Bonding of glass-based microfluidic chips at low- or room-temperature in routine laboratory

Lingxin Chen, Guoan Luo *, Kehui Liu, Jiping Ma, Bo Yao, Yongchen Yan, Yiming Wang

Department of Chemistry, Tsinghua University, Beijing 100084, China

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Abstract

A low- or room-temperature bonding method was developed for fabrication of glass-based microfluidic chips without the requirement of clean environment and programmed high-temperature furnaces. After fundamental pretreatments, the glass substrates and cover plates to be bonded were sequentially soaked in concentrated sulfuric acid, washed with high-flow-rate tap water, de-ionized water and treated using HF steam as a necessary step. Finally, the plates were bonded by bringing the cleaned surfaces into close contact either under a continuous flow of de-ionized water or directly when treated with HF steam, and annealing at low-temperature (<100 °C) or room-temperature. With the key improvements such as fabrication process optimization, gridding channel design and HF steam treatment, the glass-based chips could be obtained with fast, easy and high bonding yields (>90%). The bonding quality of the chips was evaluated by employing SEM, shear strength testing procedure and electric current measurement at different applied voltages. The mechanism for the strong bonding strength was presumably related to the formation of a hydrolyzed layer on the glass plate surfaces after soaking them in acid or water for extended periods. Microfluidic chips bonded by the above method were evaluated in the CE separation of monofuctional reactive dye Cy5-labeled bioamines.

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1. Introduction

Since the introduction of lab-on-a-chip devices in the early 1990s, glass has been the dominant substrate material for their fabrication. This is primarily driven by the fact that fabrication methods were well established by the semiconductor industry, and surface properties and derivatization methods were well characterized and developed by the chromatography industry among others. Several material properties of glass such as favorable optical properties and reproducible electroosmotic flow (EOF) properties make it a very attractive material for use in microfluidic systems; however, the cost of producing systems in glass is driving commercial manufacturers to seek other materials such as polymer-based to reduce cost and simplify manufacturing procedures [1,2]. In the past decade, various micro glass-based devices have been fabricated for chemical and biochemical applications including capillary electrophoresis (CE), capillary electrophorography (CEC), micellar electrokinetic chromatography (MEKC) and DNA separations [3,4].

The glass-based chips are usually fabricated by etching the channel manifolds into glass substrates using standard photolithographic and wet chemical etching methods, followed by the bonding of the etched plate with a flat cover plate. High-temperatures bonding processes are often employed. Glass substrate and glass cover plate are brought into close contact with each other forming closed channels under clean room conditions and are usually subjected to sophisticated temperature programming in a high-temperature muffle furnace at 620 °C [5]. A direct bonding technique has also been developed, by which the glass substrate and cover plate are first surface hydrolyzed and joined by hydrogen bonding and then anneal at 500 °C [3]. Yin et al. [6] have reported a high-temperature bonding method of glass plates under routine laboratory conditions. The disadvantages of the high-temperature bonding process are that it involves complicated manipulations requiring considerable expertise for achieving high yields and stringent laboratory conditions and hindered the fabrication of devices containing...
temperature-sensitive materials such as immobilized chemical arrays, structural features such as electrodes and waveguides, or materials with different coefficients of thermal expansion. Consequently, bonding is desired at a process temperature lower than 100 °C and manufacturing procedures simplified. In contrast to high-temperature bonding processes, low-temperature bonding of glass microfluidic chips usually involves the use of various adhesives below 100 °C.

