Detachable glass micro/nanofluidic device

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ABSTRACT

Glass is one of the most ideal materials for micro/nanofluidic devices due to its excellent optical transparency, resistance to a wide range of solvents and reagents, and easy to modify surfaces by silane-coupling reagents. From a practical point of view, glass is a hard material and is suitable for real applications. One of the advantages of glass is its reusability; however, this reusability is difficult to realize in certain conditions. Washing or re-modification of micro/nanofluidic channels is sometimes difficult due to the ultrasmall space in these channels. If the glass devices are detachable, it is easy to access the channel surface, and the channels can be cleaned and re-modified. When the substrates are bonded again, the devices are fabricated easily without repeating laborious and expensive micro/nano-fabrication processes. This technology gives researchers and users a choice of glass substrates in fundamental research studies and real-time applications. In this study, we propose a detachable glass micro/nanofluidic device by our low temperature bonding method. The surface bonding energy is controlled to realize both high pressure capacity for micro/nanofluidics and easy separation of glass substrates without fracturing. As a result, at least four times detaching and bonding is confirmed.

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INTRODUCTION

Microfluidics is a rapidly developing field with a wide range of practical applications. Currently, nanofluidics utilizing 10–100 nm space is developing.¹ In nanofluidics, unique chemical operations are realized, such as concentration,² ion rectification,^{3,4} and so on. In addition, unique liquid properties are reported. As for device applications, single cell⁵ and single molecule analytical devices⁶ are realized by taking advantage of the ultra-small space at the fL-aL scale.

The choice of the substrate material is important for fundamental research studies and device applications. Currently, polydimethylsiloxane (PDMS) is the most common material used in microfluidics.⁷ One of the reasons is that the fabrication does not require expensive instruments. However, the optical property, resistance to organic solvents and reagents, and the flexibility of surface modification, which are frequently required, sometimes pose problems for PDMS. In addition, PDMS microfluidic devices are easily deformable, and their softness will make the fabrication of ultrasmall structures (e.g., nanochannels, nanopillars) difficult. Other materials utilized for the substrate are plastics (polycarbonate, polymethyl methacrylate, and cycloolefin copolymer), and the microchannels are fabricated by injection/compression molding

processes. However, these materials have similar problems associated with PDMS.

Historically, glass has been used in general chemistry and biology and is one of the ideal materials for micro/nanofluidic devices. Glass has certain advantages in the flexible fabrication process due to its resistance to organic solvents.⁸ Optical property and mechanical strength are also advantages of glass, which allows researchers to use many optical processes (e.g., ultrasensitive detection and photochemical reaction). Moreover, the surface modification, which is necessary for functionalization of the micro/nanofluidic devices, is easy owing to the high density SiOH group on the glass surface and the well-established chemistry of silane-coupling reactions. This also makes fluidic control easy by utilizing the well-controlled surface property (e.g., Laplace pressure).⁹

One of the drawbacks, however, is that glass devices cannot be easily reused in the current fabrication technologies. Normal glass apparatuses, such as flasks and beakers, can be washed and reused, because they are large and are easy to access the surface. Normally, glass microfluidics requires laborious etching and bonding processes, and expensive fabrication instruments/equipments should be readied for such processes. This is why it is desirable to reuse glass micro/nanofluidic devices from a cost perspective. However, several issues

FIG. 1. Low temperature glass bonding method.

need to be resolved before repeated use of these devices. The first is that the functional materials need to be partially modified on the surface to realize functional chemical processes. For example, an antibody is partially immobilized on the wall, and blocking reagents are also immobilized.⁶ After the experiments, an exchange of the functional materials is necessary for recovering the analytical performance. However, partial modification is difficult after bonding due to the ultra-small space.¹⁰ For this purpose, the substrates should be detached even after bonding to access the inner surface of the micro/nanochannels. The second issue relates to washing of the micro/nanofluidic channels. Usually, small particles adsorb on the wall of the channels, and clogging easily occurs when the channel size becomes smaller, as many researchers have found in their experiments. Washing the precipitates is sometimes quite difficult, as it depends on the nature of the clogging materials or their size. In this case, it is better to detach substrates for washing.

