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Technical Note

Microfluidic PMMA interfaces for rectangular glass capillaries

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Abstract

We present the design and fabrication of a polymeric capillary fluidic interface fabricated by micro-milling. The design enables the use of glass capillaries with any kind of cross-section in complex microfluidic setups. We demonstrate two different designs of the interface; a double-inlet interface for hydrodynamic focusing and a capillary interface with integrated pneumatic valves. Both capillary interfaces are presented together with examples of practical applications. This communication shows the design optimization and presents details of the fabrication process. The capillary interface opens up for the use of complex microfluidic systems in single-use glass capillaries. They also enable simple fabrication of glass/polymer hybrid devices that can be beneficial in many research fields where a pure polymer chip negatively affects the device's performance, e.g. acoustofluidics.

Keywords: acoustophoresis, capillaries, lab-on-a-chip, PMMA, system interfacing

(Some figures may appear in colour only in the online journal)

1. Introduction

The area of lab-on-a-chip is a growing research field, and has been so since the first papers were presented in the late 1990s [1, 2]. The aim of the research field is to develop miniaturized analysis systems for applications in fields such as biomedical and clinical diagnostics, food analysis and the forensic sciences [3–5]. Low fabrication costs and simple use are often required since the devices shall be used by non-experts. Another key for the success of such devices is to enable single-use systems to avoid cross-contamination in-between patient/analysis samples. Lab-on-a-chip systems shown in literature are often fabricated from different polymers such as PDMS, PMMA or cyclic olefin polymers (COC/COP) by different microfabrication methods [6–10]. These approaches allow for the fabrication of highly complex systems with integrated valves, mixing zones and analysis sites. However, there are technologies utilized in lab-on-a-chip devices where the mechanical and technical performance may suffer if polymers are used. Acoustophoresis is such a technology that utilises acoustic waves to create forces that can be applied to cell and particle sorting medium exchange and cell and particle trapping. Due to the high attenuation of sound in polymers, it is currently difficult to realize efficient devices for acoustophoretic applications in polymer chips [11]. An alternative approach is to use glass capillaries as the resonant part of the microfluidic system while the remaining chip can be made from polymer, creating a hybrid device. Using capillaries in acoustofluidic systems has previously been a successful concept [12–14]. This is advantageous since glass is a well-known material that is chemically very inert, has low acoustic attenuation and glass capillaries are commercially available at a low cost. Hybrid devices present several further benefits, e.g. simplified fabrication, lower material costs and the possibility to be used as disposable devices. However, all commercial capillary interfaces are designed for round capillaries, which are not suitable for acoustophoretic applications. The possibility to combine rectangular capillaries with more advanced fluidic functions can open up for new applications and help move existing silicon and glass platforms to disposable devices. There are further applications where a fluidic interface for rectangular capillaries will be beneficial,

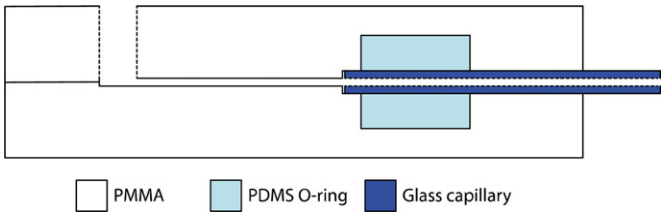

Figure 1. A schematic cross-section of the capillary fluidic interface. Two PMMA sheets are micro-milled to create the fluidics part. The capillary is inserted a short distance (0.7 mm) past the O-ring prior to bonding. The PMMA sheets are bonded together with the glass capillary and the PDMS O-ring to ensure a tight seal.