There are now a number of low-temperature techniques in commercial use for wafer scale bonding in the microelectronics industry, as alternatives to the silicon direct bonding method [7], which requires a process temperature above 1100 °C to achieve good bond strength. Among these are anodic bonding, eutectic bonding and the use of low-melting temperature glasses for bonding [8]. Anodic bonding is commonly used to bond glass to silicon, silicon to silicon, or glass to metal with electric field assistance while heat is applied (typically 300 °C). Eutectic bonding of metals is performed with applied pressure at the alloy melting temperature, which is lower than that of the pure constituents. Various glasses with low-melting points, such as boron-doped glass (450 °C) [9] and glass frit (400–600 °C) [8] have been used as an intermediate layer for silicon-to-silicon wafer bonding. Quenzer and Benecke [10] have used a spin-on sodium silicate layer to bond two wet-oxidized silicon wafers together at 200–300 °C. Yamada et al. [11] have used commercially available spin-on glass containing Si(OH)x, where 2 < x < 4, as an adhesive to bond silicon wafers with silicon dioxide or silicon nitride surface films at 250 °C. Wang et al. [12] have developed a low-temperature bonding method using a spin-on 7–10% sodium silicate layer as an adhesive at 90 °C for 1 h or room temperature overnight for the fabrication of microchip devices. Nakanishi et al. [13–16] have presented a method using dilute hydrofluoric acid as an adhesive, and bonded the SiO2–SiO2 chip at room temperature assistant with pressures 0.01–1.3 MPa. Sayah et al. [17] have described two methods for direct pressure-assisted low-temperature bonding of glass substrates, involving epoxy gluing at 90 °C in the first method and exposure of the glass stack to high pressure in the 100–200 °C temperature range in the second. Chiem et al. [18] have proposed a room-temperature bonding procedure featuring stringent washing of the plate surfaces under clean-room conditions. Fang et al. [19] have developed very recently a simple, room-temperature bonding process for the fabrication of glass microfluidic chips.

In this paper, we present a simple low- or room-temperature bonding method for glass-based microfluidic chips with high-bonding quality and yield in routine laboratory, using a spin-on HF steam treated thin water film layer as an adhesive at process temperatures lower than 100 °C or room temperature.

### Table 1

The chemical constitutes of the glass substrates

<table>
<thead>
<tr>
<th>Type of the glass</th>
<th>Main chemical constitutes</th>
<th>Major constitutes</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Boro-silicate glass</td>
<td>α(SiO2) = 74.8%, α(B2O3) = 14.7%, α(Na2O) = 4.5%, α(K2O) = 0.5%, α(BaO) = 2%</td>
<td>α(SiO2) = 74.8%, α(B2O3) = 14.7%</td>
<td>China BJ40</td>
</tr>
<tr>
<td>Quartz glass</td>
<td>α(SiO2) ≥ 99.9–99.98%, α(Fe2O3) ≤ 0.002</td>
<td>α(SiO2) ≥ 99.9–99.98%</td>
<td>HCSY01</td>
</tr>
</tbody>
</table>

### 2. Experimental

#### 2.1. Apparatus, equipment and reagents for chip fabrication

The glass substrates (substrate size: 63 mm × 63 mm × 1.7 mm; SG 2506, Shaoguang Microelectronics Corp., China) coated uniformity with 145 nm thick chromium and 570 nm thick Az-1805 positive photoresist and the polishing glass cover plates with the same size bought in the same company were used for the bonding experiments. The chemical constitutes of the glass substrates used in our experiments were listed in Table 1.

A kind of low-cost film photomasks designed with CorelDRAW 9.0 (Corel, Canada) were printed by a laser phototyping machine (Katana 5055, Japan). The designed film photomasks were transferred by ultraviolet exposure lithography onto the glass substrate using a Photolithographic Machine (JKG-2A, Shanghai Xue Ze Optical Mechanical Co. Ltd., China). The small-drilled holes were performed using a High Speed Platform Drill Machine (Tianjin Fourth Machine Tool Factory, China). Thermal bonding for glass chips was performed in a programmable Vacuum Torrefaction Chamber (DZF-6020, Shanghai Jinghong Experimental Equipment Company, China).

The mixture of 0.4 mol L⁻¹ (NH₄)₂Ce(NO₃)₃ was used to dissolve Chromium layer, 104 mL 70% HClO₄, 6400 g (NH₄)₂Ce(NO₃)₆ and 1760 mL de-ionized water mixed together; The mixture of 1 mol L⁻¹ HF/NH₄F was used as glass etching solution, 1 mol L⁻¹ HF and 1 mol L⁻¹ NH₄F mixed together; 0.7% NaOH solution or ethanol was used to dissolve the photoresist. The concentrated sulfuric acid mixture H₂SO₄/H₂O₂ (4:1) was used to cleaned the probably contaminations on the plates surfaces.

#### 2.2. Fabrication procedure

Fabrication of glass-based microfluidic chips involves microchannels fabrication using a photolithographic and wet chemical etching procedure, followed by the bonding of the etched plate with a flat cover plate. Based on the fabrication process of glass-based chips previously described by Yin et al. [6], the detailed bonding process presented in this paper was shown in Fig. 1.