However, the substrates are normally bonded tightly to avoid liquid leakage, and detachment after bonding is a challenge in glass-based micro/nanofluidics.

In this paper, we present a detachable glass micro/nanofluidic device. For this, our low temperature bonding method was utilized and modified to control the surface energy. The strong bonding and detachment was established. As a result, we could achieve at least a 4-time reuse of the glass microfluidic devices. This method will strengthen the benefit of glass micro/nanofluidic devices both in fundamental researches and in real-time applications.

EXPERIMENTAL

Low temperature glass bonding method

Various methods of glass/glass bonding have been developed, such as anodic bonding,^{14–16} thermal fusion bonding,^{17–19} bonding using hydrogen fluoride,^{20,21} and low-temperature bonding with the plasma surface activation.^{11–13} Among them, the thermal fusion method and the hydrogen fluoride method were expected to be irreversible because both methods unified two glass substrates into almost one block of SiO₂. Although anodic bonding did not completely unify two substrates, this method required a conductive interlayer. The interlayer was sometimes unfavorable if the application depended on the characteristics of glass, such as optical properties, chemical resistance, and capacities to control surface functional materials. In contrast, low temperature bonding does not require interlayers and does not unify the substrates completely. Therefore, we apply the low temperature bonding method to realize detachable devices.

The basic principle of the low temperature glass bonding method was already reported. The glass surface is treated with oxygen plasma (hydrophilization) and fluorine atom (hydrophobization), as shown in Fig. 1. The bonding energy can be large when the concentration of fluorine atoms is controlled (the contact angle

FIG. 2. Concept of detachable glass micro/nanofluidic devices.

FIG. 3. Fabrication/evaluation process and investigated bonding conditions.

of water is approximately 30° for optimum conditions). The basic mechanism remains unclear, and one of the proposed mechanisms is as follows. Fluorine atoms make a hydrophilic glass surface hydrophobic, where SiOH is converted into SiF. Glass/glass substrate bonding requires –Si–O–Si– bonding between the two substrates, and dehydration reactions occur, resulting in H₂O release. For bonding, the produced H₂O should be removed from the glass/glass interface, and SiF plays an important role in adjusting the balance between the high density of –Si–O–Si– and the amount of H₂O produced. Temperature and applied pressure are other parameters to control the bonding energy. By controlling these parameters, the bonding energy can be adjusted to realize detachable glass micro/nanofluidic devices.

Procedure to reuse glass micro/nanofluidic devices

The basic concept of the detachable glass micro/nanofluidic device is illustrated in Fig. 2. To fabricate glass micro/nanofluidic devices, microchannels and nanochannels should be formed on a glass substrate. Typically, microchannels are formed by UV lithography processes and etching processes. Nanochannels are formed by the electron-beam lithography and etching processes. Each process includes many steps, and some of them require expensive instruments/equipment, such as the electron-beam lithography system, the plasma etching system, and the sputtering system. Moreover, if the design becomes complicated,

lithography and etching processes should be repeated, thus increasing the process and cost. After forming microchannels and nanochannels, glass substrates are cleaned. Then, if necessary, functional materials can be modified on the surface of the substrates. Finally, a pair of glass substrates is bonded together.

After using the micro/nanofluidic devices, it is desirable that the devices be used repeatedly, but nowadays this has become difficult. This difficulty is related to the substrate bonding method. Most of these methods focus on how substrates bond tightly to avoid leakage, and no report focuses on detachment of substrates after bonding. In this concept, we adjust the bonding energy based on our low temperature bonding method. After utilizing the device, the substrates can be detached. Then, we can start the cleaning process and avoid the laborious micro/nanofabrication processes.

