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Simple approaches to close the open structure of microfluidic chips and connecting them to the macro-world☆

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Abstract

Microchip electrophoresis has a great potential to improve the speed and throughput of chemical and biochemical analyses. Conventional electrophoresis microchip fabrication methods comprise the main steps of channel formation, cover plate binding and access hole construction. While the fabrication of appropriate cover plates and their bonding process are quite essential to the creation of closed microfluidic networks, connection means of microchips to the macro-world is one of the most important parts of microchip fabrication. In this paper the most commonly used approaches are discussed for cover plate connector fabrication in conjunction with high and low temperature glue-less binding processes. The microchannels in the glass substrate were fabricated by sawing and powder blasting under regular laboratory settings, i.e., not necessitating the use of a clean-room environment, making in this way broader availability for electrophoresis microchip technology.
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Keywords: Microfabrication; Cover plate binding; Connections; General laboratory setting

1. Introduction

The advent of emerging microfluidics based bioanalytical methods represent a great promise for further increase in speed and throughput of electric field mediated separations [1]. Electrophoresis microchips have been developed based on well established techniques borrowed from the semiconductor industry, as channels, buffer reservoirs and other structural elements can be readily fabricated in glass substrates. Such electrophoresis microchips are then useable for electric field mediated analysis of a wide variety of chemical and biological samples [2]. The separated molecules are most frequently detected by confocal microscopy in conjunction with laser induced fluorescence, but other detection techniques, such as electrochemical are also broadly utilized. The short injection plugs and high electric field strengths used in microchip electrophoresis enable separations with very high efficiencies, just in seconds [3]. Microchip electrophoresis was reported for the analysis of almost all analyte classes [4]. Microfabrication was also adapted to liquid chromatography, referred to as chip-LC [5,6].

Traditional microfabrication procedures comprise photolithographic patterning, followed by etching the microfluidic channels. Then the patterned substrate surface had to be covered to attain a closed (sealed) channel structure. As the entire fabrication process of microfluidic chips is practically based on MEMS technology, bonding techniques of glass materials originate from silicon bonding [7]. Different types of glass substrates require different bonding conditions in terms of pre-treatment and bonding temperature programming profile. The binding strength increases with the individual chemical bond density between the substrates [8]. Increased bonding temperature form a larger number of Si–O–Si bonds, resulting in stronger attachments. One of the major factors controlling the bonding process is substrate surface smoothness. For glass substrates, high temperature fusion bonding with temperature programming is the most frequently used method [9] but room temperature bonding can also be attempted [10,11].

The way the channels are sealed also defines the type of possible chip connections, i.e., its interface to the macro-world for such steps as buffer and sample introduction, etc. The two major types of connections are lateral setting [12], and connections from the top of the chip through buffer reservoirs [13,14].

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Both configurations entail different fabrication approaches. Lateral connection of electrophoresis microchips to capillary tubes requires higher precision than producing connections simply by creating interface reservoirs on the top of a chip. The advantage of having capillary connections on a chip is that the plethora of fluid manipulation systems developed for capillary tubings can be readily applied to the micro device [12], while connections made through reservoirs require unique instrumentation for automated liquid manipulation [15].

In this paper we discuss a few approaches that can be used in any general laboratory setting (i.e. without a clean-room) for channel and cover plate connector fabrication as well as several cover plate binding options including low-temperature glue-less attachment. One of our goals with this paper is to provide an easy to use approach for general laboratories to extend their research capabilities towards microchip based separation systems.