In the procedure, the film photomasks were designed with the main channels surrounded by gridding channels intersecting with each other (refer Fig. 2). The profile of the designed film photomasks was transferred by ultraviolet exposure onto a glass substrate (refer Fig. 2). After exposure the photoresist was developed in 0.7% NaOH and hard-baked for about 10 min at 110 °C, the exposed chromium channel on the glass sub-
strate was eliminated by 0.4 mol L\(^{-1}\) (NH\(_4\))\(_2\)Ce(NO\(_3\))\(_6\) solution, and the channels were chemically wet etched in a well-stirred bath containing 1 mol L\(^{-1}\) HF/NH\(_4\)F, the length of etched time determining the channels dimensions. Then, incise the glass to the sizes needed and drill 1-mm-diameter reservoirs at each main channel terminals on the etched glass substrate using an emery drill-bit forming reservoirs, the photoresist was eliminated by ethanol and the exposed chromium out of the channels was eliminated by 0.4 mol L\(^{-1}\) (NH\(_4\))\(_2\)Ce(NO\(_3\))\(_6\) solution. The glass substrate and cover plate were dipped into concentrated sulfuric acid H\(_2\)SO\(_4\)/H\(_2\)O\(_2\) (4:1) for about 12 h (notice: this step should be handled with great care using PTFE forceps, while wearing one-time protective gloves) and rinsed using high-speed tap water succeeded with de-ionized water, then the cleaned plates were immediately put into the chamber containing 1 mol L\(^{-1}\) HF/NH\(_4\)F solution (refer Fig. 1), and opened the tap water to pump a certain vacuum of the closed chamber for a few minutes, treating the plates with HF steam for about 15–30 min. Finally, the surfaces of glass substrates and cover plates were closed positioned together directly for bonding at low- or room-temperature (notice: this step should be handled with great care using PTFE forceps, while wearing one-time protective gloves). Considering the toxicity of HF, the temperature of the chamber was keep at the room temperature, and the experiments must be carried out at a ventilating cabinet.

2.3. Chip bonding quality testing

2.3.1. Surface roughness and bonded interface image

Atomic force microscope (SPA-300 HV Seiko Instruments Inc., Japan) was used to observe the surface roughness. Scan electron microscope (SEM KYKY2000, CAS) was used for imaging the cross-sectional view of bonded interfaces.

2.3.2. Shear strength test

The shear strength of the bonded glass-based chips fabricated with a bonding area of 21 mm \(\times\) 63 mm \(\times\) 1.7 mm was measured using homely-made clamp equipment [20], as schematic diagram shown in Fig. 3. The maximum shear strength endurable by the chips was not measured until the bonding failed, i.e. one plate of the glass-based chip was pushed with the force increasing until a bonding failure occurred.

2.3.3. High-voltage test

The voltage–ampere curve was an important parameter in evaluating the bonding quality of glass chip. A range of 1–20 kV
high-voltage power supply (DW-P203-1AC, Tianjin Dongwen Power Supply Factory, China) was used for measuring electric current in the separation channel at different applied voltages, and for endurance testing of the bonded chips under high voltage. The electric current was digitally displayed in the power supply, and recorded at every 10 min intervals. The endurance to high voltage of the bonded glass-based chips with cross-channel design was tested by continuously applying a voltage of 2000 V at two reservoirs connected by a 40-mm-long channel, 500 V/cm field strength, channel dimensions: 100 μm (top width) × 60 μm (low width) × 20 μm (depth) × 40 mm (separation channel length) and 10-mm-long sample channel, filled with 4 mM borate buffer, for 12 h. Potentials of 4–6 kV were applied for identifying an electrical breakdown value.