TABLE I. Tested microfluidic devices and the bonding conditions.

	Chip 1	Chip 2	Chip 3	Chip 4	Chip 5
Surface activation	O ₂ /F plasma	O ₂ /F plasma	O ₂ /F plasma	O ₂ plasma	No plasma
Temperature (°C)	300	110	110	110	110
Pressure	0 Pa	2 MPa	0 Pa	0 Pa	0 Pa

FIG. 4. (a) Pressure capacity test and (b) detachment of substrate and surface energy calculation.

Repeated use experiments

Here, we designed experiments shown in Fig. 3. A pair of glass substrates with microchannels was cleaned and bonded together. With the fabricated microfluidic device, the pressure capacity of the microchannels was tested. Then, we tried to detach the substrates by inserting a razor blade between the two substrates

in water. If the substrates are successfully detached without any fractures, the substrates can be bonded again. To realize the detachable glass device, bonding conditions are the most significant factor. If the bonding between substrates is very tight, it is difficult to detach substrates without any fractures. If the bonding is very weak, it means that the microfluidic device has a low pressure capacity, making it difficult to drive liquids. Therefore, we investigated the bonding conditions.

To investigate the proposed fabrication processes experimentally, 10 pieces of glass substrates (fused-silica glass; 70 mm × 30 mm, 0.70 mm thick; VIOSILSX, Shin-Etsu Quartz Co., Ltd., Tokyo, Japan) were prepared. Five of those substrates had inlet holes and rectangular microchannels of 400 μm width and 4 μm depth. The others were plane substrates without channels. The microchannels were fabricated by UV lithography and plasma etching, as described previously.¹ Inlet holes were fabricated with a milling machine. Then, substrates were cleaned for 8 min in the piranha solution prior to bonding steps. Five pairs of plane substrates and substrates with microchannels were bonded together with various conditions shown in Table I. Chip 2 was bonded with a typical condition of low-temperature bonding methods. As described in a previous research,¹³ the cleaned substrates and Teflon pieces of 2000 mm³ were placed in a RIE (reactive-ion etching) plasma chamber. The oxygen gas was introduced into the chamber at a pressure of 60 Pa. The oxygen RIE plasma was generated with a 250 W discharge power for 45 s. The Teflon pieces induced fluorine gas to activate the surface. Then, the substrates were contacted and pressed with 5000 N force (2 MPa pressure). This condition was used in several previous studies and was known to bring sufficient pressure capacity.^{6,22} Based on this condition, we also investigated various conditions, including one with a higher temperature instead of no

TABLE II. Summary of results after first cycle bonding.

	Chip 1	Chip 2	Chip 3	Chip 4	Chip 5
Photograph of bonded substrates					
Fluorescent image before applying the pressure					
Fluorescent image after applying the pressure					
Intensity profile

FIG. 5. Results of detachment processes after the 1st cycle: (a) photo of chip 1 broken by inserting a razor blade, (b) photo of chip 3 detached without visible fractures, and (c) surface energy calculated by measuring the crack propagation length.

pressure (chip 1), a condition without pressure (chip 3), one without Teflon pieces (chip 4), and one without the whole plasma surface treatment (chip 5).

Measurement of the pressure capacity and substrate detachment

The setup to test the pressure capacity of fabricated microfluidic devices is shown in Fig. 4(a). The microfluidic devices were embedded in an aluminum chip holder (ICH-01K, IMT Co., Ltd., Kawasaki, Kanagawa, Japan). The vial including the sample solution (fluorescein sodium 20 μM) was connected to the inlet hole of the microchannels via a poly (ether-ether-ketone) tube (100-μm i.d., Upchurch Scientific, Oak Harbor, WA, USA) and a Teflon joint (0.6, Nihon Seimitsu Kagaku Co., Ltd., Tokyo, Japan). The sample solution in the vial was pressurized at 10 kPa using an air pressure controller (PC20, Nagano Keiki Co., Ltd., Tokyo, Japan) and delivered into the microchannels.