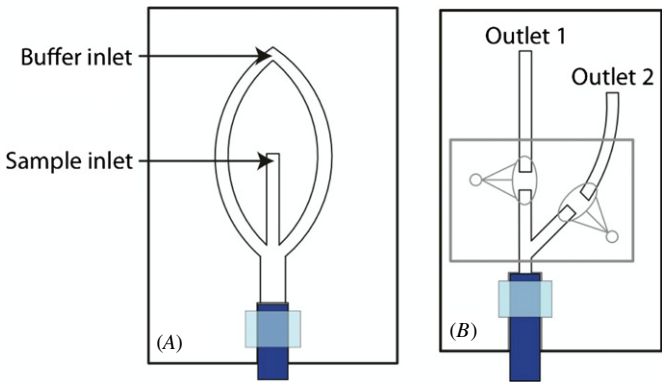

Figure 2. A schematic top-view of the two different capillary interface designs that were fabricated and tested. (*A*) has two inlets/outlets for hydrodynamic sample focusing or flow splitting of the capillary outlet while (*B*) has two inlets/outlets that can be individually addressed through pneumatic valves. The valve seats (drawn in grey) are situated in a separate PMMA layer, reversibly bonded to the top of the interface substrate through a 0.25 mm thick PDMS film.

e.g. in systems where optical imaging possibilities from the top/bottom are desirable.

Our main research focus is on acoustic trapping, as a means for sample concentration and/or sample handling and separation [15]. Acoustic trapping is obtained by forming an ultrasonic standing wave of ultrasound inside a micro-channel. As most particles and cells in standard buffer solutions have a positive acoustic contrast factor, they are attracted and retained at the potential energy minima and the kinetic energy maxima of the acoustic field as the solution passes through the micro-channel [16]. The generation of a standing wave inside a micro-channel requires very precise dimensions that need to be matched to the frequency of the ultrasound transducer. We have previously shown that glass capillaries with rectangular cross-sections are highly suitable for this application [17, 18]. The challenges met when working with rectangular capillaries is how to introduce a fluid flow in a repeatable and stable manner. There are several types of tubing connectors available on the market for round capillaries but not for capillaries of other cross-sections. These connectors are also limited to a single inlet/outlet.

This paper describes a method to create a microfluidic system using PMMA interfaces irreversibly coupled to rectangular borosilicate capillaries (VitroTubes, VitroCom, Mountain Lakes, NJ, USA). The PMMA interfaces can, however, be fabricated to fit the cross-section and dimensions of *any type* of glass capillary. The combination of PMMA interfaces with glass capillaries will result in a microfluidic system with high functionality, yet composed of cheap or disposable components. Two different designs of interfaces are shown in this work; one with multiple inlets/outlets and one with integrated pneumatic valves.

2. Materials and methods

2.1. Design

The functionality of the chip is determined by the combination of capillary interfaces that are connected to the capillary. The capillary can either be used open-ended, to allow for simple aspirate–dispense experiments, or it can have a PMMA interface attached at each end to allow for a more advanced microfluidic platform.

A moulded O-ring is fabricated to fit the specific capillary to ensure a tight fluidic seal. This O-ring is combined with a fluidics design micro-milled in PMMA. We have chosen to work with PMMA as it is a commonly used material in microfluidic systems. The principle of our design is however equally applicable to other polymer materials e.g. polycarbonate or cyclic olefin polymers. The O-ring is positioned in a recess in the PMMA chip that compresses the O-ring a distance of 70 μm when the two PMMA pieces are bonded. Due to the compression, the O-ring is allowed to expand slightly (50 μm) in the other two dimensions. The fluidics part of the PMMA chips can be designed in any desired way, see figure 1.

To demonstrate some of the possible designs, two different interfaces were fabricated and tested, see figure 2. The first design shows an interface with two inlets/outlets that enable hydrodynamic focusing or the use of multiple buffers in the same capillary. Hydrodynamic flow focusing has been shown to increase the acoustic trapping efficiency when working with cells and particles [19] and even more when working with sub-micron particles and bacteria [20, 21]. If the interface is used at the outlet of the capillary, it can be used to separate the flow into sample/waste collection.

The second interface is designed with two inlets/outlets that can be individually addressed using pneumatically actuated micro-valves [22]. The valves can be used either to select two different inlets (e.g. sample injection and washing buffer) or to collect the capillary outlet sample in different fractions (e.g. sample and waste).