2.4. Chip performances in CE separation

An 8-channel high-voltage power supply (Shandong Academy of Chemical Engineering, China), variable in the range of 0–5000 V, was used for on-chip sample injection and CE separation. A 635 nm LIF detection device was homely-made for detection. Monofunctional reactive dye Cy5 hydrolysate (0.30 mmol L⁻¹, Cy5-OH) labeling reagent was prepared by dissolving a pack (labeled for 1 mg proteins) obtained from Amersham Pharmacia Biotech Inc (NJ, USA) in 1 mL dimethyl sulphoxide (DMSO), gridding nine times volume of 20 mmol L⁻¹ borate buffer (pH 9.3) reacting for 30 min at a room temperature. The concentration of Cy5 hydrolysate can be estimated by the [Cy5] = A₆₅₀ nm/250,000, where A₆₅₀ nm is the absorbency of the Cy5 at 650 nm. Stock solutions of 0.4 mmol L⁻¹ bioamine sample were prepared from putresine and cadaverin purchased from Sigma, by dissolving the two bioamine samples in sodium borate buffer (pH 9.3), respectively. The Cy5-labeled bioamines solutions containing 0.2 mM of each bioamine were prepared by mixing 1.0 mL of the stock solutions of each of the bioamines with same volumes Cy5 DMSO solutions, reacting for 30 min in the dark at room temperature. A mixture of the sample was prepared by mixing 0.5 mL of each of the labeled bioamine solutions and/or diluting to 20 mmol L⁻¹ borate buffer according the requirement. Sodium borate buffer (20 mM, pH 9.3) was used as the working electrolyte for micellar electrokinetic chromatography (MEKC) separation, by adding the lauryl sodium sulfate (SDS, Acros) and methanol (Merck) as additives. All the solution and de-ionized water were filtrate through 0.20 μm membrane.

The room-temperature bonded chip performances was carried out using a LIF detection and a multichannel high-voltage power supply. The microchannels on chips were treated carefully with 0.1 M NaOH, 1 M H₂SO₄, rinsed with de-ionized water and finally the buffer solution, before use. Buffer solution was filled into the buffer and waste reservoirs, and a sample with Cy5-labeled bioamines was filled into the sample reservoir. Platinum wire electrodes were inserted into the reservoirs. The sample was injected into the CE separation channel by pinched injection mode. A field strength of 300 V/cm was applied for CE separation, with an effective separation length of 35 mm.

3. Results and discussion

3.1. Key characteristics of chip fabrication process

3.1.1. Optimized chip fabrication process

The successful bonding chips are depend on stringent pre-bonding treatments. There are many parameters affecting the bonding process and, therefore, the bonding quality [12,18,19], for example, the cleanliness of the glass plates, the surface flatness of the glass plates, bonding temperature and pressure. The basic requirement for strong bonds to be achieved between the two surfaces of glass is the two surfaces must be brought into excellent quality intimate contact. To fulfill the requirement, the surface must be free of solid particulate matter or organic material remaining and thus, the bonding must be performed in a clean room environment. Surface roughness and flatness also affect the contact of the surfaces. Our experiences demonstrated that the effective protecting surfaces from any probably damage was the most important among these parameters.

Compared with the usually fabrication process described by Yin et al. [6], the chip fabrication process presented in this experimental work has put the steps of glass incising, hole drilling before the steps of photoresist eliminated and exposed chromium eliminated (refer Fig. 1). If the exposed chromium out of the channel and photoresist were eliminated by 0.7% NaOH or ethanol and (NH₄)₂Ce(NO₃)₆ solution, respectively, before the glass incising and hole drilling, the surfaces of the glass would probably have been hard damned even at a discreet operation. By only these minor arranged steps could protect effectively the surfaces of the glass plates from the probably mechanical damage. Moreover, the probably surface contaminants and those particulate remaining in the microchannel structures should be cleaned and removed using detergents, water-soluble organic solvents (e.g., ethanol and acetone), concentrated sulfuric acid, and high-flow-rate tap water [19]. These were important pretreatment steps in successful bonding the glass-based microfluidic chips.

3.1.2. Designed gridding channels for ventilation

The other important step involved the chip fabrication process was the design of cross-linking gridding channels surrounding the main channels (refer Fig. 2). The size of the intersected chan-

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**Fig. 3. Schematic diagram of the shear strength test experiments.**
nals was generally designed at about 1/10–1/3-fold of the main channels. These channels were effective in ventilating the water moisture during the chip bonding process, preventing the water blasting suddenly and sharply when at high-temperature bonding and/or accelerating the stabilized bonding process speed when at low- or room-temperature bonding, as shown in Table 2.

The small quantity of water moisture between the pair of glass substrate and cover plate could also generate blasting easily and finally effected the chips product ratio and quality when bonded at high temperature. Although by prolonging the preheating time at heating program could reduce the damage blasting, this was not an effective method. It was also found that excess water between the two surfaces of the glass resulted in the failure of the low-temperature bonding process, presumably due to the need for higher temperatures or longer annealing times for its removal. So the gridding channels design were an effective method in accelerating the bonding process speed at low- or at room-temperature bonding, shorten the bonding time.