After the microchannels were filled with the sample solution, a stop valve was connected to the outlet of the microchannels. Then, microchannels were observed using a fluorescence microscope (IX71, Olympus) with a high-resolution charge-coupled device camera (Retiga EXi, QImaging). While observing the microchannels, the pressure applied to the sample solution was increased from 50 kPa to 500 kPa by 50 kPa steps every 10 min. By comparing the fluorescence profile before and after applying the pressure, the leakage around the microchannels was evaluated. Microchannels have a larger area than nanochannels and suffer from higher forces with the same applied pressure and suitable for a pressure capacity test.

To detach the substrates, a 100-μm-thick razor blade was inserted into the bonding interfaces of microfluidic devices dipped in water, as shown in Fig. 4(b). During the detachment process, the crack propagation length was measured to estimate the surface energy. This crack-opening method is widely used to evaluate the bonding strength.²³ The bonding energy can be calculated using the following equation:

$$\gamma = \frac{3Et^3y^2}{8L^4}, \tag{1}$$

where *E* is Young’s modulus for the fused silica substrate (6.6 × 10¹⁰ Pa), *y* is the half of the blade thickness (0.05 mm), *t* is the substrate thickness (0.7 mm), and *L* is the crack propagation length.

RESULTS AND DISCUSSION

The 1st cycle of the experiment

At the 1st cycle of experiment, all the substrates were successfully bonded. The photos of bonded microfluidic devices are shown in Table II. The inserted graphs describe the fluorescence intensity profiles along the cross section of the microchannels. In all conditions, no significant differences were observed between the intensity profiles around the microchannels before and after applying the pressure, indicating that there were no critical leaks at 500 kPa pressure, which was sufficient for various micro- and nanofluidic experiments. Then, we tried to detach the substrates of microfluidic devices.

TABLE III. Result of repeating bonding, pressure capacity test, and detachment.

		Chip 1	Chip 2	Chip 3	Chip 4	Chip 5
1st cycle	Bonding	Success	Success	Success	Success	Success
	Pressure capacity	>500 kPa	>500 kPa	>500 kPa	>500 kPa	>500 kPa
	Detachment	Broken	Success	Success	Success	Success
2nd cycle	Bonding		Success	Success	Success	Success
	Pressure capacity		>500 kPa	>500 kPa	>500 kPa	<450 kPa
	Detachment		Broken	Success	Success	
3rd cycle	Bonding			Success	Success	
	Pressure capacity			>500 kPa	>500 kPa	
	Detachment			Broken	Success	
4th cycle	Bonding				Success	
	Pressure capacity				>500 kPa	
	Detachment				Success

FIG. 6. Result of the pressure capacity test for chip 4 (4th cycle) and chip 5 (2nd cycle).

Although chip 1 fractured when inserting a razor blade, as shown in Fig. 5(a), chips 2, 3, 4, and 5 were successfully detached without visible fractures shown in Fig. 5(b). During the detachment process, the surface energies of the bonding of chips 2, 3, 4, and 5 were calculated by measuring the crack propagation lengths (Fig. 5).

Chip 2 was bonded with a typical condition of the low-temperature bonding method. Although chip 3 was bonded without applying pressure, the surface energy of chip 3 was similar to that of chip 2. The surface energy of chip 4, bonded without inducing fluorine, was lower than that of chip 2. The surface energy of chip 5, without plasma treatment, was even lower, as expected. The difference of the bonding energy was reasonable considering the surface treatment.

The 2nd or later cycle of the experiment

At the 1st cycle of the experiment, chips 2, 3, 4, and 5 were successfully detached. At the 2nd cycle, these substrates were bonded again with the same conditions as those of the first cycle, followed by the pressure capacity tests and detachment processes. The results of the 2nd or later cycle of the experiment are summarized in Table III. Chip 4 was successfully bonded and detached for at least 4 times (the maximum number was not investigated).

