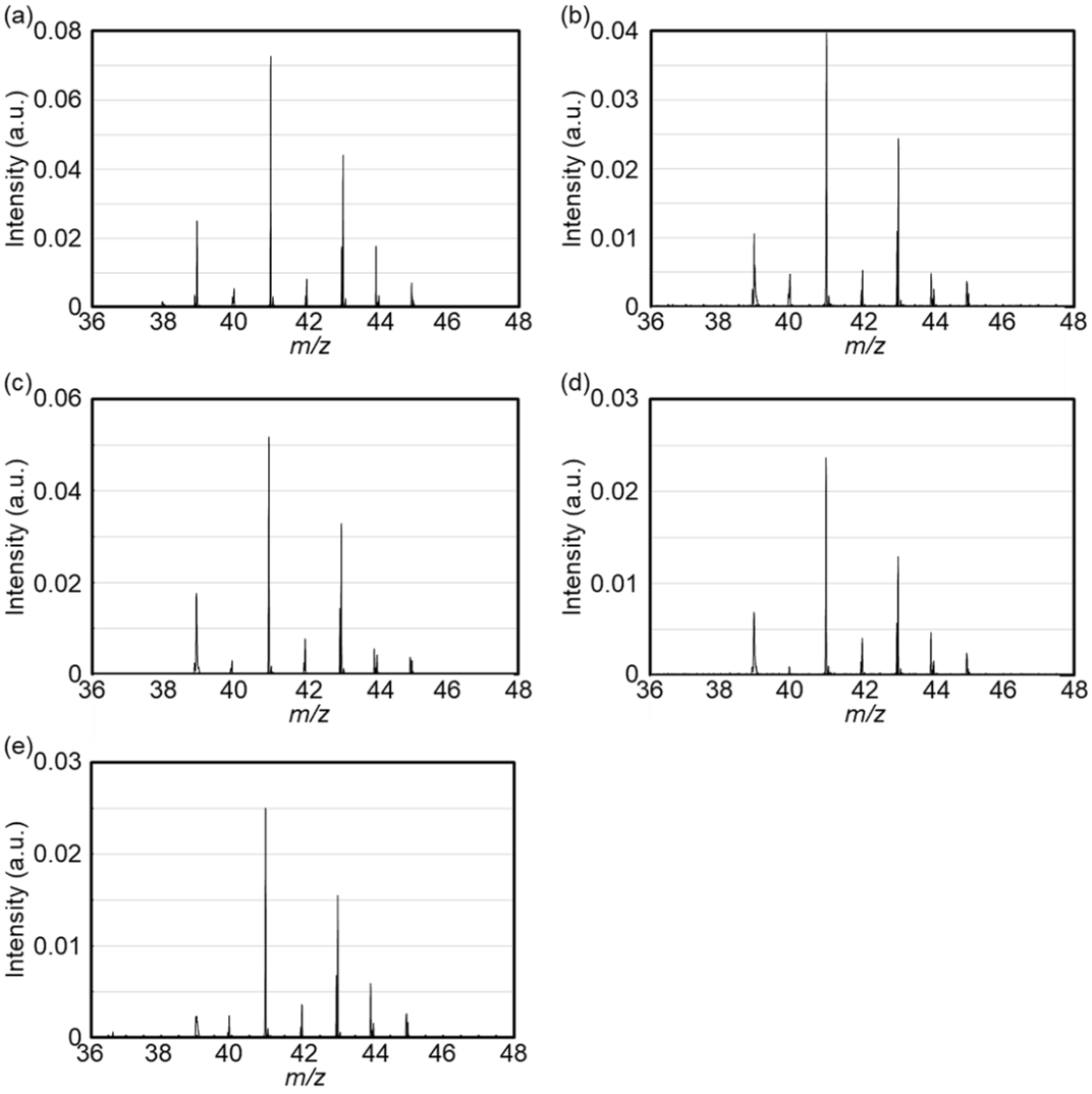

Fig. S2 Mass spectra in absolute intensity for glass surfaces with different conditions, obtained by sputtered neutral mass spectrometry (SNMS). (a) An untreated glass surface, (b) a glass surface rinsed by water only, (c) a glass surface cleaned by detergent, (d) a glass surface cleaned by piranha solution, and (e) a glass surface cleaned by oxygen plasma.

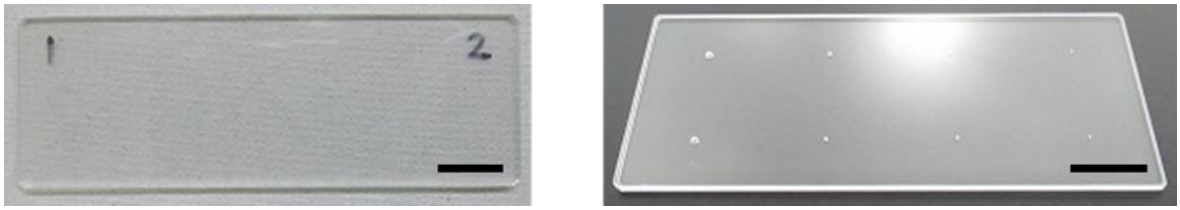

Fig. S3 Photographs of soda-lime glass plates (left) and fused silica plates (right) after detergent treatment and glass-glass bonding. The black bars indicate 1 cm.