### 3.1.3. No requirement of clean environment

In routine laboratory, there are thousands upon thousands micron and nanometer grains suspended in the air, and these grains adhere to the surface of the glass would affect the bond quality greatly, so the cleaned and hydrolyzed glass surfaces should not be exposed to an unclean environment and the chips bonding must be bonded in super clean room condition. In order to avoid the requirement for clean-room facilities, the effective methods, whether a high-temperature bonding process or room temperature bonding, glass substrates and cover plates were brought into contact under a continuous stream of de-ionized water flow to avoid contamination from the environment, before bonding [6]. Then the closed contact glass chips could anneal at a high- low- or room-temperature treatment, reliable bonding was achieved at low- or room-temperature with a much slow speed (refer Table 2). Although the room-temperature bonding procedure is sufficient for most chip-based applications, a stronger permanent bonding may be achieved by a subsequent high-temperature treatment if required. In that case, the present approach may be used as a pretreatment before the furnace heating process, i.e. with the room-temperature bonding method and then high-temperature treatment, and almost 90% bonding yield could be obtained. A more than 90% high-bonding yield was achieved in our routine laboratory using low- or room-temperature bonding method after the following step.

#### 3.1.4. Necessity of HF steam treated hydrolyzed plates surface

To achieve strong bonds between the two surfaces, the contacting surfaces must react with each other. After the treatment with the concentrated sulfuric acid, the surfaces would have a high density of Si–OH groups suited for hydrogen bonding when brought the two surfaces together during the high-temperature curing process [6] or room temperature process [19]. In our research, we found that the glass substrate and cover plate treated with HF steam was an important pretreatment step to enhance the glass-to-glass bonding product ratio.

When the surfaces of the glass substrate and cover plate were carefully treated according to the chip fabrication process, in the last stage, i.e., the plates were soaked in concentrated sulfuric acid overnight at room temperature and cleaned with high-speed-tap water and de-ionized water, the plate surfaces would be hydrolyzed with a thin layer of water. Then such plate surfaces were positioned discreetly into the closed chamber to be treated with HF steam (refer Fig. 1), the quality as well as the product ratio would improve greatly, as shown in Table 2. When the glass plate surfaces were put into the closed chamber containing 1 mol L$^{-1}$ HF/NH$_4$F solution, the very small amount of HF steam in the air within the chamber dissolved into the thin layer water of the glass surfaces, forming a hydrofluoric acid (H$_2$O-HF). The glass substrate and cover glass plate were brought close contact with each other, a spin-on HF thin water film layer would create between them and reaction taken place, as schematically shown in Fig. 4. The device could be annealed at either low temperature (<100°C) or room temperature. During the room temperature bonding, a bonding wave was observed once the two glass surfaces were brought into initial contact, and within a few seconds, the contacting wave had spread over the entire area. The cover plate could be removed using a thin blade after the initial bonding, but was very difficult to remove after the annealing process. Applying pressure of about 0.1–1 MPa during the annealing process by clamping the two pieces together also gave better bonding.

### Table 2: Effectiveness of gridding channels design and HF steam treatment

<table>
<thead>
<tr>
<th>Bonding temperature</th>
<th>Gridding channels design</th>
<th>HF steam treatment</th>
<th>Shear strength (N/cm$^2$) with three trials</th>
<th>Product ratio (%)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-temperature at 620 °C$^a$</td>
<td>No</td>
<td>No</td>
<td>0.8–1.2 × 10$^3$</td>
<td>~35</td>
<td>Water blasting easily</td>
</tr>
<tr>
<td>Low-temperature at 70–90 °C$^b$</td>
<td>Yes</td>
<td>No</td>
<td>3–8 × 10$^2$</td>
<td>&gt;40</td>
<td>No water blasting</td>
</tr>
<tr>
<td>Room-temperature at 20 ± 5 °C$^b$</td>
<td>Yes</td>
<td>Yes</td>
<td>2–6 × 10$^2$</td>
<td>&gt;96</td>
<td>No water blasting</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Bonding temperature</th>
<th>Gridding channels design</th>
<th>HF steam treatment</th>
<th>Shear strength (N/cm$^2$) with three trials</th>
<th>Product ratio (%)</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>High-temperature at 620 °C$^a$</td>
<td>No</td>
<td>No</td>
<td>60–100</td>
<td>&gt;90</td>
<td>2–3 h</td>
</tr>
<tr>
<td>Low-temperature at 70–90 °C$^b$</td>
<td>Yes</td>
<td>No</td>
<td>&lt;5</td>
<td>7–30 days</td>
<td></td>
</tr>
<tr>
<td>Room-temperature at 20 ± 5 °C$^b$</td>
<td>Yes</td>
<td>Yes</td>
<td>30–60</td>
<td>&gt;92</td>
<td>1–3 days</td>
</tr>
</tbody>
</table>