2. Experimental

2.1. Chemicals and materials

For creating chips with sawn and/or powder blasted channels, simple microscope slides were used: 50 Lames Porte-Object from Menzel Glaser GmbH (Braunschweig, Germany) and Schott Ag (Feldbach, Switzerland). The sawn glass microchips were made of pre-cuts unbeveled float glass sheets with the dimensions of 76 mm × 26 mm (thickness 2 mm ± 0.2 mm) from Precision Glass and Optics (PGO, Iserlohn, Germany). The needles connected to the sandblasted microchip were from H. Sigrist + Partner AG (Uster, Switzerland). The fused silica capillary (50 μm i.d., 360 o.d.) was from Polymicro Technologies (Phoenix, AZ, USA). 353 NDT Epoxy glue from Epoxy Technology, Poliscience AG (Cham, Switzerland) was used to make irreversible bonding between the capillaries and the chips for the lateral connections. In case of for high temperature bonding, the microchips were placed on a 1 mm thick 100 mm × 100 mm 99.8% Al_2O_3 ceramic support (Metoxit AG, Thayngen, Switzerland). Sulfuric acid and hydrogen peroxide were from Fluka (Buchs, Switzerland).

2.2. Instrumentation

Fabrication of microchannels by sawing was accomplished by means of a Disco DAD 321 saw (Disco Hi-Tec Europe GmbH, Munich, Germany) equipped with a 70 μm resinoid blade. Sandblasting was made by means of a Model HP-2 system from Texas Airsonics Inc., Abrasive Jet Technology Blast (Indianapolis, IN, USA). The sandblasting, a mask was fabricated using a Nd-YAG ZigZag laser (1064 nm pulse length 100 μs-5 ms, 300 Hz, energy 10–500 mJ, focus diameter 12–60 μm, 500 W/cm^2) as a courtesy of Prof. R.-P. Salathé's group at EPFL (Lausanne, Switzerland). For all high temperature bonding processes a Heraeus K 114 programmable furnace (Kendro Laboratory Products AG, Geneva, Switzerland) was used. The microchips' quality was evaluated by a scanning electron microscope (SEM), model XLF 30 FEG from (Phillips, 22335 Hamburg, Germany) and X-ray Photon Spectrometry, Kratos Axis Ultra (Eppstein, Germany). X-ray Photon Spectrometry was used to measure glass surface composition and if the chip was not transparent after bonding, a polishing system Mecapol P220 U (Presi, Grenoble, France) was used for transparency improvement. For liquid manipulation testing, a syringe pump (kdScientific model 100, Fischer, Wholen, Switzerland) and an Agilent 1100 series G1312A Bin Pump (Agilent, Waldorf, Germany) were employed.

2.3. Cover plates and connections

Glass microchips with appropriate microchannels were sealed with a cover plate possessing the necessary access holes. Access holes were fabricated by sandblasting using a stainless steel mask. This approach provided a simple process with high speed drilling rate, attractive for rapid prototyping. A stainless steel mask was applied in the fabrication process of the lateral connections. Before the bonding process, the end sections of the channels were enlarged to accommodate lateral connections with fused silica capillaries. This was accomplished by sandblasting using a 1 mm thick stainless steel metal mask with the instrument parameters of 9.5 cm nozzle distance, 1.5 bar working pressure and 45 s duration (Texas Airsonics). Exact positioning was attempted by placing the cover plate on top of the channel structure and marking the connection positions. Each plate was subsequently patterned by sandblasting and the structured slides were assembled for bonding. Finally, the capillaries were attached to the connection spot by 353 NDT Epoxy glue.

2.4. Metal mask for reservoirs

First, holes were drilled in the protecting metal mask at the desired reservoir positions. The substrate was then protected with scotch tape on each side to avoid any scratch on the surface and the metal mask was fixed on the substrate with another tape prior to sandblasting. Sandblasting was accomplished by applying 3.5 bar pressure and 2 cm working distance (i.e., the distance between the nozzle and the glass plate) for 90 s to ensure appropriate mechanical etching. Once the hole was created, the tapes were removed.

2.5. Cover plate bonding

For surface cleaning and activation, a 5:1 sulfuric acid – hydrogen peroxide mixture was applied that also increased the hydrophilicity of the glass substrates [16]. This step was carried out at room temperature for 30 min. The slides were then thoroughly rinsed with de-ionized water and dried. The dry surface was carefully inspected for cleanness and the ones with no homogeneous transparency were discarded. Next, the two slides were assembled under distilled water in a tank, creating in this way dust free contact and ensuring the presence of hydrate layers on the surfaces. The assembled slides were then dried by means of forced air stream.

