Electronic Supplemental Information

A packaging method of molecule/cell-pattern in an open space into a glass microfluidic channel by combining pressure-based low/room temperature bonding and fluorosilane patterning

S. Funano,‡ N. Ota,‡ A. Sato, and Y. Tanaka*

Quantitative Biology Center (QBiC), RIKEN, 1-3 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: yo.tanaka@riken.jp

‡ These authurs are equally contributed to this article.

● The detailed experimental procedures

Channel fabrication

Glass plates (TEMPAX Float, 30 × 70 × 0.7 mm, Matsunami Glass Industry, Osaka, Japan) 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, Tokyo, Japan). The exposed photoresist layer was developed with a developer (NMD-3, TOKYO OHKA KOGYO, Kanagawa, Japan). 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, Wako Pure Chemical Industries, 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.

Glass bonding

Glass plates were treated with piranha solution, vacuum oxygen plasma at an intensity of 25 W and oxygen flow rate of 5 mL min^{-1} for 1 min in the chamber of the compact etcher, or 35% HCl to activate the glass surfaces. After the glass plates were dried with blowing by nitrogen gas, two glass plates were aligned manually for pre-bonding. Pre-bonded glass plates were set on a bonding machine (WA-100R, Bondtech, Kyoto, Japan) in order to apply 450 N to form glass-glass bonding.

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 or ink 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 non-hydrophobic areas

was transcribed on the glass plates from a photomask with the mask aligner. The exposed photoresist layer was developed with the developer. In case of using ink, pattern was drawn with a pen on the glass plates. The glass plates coated with photoresist or ink 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 30 min.

Protein patterning

10 μg mL^{-1} fluorescent dyes (Alexa Fluor 555 or Alexa Fluor 488) conjugated with bovine serum albumin (F-BSA) solutions were deposited on non-hydrophobic areas of a glass plate with a micromanipulator (MMN-1 and MMO-203, NARISHIGE, Tokyo, Japan) and a microinjector (CellTram Air, Eppendorf, Hamburg, Germany). After deposition, the glass plate was bonded. Bright field and fluorescence images were observed with an inverted system microscope (IX71, Olympus, Tokyo, Japan) equipped with a charge-coupled device (CCD) camera (24-bit RGB color) and filters (filter combinations of excitation/emission are 530-550 nm (bandpass)/575 nm (longpass) and 460-495 nm (bandpass)/510 nm (longpass)).

Cell patterning

A glass plate was placed on a heater set at 37 °C. Polydimethylsiloxane (PDMS) (SILPOT 184, Dow Corning Toray, Tokyo, Japan) pools were placed on non-hydrophobic areas of the glass plate. Myoblast cell line C2C12 cells and fibroblast-like cell line 10T1/2 suspensions in Dulbecco's modified Eagle's medium (D6429, Sigma-Aldrich, St. Louis, MO, USA) with 10 v/v% calf serum (12133C, Nichirei Biosciences, Tokyo, Japan) and 1 v/v% penicillin and streptomycin antibiotics (168-23191, Wako Pure Chemical Industries, Osaka, Japan) were introduced into the pools. After cell attachment (3 h later), most of medium was aspirated while small amount of medium was left on the cells. Then PDMS pools were removed from the glass plate to bond with another glass plate treated by piranha. After bonding, medium was introduced into a channel. The cultivation was conducted at 37 °C under humidified conditions with 5% CO_2 for 5 d. The supernatant was changed with fresh culture medium every day during the subsequent cultivation. Cells were observed with the inverted system microscope.

● Table S1

Treatment	Bonding strength measured by	Bonding strength (MPa)	Fluorosilane patterning	Protein/cell patterning	Surface cleanliness	Reference
Oxygen plasma	Separating glass plates (crack-opening)	0.28 ± 0.06	×	×	○	19
Piranha + HF gas	Separating glass plates (shear force)	0.30 - 0.60	×	×	○	20
Sulfuric acid (Wet)	Separating glass plates (shear force)	0.72 ± 0.04	○	×	○	22
Oxygen plasma + Pressure (Dry)	Water leakage (water pressure)	0.17 ± 0.03	×	×	○	This study
HCl + Pressure (Dry)	Water leakage (water pressure)	0.13 ± 0.01	○	○	×	This study
Piranha + Pressure (Dry)	Water leakage (water pressure)	0.11 ± 0.02	○	○	○	This study

Table S1 Characteristics of glass treatments for production of glass microfluidic devices at room temperature. Only piranha treatment in our study was suitable for chemical patterning and cell culturing on a glass microfluidic device. The values of bonding strength of glass microfluidic devices in these studies were in the same magnitude of the order, regardless of chemical treatment. Meanwhile, different tests applied for checking bonding strength of glass plates could be a major cause of the difference in the values of bonding strength. The values of bonding strength in the present study were obtained when it observed local leakage of water while the glass plates were still bound strongly at the area away from water leakage. Thus, the values given in this study could be lower in general than those in the other studies that employed shear force test or crack-opening test for separating the glass components entirely.