$^a$ Heating program: pre-heating up to 150°C, keep 150°C for 50 min; increase temperature up to 300°C, keep 300°C for 60 min; increase temperature up to 620°C, keep 620°C for 360 min and then decrease temperature down to 400°C, keep 400°C for 60 min.

$^b$ When the chips were bonding at low- or room-temperature, the chips should be positioned in a ventilated place.

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Compared with the sodium silicate adhesive [10–12], the advantage of the method presented here was that the HF steam could be controlled easily and conveniently, and more important was that the surfaces treated with HF steam were uniformity without any probably putridly fine particles accompanied by sodium silicate leading a liquid-logged in the channel. The direct using hydrofluoric acid as the adhesive could also lead the liquid-logged in chip with fine channels design. The surface of boro-glass surface after the cleaning described above was as smooth as that before the cleaning with the surface roughness of about 4–5 nm. When the boro-glass surface cleaned and treated with HF steam, the surface roughness was only a little rougher (refer Fig. 5). Moreover, the reactivity of the surface was proportional to the dilute concentration of the hydrofluoric acid. The concentration of hydrofluoric acid must be sufficient for the condensation reaction to occur, but not so much as to etch the channel, which increases the roughness and reduces the bonding efficiency.

3.2. Mechanism of low- or room-temperature bonding chips

The bonding mechanism for direct bonding of quartz glass (SiO$_2$–SiO$_2$) is now clear and commonly accepted. The two hydrolyzed quartz glass plate surfaces were initially held together by hydrogen bonding and then joined by siloxane bond formation during the annealing process either at high or low temperature, the Si–OH groups gradually dehydrated, forming siloxane bonds and terminating with a condensation–polymerization reaction [19]. When the quartz glass plates were treated with HF steam, there was chemical reaction,

$$\text{SiO}_2 + 4\text{HF} \rightleftharpoons \text{SiF}_4 + 2\text{H}_2\text{O}$$  \hspace{1cm} (1)

Accompanying by the SiF$_4$ and H$_2$O dried or disappeared, the two glass plates first dissolved and then unified immersed into one block of SiO$_2$. That was to say, the adhesion of the two pieces of quartz glass surfaces would reopen with the high-energy bond form. Low temperature condensation reactions between Si–OH groups to form Si–O–Si bonds had been appeared in the bonding process taken place between the two pieces of glass. The natural dehydration process was slow, apparently being determined by the speed of water evaporation from the bonded surface, so the design of gridding channels intersecting with each other was a necessary step.
The major raw materials of boro-silicate glass are SiO2, B2O3 and Na2O, and boro-silicate glass is generally consisted of \(\omega(\text{SiO}_2) = 70-80\%\), \(\omega(\text{B}_2\text{O}_3) = 6-15\%\), \(\omega(\text{Na}_2\text{O}) = 4-10\%\), \(\omega(\text{Al}_2\text{O}_3) = 0-5\%\), \(\omega(\text{BaO}) = 0-2\%\) and \(\omega(\text{CaO}) = 0-2\%\). For example, Pyrex-7740 glass (one kind of boro-silicate glass) is consisted of \(\omega(\text{SiO}_2) = 80.5\%\), \(\omega(\text{B}_2\text{O}_3) = 12.6\%\), \(\omega(\text{Na}_2\text{O}) = 4.5\%\), \(\omega(\text{Al}_2\text{O}_3) = 2.1\%\), \(\omega(\text{CaO}) = 0.1\%\). The boro-silicate glass used in this paper is \(\omega(\text{SiO}_2) = 74.8\%\), \(\omega(\text{B}_2\text{O}_3) = 14.7\%\), \(\omega(\text{Na}_2\text{O}) = 4.5\%\), \(\omega(\text{K}_2\text{O}) = 0.5\%\), \(\omega(\text{BaO}) = 2\%\) (refer Table 1). In boro-silicate glass, the trigonometry structure of boron oxide \([\text{BO}_3]\) is transformed into the tetrahedron structure of boron oxide \([\text{BO}_4]\) with the aid of the free oxygen supplied by sodium oxide \((\text{Na}_2\text{O})\) and the structure of boron is changed from the layer-shape to the frame-shape. The uniform and consistent glass can be easily formed with \(\text{B}_2\text{O}_3\) and \(\text{SiO}_2\) based on above changes. The integrality and tightness of the bora-silicate structure network would be enhanced when the \(\text{B}_2\text{O}_3\) is immerged into the network as the form of \([\text{BO}_4]\) or \([\text{BO}_3]\), especially, the network would be enhanced when the \(\text{B}_2\text{O}_3\) is immersed into the glass interfaces shown by the arrows were unified and by measuring electric current at different applied voltages.