2.2. PMMA interface fabrication

The PMMA interface was micro-milled from a 2 mm thick PMMA XT piece using an isel ICP 4030 milling machine (isel Germany AG, Eiterfeld, Germany) and a 2-flute 0.5 mm carbide milling tool (Performance Micro Tool, Janesville, WI, USA). The outer dimensions of the bonded chip were 22 $\times$ 31 mm^2 for the sample focusing interface and 17 $\times$ 27 mm^2 for the valve interface. The dimensions of the channel of the PMMA interface are designed to perfectly match the dimensions of the glass capillary but due to

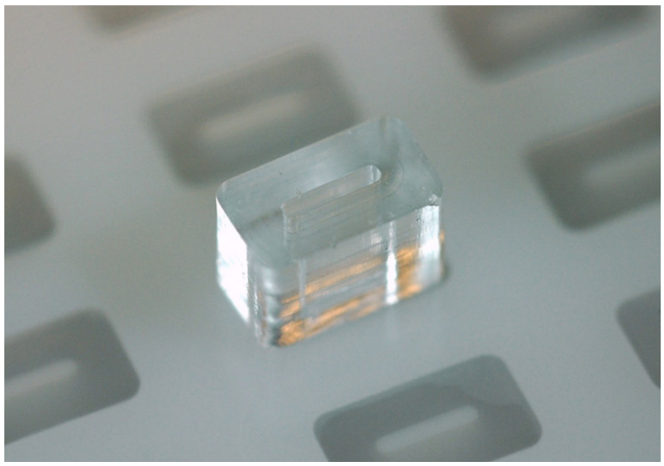

Figure 3. Picture of moulded PDMS O-ring on the milled O-ring mould. The external dimensions of the O-ring are $4.35 \times 2.53 \times 2.95$ mm^3.

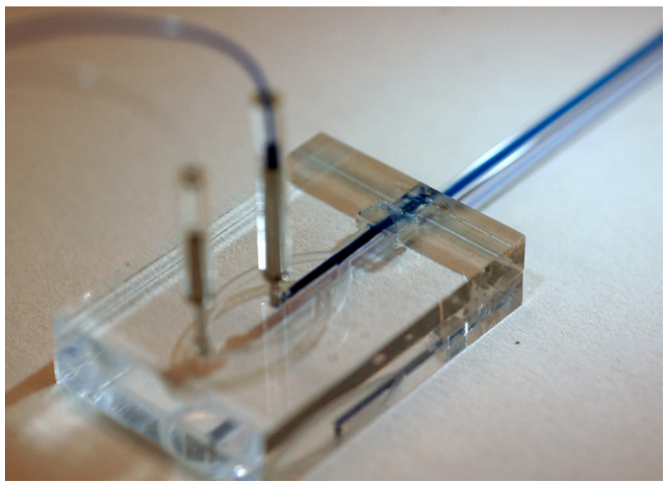

Figure 4. A PMMA capillary interface with two inlets connected to a rectangular glass capillary. A solution containing Evans blue is injected in the first/centre inlet and deionized water is injected through the second/side inlet. Here, the deionized water is used as a shear liquid for hydrodynamic focusing of the Evans blue solution.

variations in the capillary dimension, a maximal dead volume of 1 μL is possible. The capillaries used here were rectangular borosilicate capillaries with an inner dimension of 2×0.2 mm^2 (Vitrotubes 3520, Vitrocom, Mountain Lakes, NJ, USA).

2.3. O-ring fabrication

The inner dimensions of the O-ring are designed to be slightly smaller than the outer cross-section dimensions of the capillary in use (2.34×0.52 mm^2 for the 2.4×0.6 mm^2 capillary used here) to create a tight seal against the glass surface. The outer dimensions of the O-ring ($4.35 \times 2.53 \times 2.95$ mm^3) are decided by the thickness of the PMMA chips and the fabrication method used. For these O-rings, a mould was micro-milled in polyoxymethylene (POM), filled with PDMS 1:10 (Sylgard 184, Dow Corning, Midland, MI, USA), placed in a vacuum chamber for 20 min and cured at 80 °C for 1 h. Figure 3 shows a cured PDMS O-ring on top of the mould.