In high temperature bonding, the chips were placed on a 1 mm thick 100 mm × 100 mm 99.8% Al_2O_3 ceramic support usually in the middle of a Heraeus K 114 programmable furnace. Placing the chips close to the furnace wall resulted a high tendency of

chip breakage probably due to higher temperature gradient. If necessary, in some instances, the chip was covered with a second ceramic plate of a similar kind. Fusion bonding was carried out at 600 °C, slightly above the softening point of 585 °C. The following temperature profile was used for high temperature bonding: 25 to 110 °C in 8 min, 110 °C for 30 min, 110 to 500 °C in 34 min, 500 °C for 30 min, 500 to 628 °C in 11 min and 4 h at 628 °C. A 3 h cool-down period was programmed to reach room temperature.

For low temperature glue-less attachment utilizing simple adhesion forces, the glass slides were prepared the same way as described above, but with no surface activation step. The two slides were assembled under distilled water in a tank and dried at room temperature. In this process the slides were handled even more carefully, especially avoiding any possible surface scratching. The two slides were brought into contact without shifting once the first contact had been established, as moving the two slides already attached by adhesion could result surface damage. The bonding was visually tested by inspecting homogeneous transparency without interference fringes.

3. Results and discussion

3.1. Cover plate with lateral connections

Microchip preparation with lateral connections is a complex process including channel etching, access hole fabrication and wafer cutting, all requiring high precision. This latter was very critical to make sure of precise alignment of the capillaries with the access holes at the end of the microchannels. Access hole and capillary dimensions are depicted in Fig. 1, Panel A. To ensure good fluidic coupling between the microchip and the connection capillary, the microchannels had to be centralized. The three critical points of the connections are shown in Fig. 1, Panel B. (1) Connection section of the microchip with the sandblasted channel; (2) sandblasted connection aligned with the channel and on the top and bottom slide, (this alignment is fixed later by high temperature bonding); (3) capillary inserted from the side. The first step was the creation of the access holes on the chip (each slides top and bottom part have to be manufactured). This was followed by the alignment of the two slides and the insertion of the capillary. To get a higher number of channels in a single process, a metal mask was created with three channel patterns and another mask for the connections, having three connections in one mask. To use the same mask for the channels and connections was not advisable because each process needed different etching depths. The two masks must have had perfect overlay to ensure that the connections were exactly above the channel ends. In this process a slight misalignment of the two masks could result in failure of the entire process. To alleviate this problem, we focused on single channel configuration.

Gluing the capillary to the microchip can easily block the microchannels, as capillary forces may act on the glue. In other words, by simply placing the glue at the connection spot can easily result in channel clogging. To obtain strong and irreversible bond, the epoxy glue was cured at 100 °C. During the curing step, there is a certain transition stage in which the originally highly viscous epoxy becomes more fluid and enters into all small cavities. To overcome possible clogging, two gluing methods were developed: (1) the epoxy glue was placed on the tip of the capillary and cured either for 45 min at room temperature; or (2) only 3 min at 100 °C before the insertion of the capillary into the connection hole. Following this, the capillary and the chip were brought into close contact and placed into a 100 °C oven for 1 h. In this way, strong and irreversible bond was attained between the chip and the capillary. Note that over-cured glue does not provide good bonding. The above given temperatures and curing intervals are only indicative and should be optimized with regard to batch type, humidity, etc. Carrying out this process manually, i.e., alignment of the capillaries within a few micron tolerance may lead to a certain failure rate. Even when the alignments were fitted correctly, the final step to fix the capillary by gluing can represent a problem. It is important to note that epoxy gluing makes the capillary less flexible, and concomitantly more fragile. Also, due to the sandblasting process, the access holes always differed a little in size and shape, therefore, capillaries were fitted with or without the polyimide coating on their tips to accommodate actual access holes sizes.