### 3.3. Bond quality evaluations and practical application

The bonding quality of glass chips using the above process was evaluated by employing SEM. The cross-sectional view of bonded interfaces was shown in Fig. 6. As shown in the images of the figure, the glass interfaces shown by the arrows were unified immersed. The bonding quality of the chips were evaluated by employing shear strength testing, compared with that using a conventional high-temperature bonding method (refer Table 2), and by measuring electric current at different applied voltages. The bonding strengths of room-temperature (25 ± 5°C) bonded chips following different anneal periods were evaluated using a shear force testing technique. The effects of postbonding anneal time on bonding strength for chips treated with HF steam or only soaked in sulfuric acid then cleaned with water were studied. The measurements were made 1, 3, 10, 20 and 25 h after initial bonding, a total number of 36 separate chips were subjected to the tests, and the results were shown in Fig. 7. The final tests were conducted until a several days duration period, and then the maximum bonding strength were measured. Since each chip was destroyed during the test, the data for varying anneal times were collected from different chips.

On the one hand, for chips treated with HF steam of the pretreatment, bonding strengths increased on anneal times and gradually stabilized at a maximum value about 60 N/cm² only 1–3 days after bonding. About 90% of maximum bonding strength could be achieved after 24 h bonding, when the strength was sufficient for most applications. On the other hand, for chips soaked in sulfuric acid then cleaned with water of the pretreatment, bonding strengths increased also on anneal times and gradually stabilized at a maximum value about 50 N/cm² 3–7 days after bonding (refer Table 2). About 70% of maximum bonding strength could be achieved after 24 h bonding. Although the bonding strengths of chips treated with HF steam or soaked in water did not differ significantly, the yield for successful bonding of HF-steam-treated chips was significantly higher.

Endurances of two room-temperature bonded chips with similar channel dimensions to high voltage were tested by measuring electric current at different applied voltages, as shown in Fig. 8. With an applied voltage less than 2000 V, 500 V/cm field strength, the two voltage–ampere curves were a straight linearity relationship. With an applied voltage of 1000 V, the observed variation in current during a total testing time of 24 h for the two chips showed an average reproducibility of 3.5–4.0% R.S.D. The largest R.S.D. for a single chip was 4.0% \((n = 10)\). The longest continuously operated period during this study was 30 h, during which the current R.S.D. was 3.7% \((n = 30)\). Under an applied voltage of 2000 V \((500 \text{ V/cm field strength})\), results similar to those obtained under 250 V/cm field strength were obtained. Under applied voltages of 4–6 kV \((1.0–1.5 \text{ kV/cm field strength})\), constant currents were maintained for about 3–5 h. When higher voltages (>10 kV, 2.5 kV/cm field strength) were applied, excessive current fluctuations occurred.
Although the mechanical strength of low- or room-temperature bonding glass-based chips was a little lower than that of the high-temperature bonding, strong bonds between glass surfaces and good channel sealing had been achieved, which were sufficient for most chemical microchip applica-

Fig. 6. SEM images of the cross-sectional bonded interfaces. The arrows in images showed the glass interfaces and the lowest was the enlargement of the dashed rectangle in the middle image.

Fig. 7. Effect of annealing time vs. shear strength of glass-based chips using room-temperature (25 ± 5 °C) bonding method. The glass substrates and cover plates were (upper) all treated with HF steam and (lower) not treated with HF steam, before bonding.