Moreover, no critical leakage was observed during the all pressure capacity test of the chip 4, while leakage was observed for chip 5 at the 2nd pressure capacity test (Fig. 6).

In contrast, chips 1, 2, and 3 were broken at the 1st, 2nd, and 3rd detachment process, respectively. After all, chip 4 allowed the largest number of repeating the cycle of bonding and detachment. No visible fractures were observed on the substrates of chip 4 after the 4th detachment. Therefore, this chip 4 might be bonded and detached again. Reproducibility of these results was confirmed by at least 2 repeated experiments for each chip.

DISCUSSION

Among the five bonding conditions, only chip 5 leaked during the pressure capacity test. Because chip 5 had the lowest surface energy, leakage was likely to be caused by very weak bonding. It was difficult to judge the reason of different pressure capacities of chip 5 between the 1st and 2nd cycles. In this condition, the surfaces were not treated by oxygen plasma, which would reactivate native oxides. Thus, the amount of native oxides on surfaces might depend on the initial state before the bonding. The unstable amount of native oxides might produce the different qualities of bonding.

Chips 1, 2, and 3 were broken during their detachment process. According to the fracture mechanics, the fracture of brittle materials including glass occurs when the stress intensity factor reaches the value specific to materials, referred to as the fracture toughness. The stress intensity factor K was given by the following equation:²⁴

$$K = F \sigma \sqrt{\pi a}, \quad (2)$$

where σ is the applied stress, a is the length of a crack existing on the surface of the specimen, and F is a parameter dependent on the specimen and on the crack geometry. When higher stress was applied or larger cracks existed on the surface, the substrates fractured more easily. According to the mechanics of materials, the maximum stress σ applied to the substrates during blade-insertion could be calculated by the following equation:

$$\sigma = \frac{3Ety}{L^2}. \quad (3)$$

Here, E is Young's modulus for the fused silica substrate (6.6×10^{10} Pa), y is the half of the blade thickness (0.05 mm), t is the substrate thickness (0.7 mm), and L is the crack propagation length. Although this equation was the function of L , we could rearrange the equation to the function of surface energy γ by using Eq. (1),

$$\sigma = \sqrt{24E \frac{\gamma}{t}}. \quad (4)$$

This equation indicated that, to detach the microfluidic device bonded with a higher surface energy, higher stress should be applied to the substrate, which increased risks of fracture. The surface energies of chips 2 and 3, which fractured during detachment processes, were higher than those of chips 4 and 5. Although chip 3 was successfully detached at the first cycle, the detachment failed at the third cycle. This difference might be due to the accumulation of cracks on the surface of glass substrates by repeating bonding and detachment.

The leakage of chip 5 indicated that very weak bonding was not suitable for micro/nanofluidic devices. The fractures of chip 1, 2, and 3 indicated that very stiff bonding was not suitable for detachable micro/nanofluidic devices. Therefore, controlling the stiffness of bonding was important to realize the detachable glass micro/nanofluidic devices.

CONCLUSION

We proposed and realized a detachable glass micro/nanofluidic device for the first time by modifying our low temperature bonding method. The bonding strength should be controlled to simultaneously achieve the sufficient pressure capacity and the detachment without any fractures. In the best condition, we could repeat the bonding and detachment of the glass device for at least 4 times. High

pressure at 500 kPa could be applied, which was sufficient for most micro/nanofluidic experiments. The detachable bonding method can compensate for one of the most serious drawbacks of glass devices, which is the laborious fabrication process, and also allows new applications by accessing the inside of the channel after chemical processes (e.g., chemical synthesis, tissue engineering). This method will strengthen the benefit of glass micro/nanofluidic devices both in fundamental research and in real-time applications.

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