To make the capillary connection process easier, a new generation of chips were prepared with reservoirs and only one side channel with a syringe connection (Fig. 2) to enable various filling process (such as capillary electrophoresis buffers and capillary electrochromatography stationary phases). The simple straight channel configuration with an injection cross was accomplished by sawing, which simplified the fabrication process by not necessitating clean-room environment. Using only one side channel with a needle connection also offered better tolerance with its larger inner diameter (300 μm i.d. needle versus 50 μm i.d. capillary).

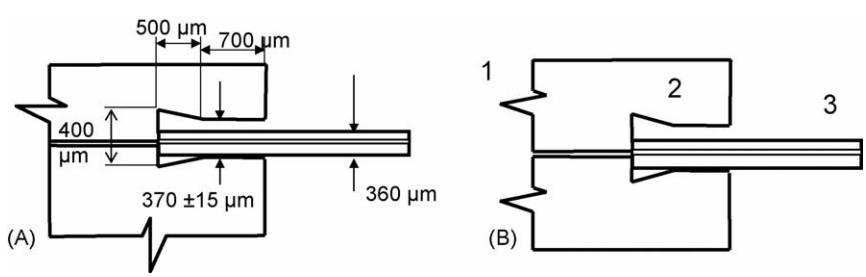

Fig. 1. Three critical points of lateral chip-capillary connections showing the top (left panel) and side (right panel) view of the microchip-capillary connection section.

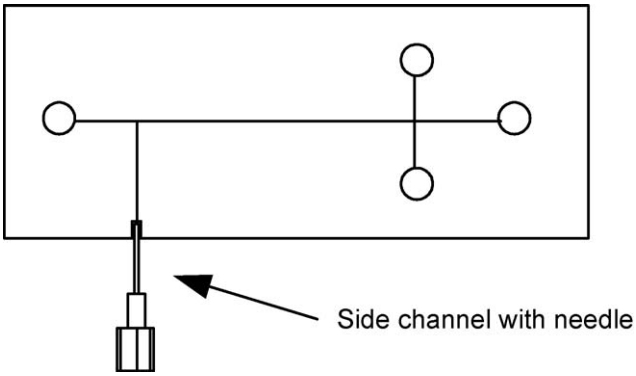

Fig. 2. Schematic representation of a microchip with reservoirs and one side channel connector.

3.2. Cover plate with reservoirs

Producing chips with reservoirs is much simpler than accommodating lateral connections. Reservoirs can be made by drilling or powder blasting the cover plate with appropriate alignment with the microfluidic channels. The same preparation method was used as described above, however, the access holes also served here as buffer reservoirs, therefore, were relatively large (2–3 mm in diameter) providing greater tolerance for alignment. Please note that powder blasting resulted in conically shaped holes with typical diameters of 3 and 1.5 mm on the top and bottom side, respectively (provided a 2 mm thick cover plate), as shown in Fig. 3. Using greater than 2 mm thick cover plate provided the option for larger reservoir volumes, although for capillary electrophoresis and capillary electrochromatography this volume may still not be sufficient. Another important fact is that sawn channels end in round longitudinal sections due to the circular shape of the rotating blade. Creating the reservoirs or lateral connections above this section easily solves the geometrical handicap.

3.3. Bonding procedure

The open channel structure generated by standard microfabrication procedures must be sealed at the top to create closed microfluidic networks. For adequate optical transparency, in most instances another glass slide (cover plate) is attached to the top of the channel structure. One of the most common techniques to provide a liquid-tight seal for microchips is high temperature bonding. The advantage of such thermal bonding process is uniform channel attachment accommodated by the same type of glass (Fig. 4, Panel A). Different ceramic plates were tested for surface inertness of the chip to be placed on for thermal bonding and the best results were obtained using Al_2O_3 ceramic support (there was no detectable contamination after bonding, measured by X-ray Photon Spectrometry). The fusion bonding temperatures were around 600 °C for the microscope slides, close to the melting temperature. A temperature program was used to heat up the oven to the bonding temperature. The heating speed was approximately 10 °C/min, with a 10 min stop at 110 °C for residual water removal. Some chips broke when the cooling period was not controlled properly. Similar effect was observed when a second ceramic plate was placed on the top of the chips during thermal bonding to apply a slight pressure and homogenous heat transfer on each side of the chip. However, this latter approach probably induced some temperature gradient during the bonding process, resulting in accumulated stress and concomitant cracking. As a first approximation we consider that the two slides were at different temperature when the fusion occurred resulting in different expansion characteristics. Also, after thermal bonding when the chips were at the same temperature, some slight bending was observed. To alleviate these problems, instead of using a second ceramic plate, the bonding temperature was increased with a few degrees and the ramp time was also increased for the heating and cooling periods. Even after these optimization attempts, non-homogenous bonding was observed in some cases for chips made of microscope slides as one can see the cloudy areas in Fig. 4, Panel B. The transparency of non-bonded parts were different with observable interferences. This defect was caused by the quality of the microscope slides as their flatness and composition is usually not strictly controlled during industrial production. Also, in some cases even under optimized conditions, the chips got completely destructed during the bonding process such as certain parts were bonded to the ceramic support. This phenomenon turned out to be due to the fact that the ceramic plate