Fig. 8. Electric current in separation channel measured as a function of the applied electric field strength.
4. Conclusion

We have developed an effective bonding method at low- or room-temperature for fabrication of glass-based microfluidic chips without the requirement of clean room environment and high-temperature furnaces. The optimized chip fabrication process can effectively prevent the probably damaged parameters from successful bonding, especially the mechanical damage. The strong bonding of glass-based chips can be obtained with easy and fast (only tens of hours are required) for stabilizing the bonding quality when the hydrolyzed glass-based plates are treated with HF steam, and with the help of the gridding channel design. The above low- or room-temperature bonded chips can certainly be subjected to a high-temperature treatment. The evaluation of the fabricated glass microfluidic chips by this method has shown that their performances could comparable to high-temperature bonded chips. The lower bonding product ratio and the high cost of the glass chip are the bottleneck in most laboratories. The simple and effective method for low-temperature fabrication of glass microchip devices described in this paper will be sufficient for most microfluidic operations, and useful for those who are engaged in glass-based chips fabrication.

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Biographies

Lingxin Chen received a B.Sc. from Department of Chemistry at Qufu Normal University in 1990, a M.Sc. from Department of Chemistry at Shandong University in 2000 and a Ph.D. from Dalian Institute of Chemical Physics, Chinese Academy of Sciences in 2003. Then he was a postdoctor at Department of Chemistry at Tsinghua University in 2005. His major research interests include micro chromatographic separation techniques (capillary HPLC, CE, CEC), microfluidic analysis systems, nanochannel techniques and chemical sensors.

Guoan Luo received a B.Sc. from Department of Biochemical Engineering at the China East Technology University in 1969 and a M.Sc. from Department of Chemistry at the same university in 1982. Then, he worked as associate professor and deputy director in the Lab of Chemical Analysis at China Medicine University. In 1990, he was invited to work as a Visit Professor and later Research Scientist in Penn. State University in U.S.A. Four years later, he returned to China and resumed his teaching career in Department of Chemistry at Tsinghua University as a professor and Ph.D. director. His research fields are the chromatographic analysis and the research and development of new drugs, mainly focusing on the research of TCM modernization theories as well as the researches of CE’s new theories, new methods and new applications. He is also researching on CE and its new methods and their application in bio-medicine applications, mainly about the theories of CEC and its application, immunoaffinity CE and CE chips.

Kehui Liu received a B.Sc. from Nanjing University of Science and Technology in 2000 and a M.Sc. from Department of Chemistry at Tsinghua University in 2004. Now he is a graduate student of Ph.D. in Beijing (National) Proteome Research Center, Chinese Academy of Military Medical Sciences. His research interests include micro total analysis systems, mass spectrometry and proteomics.

Jiping Ma received a B.Sc. from Fushun Petroleum Institute in 1993, a M.Sc. from Daqing Petroleum Institute in 1996 and a Ph.D. in Dalian Institute of Chemical Physics, Chinese Academy of Sciences in 2003. Then she was a postdoctor at Department of Chemistry at Tsinghua University in 2005. Now she is an associate professor in Qingdao Institute of Technology. Her research interests include spectroscopy analysis, capillary column LC, preparation of HPLC or IC columns and SPME sample preparation technique.

Bo Yao received her B.Sc., M.Sc. from Department of Chemistry at Northeast University (China) in 1999 and 2002, respectively. Now she is studying in Department of Chemistry at Tsinghua University as a Ph.D. candidate and expects to get the degree in 2006. Her research interests include analysis of biomolecular such as DNA, proteins and cells in microfluidic chip systems.

Yongchen Yan received his B.Sc. from Department of Chemistry at Jinan University of China in 2003. Now, he is a graduate student of Qingdao University, and doing his research work of Chiral Functionalization and Application of Carbon Nanotubes in Department of Chemistry at Tsinghua University. His research interests include organic functionalization of carbon nanotubes and its applications in electrochemistry.

Yiming Wang received a B.Sc. from China East Science & Technology University in 1970. She was a lecturer in the Analysis Center of Chinese Medicine University from 1984 to 1991. She also worked as a visiting professor in the Department of Chemistry in Penn. State University from 1991 to 1994. In 1994, she returned to China, and is now a professor in the Department of Chemistry in Tsinghua University. Her research fields are the chromatographic analysis and the research and development of new drugs, mainly focusing on the research of TCM modernization theories.