2.4. Interface bonding

Two different bonding techniques were tested; thermal fusion bonding and adhesive bonding. The thermal fusion bonding was performed by first subjecting the PMMA pieces to a 20 min cleaning/activation in a UV/ozone chamber (UV/Ozone Procleaner Plus, BioForce Nanosciences, Inc., Ames, IA, USA) before aligning the two PMMA pieces with the O-ring and capillary in place and clamping them between two microscope cover slides. The clamped chips were then bonded in an oven at 120 °C for 10 min and allowed to slowly return to room temperature. For the adhesive bonding, the PMMA pieces were bonded using a UV-curing adhesive (Norland Adhesives 68, Norland Products, Cranbury, NJ, USA). A thin layer of glue was applied to one of the milled PMMA interfaces and on the outside of the O-ring before it was positioned in the O-ring recess. It is, however, important not to use a too thick layer as this will block the fluidic channels. After applying the glue, the second PMMA interface was aligned and brought in contact with the other chip and clamped in place. The interface was visually aligned and cured in the UV/ozone chamber for 10 min. Successful bonding was determined as a bonding that was not showing any leakage at a flow rate of 3 ml min^{-1} at the PMMA-capillary interface or at any point of the microchannel in the PMMA interface. Debonding of the chips was also not possible without breaking the chips.

If the fluidics design consists of very shallow channels, thermal fusion bonding is recommended to avoid the risk of getting adhesive in the channels. Adhesive bonding requires a skilled technician since it is very easy to get surplus glue into the channel. Placing the capillary in place in the O-ring during the bonding ensures a better fluidic seal. Including a very thin layer of adhesive in the O-ring recesses in the PMMA sheets ensures a better contact between the PDMS O-ring and the PMMA sheets, additionally improving the bonding strength. A PMMA chip that was thermally bonded with a capillary in place was tested for leaks and no leaks or failure could be seen during tests with flows up to 3 ml min^{-1}—a flow rate span that should cover most microfluidic applications.

2.5. Fluidic connections

Fluidic connections to the PMMA interfaces were achieved through 20 gauge steel tubing that was inserted into the PMMA. It is, of course, also possible to machine the inlet/outlet holes to accept standard fluidic ferrules. However, since ferrules normally require rather thick PMMA substrates, we decided against this approach to avoid a bulky result.

2.6. Pneumatic valve control

The valves presented here were controlled via a pressure terminal (VEMA, Festo AG & Co., Esslingen, Germany) that allowed precise and fast pressure control on multiple outlets. The pressures used to control the valve membrane were 50 mBar for a closed valve and -400 mBar for an open valve. With a slew rate of <25 ms for 35 mBar, the switching time for the valve system is in the range of 300 ms.

Figure 5. Demonstration of hydrodynamic focusing using a blue dye in the capillary. The images are taken 2 cm downstream of the focusing interface. The flow ratio between the sheath flow and the dye flow in the images varied between 100:100 μl min^{-1} (*A*), 100:50 μl min^{-1} (*B*) and 100:10 μl min^{-1} (*C*). The width of the capillary is 2 mm.

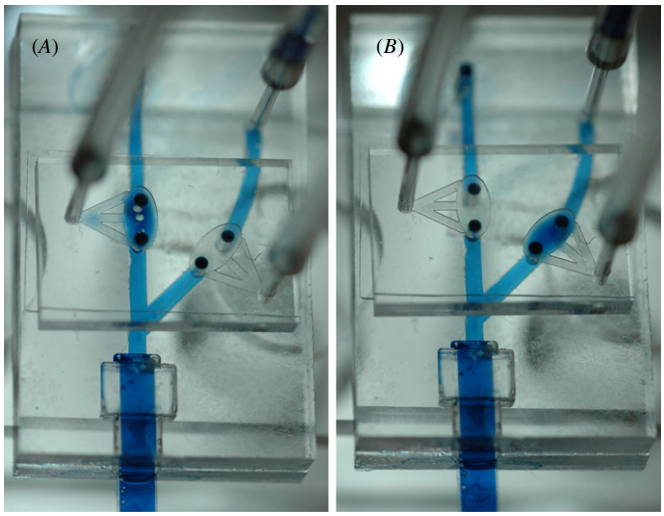

Figure 6. A demonstration of the capillary interface with integrated pressure controlled valves. In (*A*), the left valve is open and the liquid can flow from the capillary to the left outlet. In (*B*), the right valve is open and liquid can now exit through the right outlet.