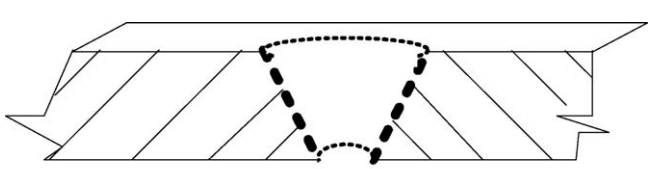

Fig. 3. Cross-section of the reservoir drilled by sandblasting.

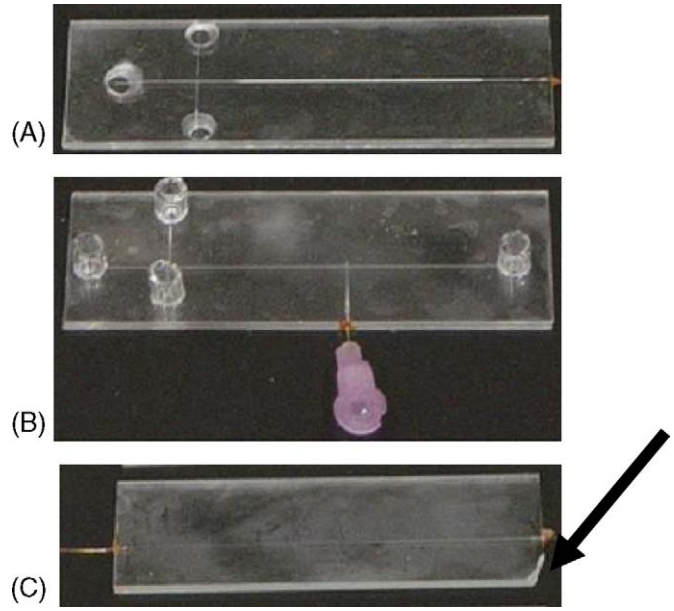

Fig. 4. Microscope slides with sandblasted channels and access holes attached by high temperature fusion bonding. (A) Uniform channel attachment; (B) non homogenous bonding; (C) effect of inappropriate ceramic support (breakage is shown by the arrow).

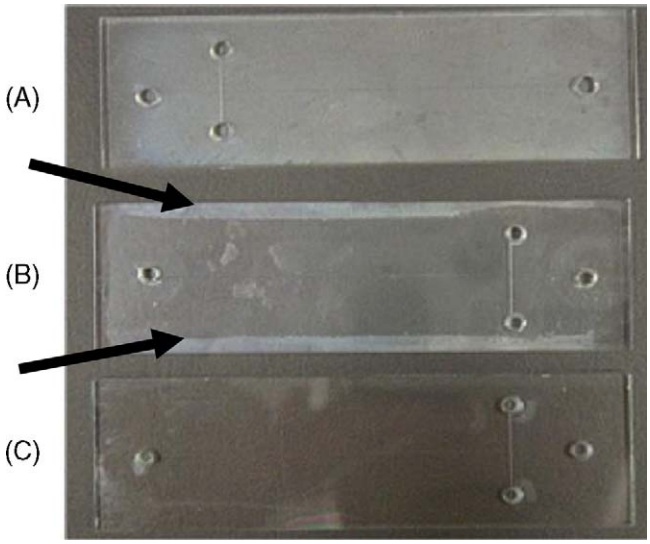

Fig. 5. Sawn channels on optical quality glasses. (A) The bonding results in opaque chips sometimes; (B) the side of the chip is not bonded (non bonded parts are shown by arrows); (C) good optical quality chip with low temperature glue-less adhesion.