● Figure S1

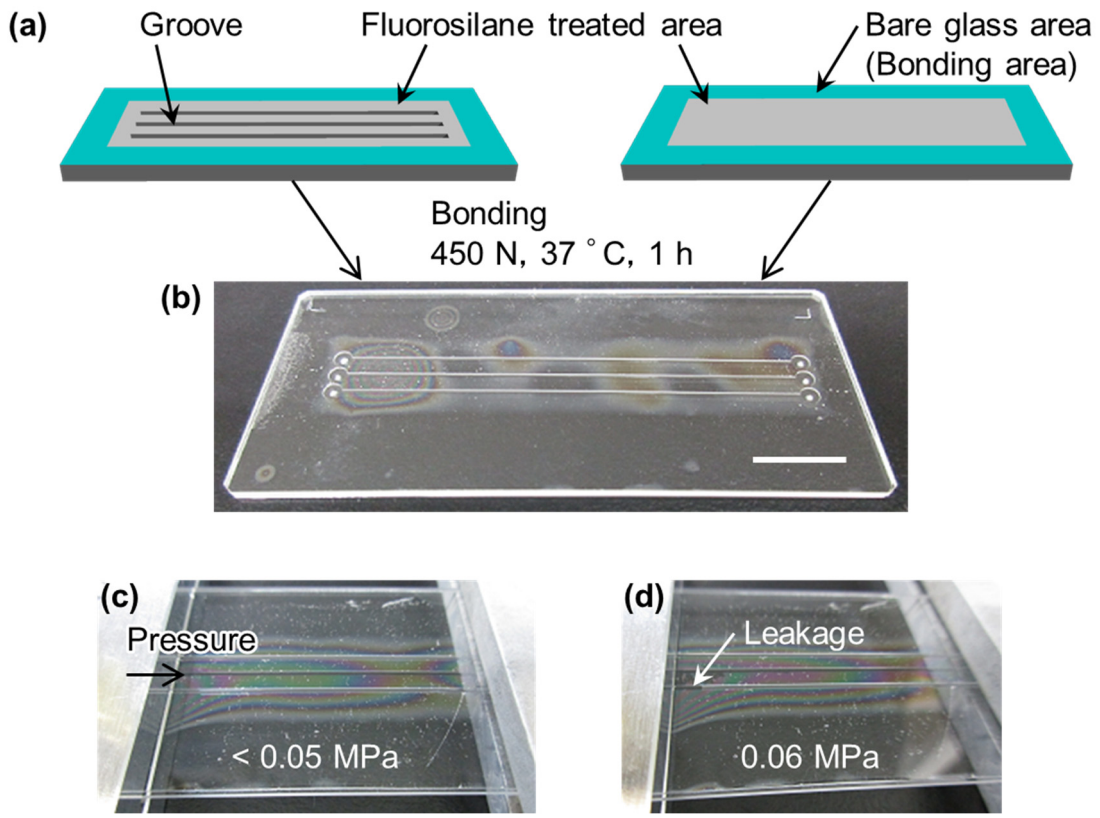

Fig. S1 Investigation of the pressure durability of glass microchip when the surfaces around microchannels were treated with fluorosilane. (a) The images of the glass plates before bonding. White areas indicate fluorosilane treated areas. The left side plate has grooves. A glass plate with micro-grooves fabricated by HF etching (0.5-mm width and 0.25-mm depth) and a cover plate were prepared. The bare glass parts were bonded. (b) The photograph of the glass microchip after bonding. It was observed that the glasses were not bonded together around microchannels. Part of the surfaces of the above two glass plates were treated with fluorosilane and two glass plates were treated by piranha solution. The two glass plates were bonded by applying 450 N for 1 h at 37 °C. The white bar indicates 1-cm length. (c) The photograph at the time of pressure test. For pressure test, water was loaded into the central channel and pressure was applied to the central channel through pressure controller. This detailed operation is indicated in the main text and Fig. 2. (d) The photograph at 0.06 MPa. Water leakage was found in next channel by applying 0.06 MPa. Although the pressure durability in this method is weaker than the case of usual glass bonding, it is sufficiently stronger than the case of PDMS bonding. This pressure durability is sufficient for cell experimental applications.