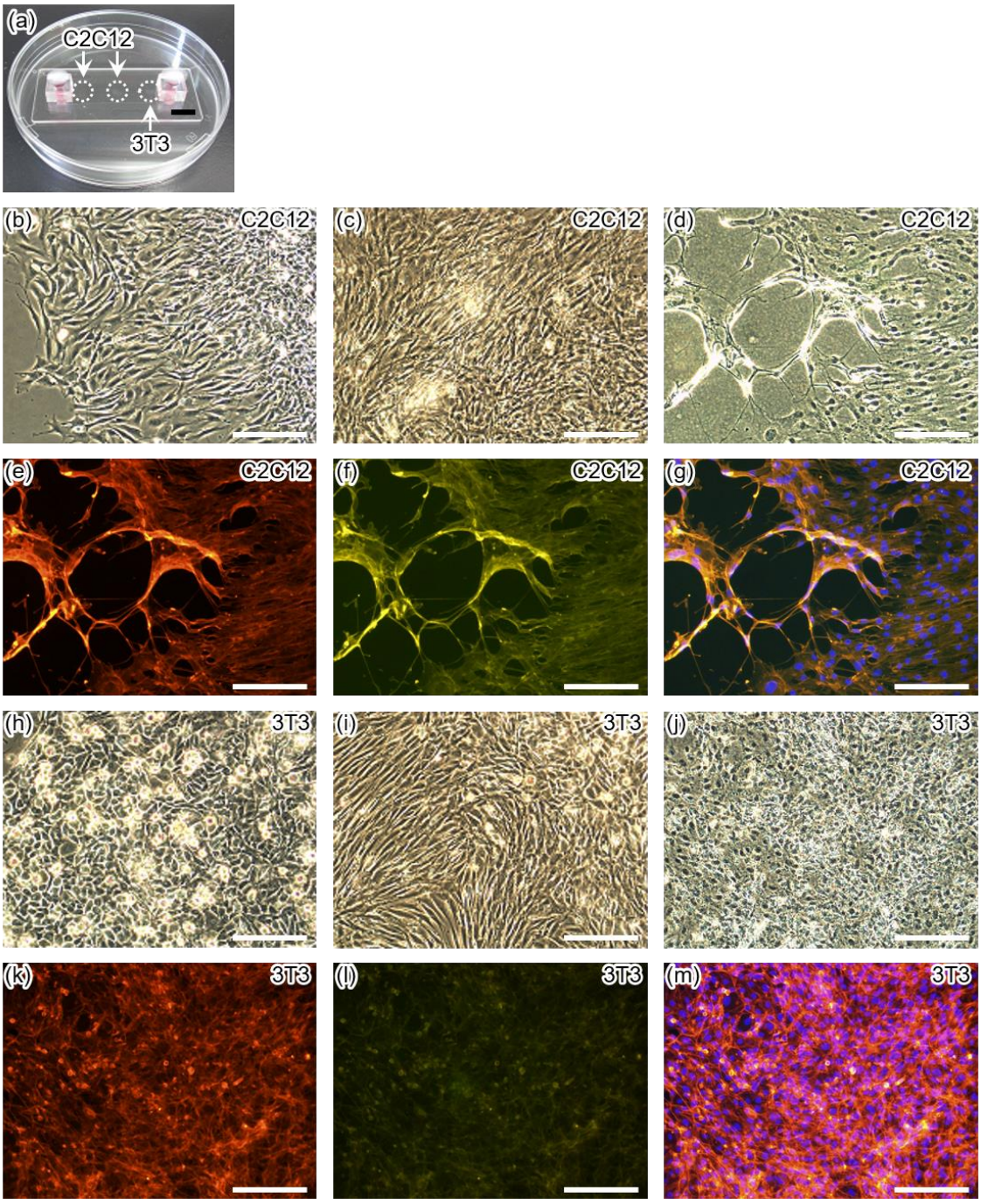

Fig. S4 Cell cultivation and differentiation induced in a channel. (a) A photograph of a glass microfluidic device containing C2C12 and 3T3 cells. (b-d) Microphotographs of C2C12 cells after (b) 1-day, (c) 4-day, and (d) 10-day cultivation. (e, f) Fluorescence images of C2C12 cells with stained (e) actin and (f) myosin. (g) A merged image generated from fluorescence images of C2C12 cells with stained nuclei (blue), (e), and (f). (h-j) Microphotographs of 3T3 cells after (h) 1-day, (i) 4-day, and (j) 10-day cultivation. (k, l) Fluorescence images of 3T3 cells with stained (k) actin and (l) myosin.

(m) A merged image generated from fluorescence images of 3T3 cells with stained nuclei (blue), (k), and (l). The black and white bars indicate 1 cm and 200 μm, respectively.