was contaminated with another type of glass of a different melting point. The expansion characteristics may resulted in glass breakage due to the bonding between the contaminated surface and the chip having different thermal expansion. This suggests that the same ceramic support plate cannot be used with different type of glasses without appropriate surface renewal (achieved by polishing), as depicted in Fig. 4, Panel C, the microscope slides. The glass composition for the base and the cover plate must be the same to ensure that the two slides are at the same transition phase during temperature treatment. The stability of the furnace temperature was also very important, in our experiments ±2–3 °C. The spatial temperature homogeneity was also important to obtain good bonding quality. After several bonding processes without polishing the ceramic support unit the bottom part of the chip become opaque Fig. 5, Panel A. Even with the use of high optical quality glass (float glass sheets from PGO) there were cases, when the sides' top and bottom parts were not in contact, leaving the edge unbonded (Fig. 5, Panel B).

During low temperature glue-less attachment, when the two cleaned parts of the chip were simply put together under distilled water there was no apparent optical interference fringe observable after the cover plate assembly as depicted in Fig. 5, Panel C. This means that the entire chip – cover plate surface had good contact, therefore, using this chip without any high temperature bonding was possible. In general, we observed that it was easier to achieve good bonding with good optical quality glass, where manufacturer assured the appropriate flatness. Cleanness of the two surfaces was also crucial.

3.4. Comparison of the different connections

The different microchip connection approaches were compared by means of a pumping system. The microchip with reservoirs was connected to a high pressure support unit shown in Fig. 6. The capillary connection and the support unit were connected to an Agilent 1100 series pump for pressure testing. The syringe connection was connected only to a syringe pump. The lateral connection was able to stand approximately 60 bar, while the chip with the support unit did work up to more than 150 bar. The capillary connection proved to be more useful for open tubular separations than for packed or polymer based columns, where high pressure rinsing was important. One drawback of using small diameter connecting capillary (50 μm) is the bubble formation observed during liquid manipulation. This bubble formation was not observable with the 300 μm syringe connection.

Fig. 6. High pressure microchip connection module for the microchips with reservoirs.

4. Conclusions

In this paper we report on controlled microchip channel and cover plate fabrication without the necessity of a clean-room environment, using sawing and sandblasting for channel preparation and sandblasting for reservoir drilling. The drilled holes had conical shapes and rather rough surfaces. Using microchips with add-on reservoirs had the advantage of simple manufacturing but needed special connection units for liquid manipulation. The connections with capillaries and syringes were more complex and could stand lower pressure, but standard liquid manipulations could be readily applied. Both high temperature bonding and simple adhesion force approach were used for channel closure. Employing different glass qualities required different bonding temperatures and gave different results as shown in Table 1. In special cases, when the two glass slides (the top with the reservoirs and the bottom with the channels) were appropriately flat and smooth to assure homogenous contact of the whole surface, simple adhesion was used for binding. Bonding achieved without high temperature process should be advantageous in certain applications where surface modification or special structure integration is necessary, such as heat sensitive bio-components or special sensors, which do not stand the heat exposure during high temperature bonding. In microflu-

Table 1
The output of bonding for different glass types

Glass Manufacturer	Bonding temperature (°C)		Surface quality	Bonding output (%)
	No Top plate	Top plate		
MGG	595	590	Non controlled	50
Schott	603	595	Non controlled	65
PGO	628	620	Optical quality	90

Top plate means the Al_2O_3 ceramic support.

idics channel structure design, special attention should be paid to micro scale alignments and fittings.

Acknowledgments

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