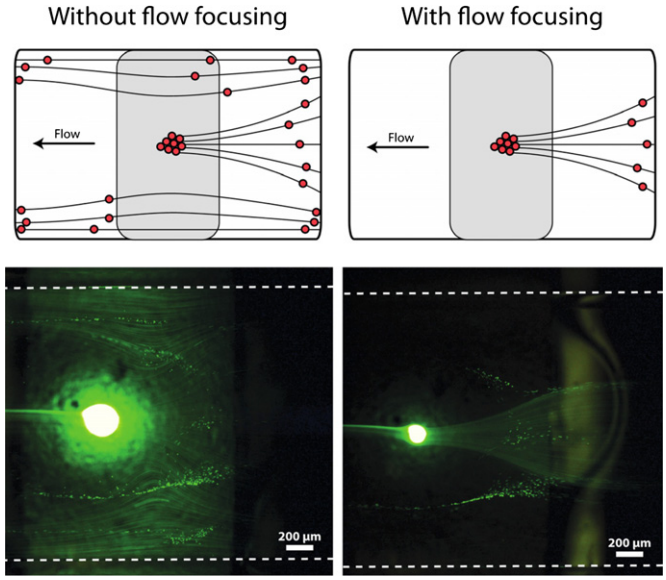

Figure 7. Acoustic trapping of 4 μm polystyrene particles without and with flow focusing. Without flow focusing, particles can be seen evading the trap by flowing along the walls of the capillary (marked with a white, dotted line). A focused particle sample is instead directed to the centre of the trap and a higher trapping efficiency can be obtained.

2.7. Acoustic particle trapping using flow focusing

A 4 MHz piezoelectric transducer was coupled to the capillary through a thin layer of glycerol and actuated using a sinusoidal signal at 10 V_{pp}. The resulting standing wave was used to trap 4 μm fluorescent polystyrene particles for two different flow conditions. For the unfocused particle trapping, a flow of 100 μl min^{-1} was used for the particle solution. For the particle trapping with flow focusing, a buffer sheath flow of 50 μl min^{-1} together with a sample flow of 50 μl min^{-1} was used, creating a total flow rate of 100 μl min^{-1}.

3. Results and discussions

3.1. Double-inlet interface

The double-inlet interface can, for example, be used when two different liquid samples are laminated side-by-side in a capillary [23]. The interface can also be used at the outlet side of the glass capillary to separate two solutions from each other into different fractions.

Hydrodynamic focusing is demonstrated by introducing an Evans blue solution in deionized water in the first/centre inlet of the capillary interface and deionized water with 1% Tween through the second/side inlet, see figure 4. Figure 5, shows snapshots 2 cm downstream of the capillary interface when the ratio between the flow rate of the sheath flow (clear) and the sample flow (blue) is varied between 100:100 μl min^{-1} (*A*), 100:50 μl min^{-1} (*B*) and 100:10 μl min^{-1} (*C*). The effect of the hydrodynamic focusing is clearly seen and the results correlate well with the expected levels of focusing. The grey lines at the top and bottom of the images are the inner walls of the glass capillary, corresponding to a width of 2 mm.

3.2. Interface with integrated valves

A capillary interface with integrated pneumatic valves was also fabricated. This interface can be used to collect different sample fractions of a sample by connecting the interface to the outlet side of the capillary. The action of the valves is shown in figure 6 where the blue liquid is seen to pass through the open valve. Depending on the pressure of the valve sites, the fluid is either allowed to continue straight forward, figure 6(*A*), or be directed into the side channel, figure 6(*B*).

Combining the capillary with a PMMA interface containing an integrated valve simplifies fraction collection from the capillary micro-channel considerably. Previously, fractions have been collected by a stop-flow-principle where the fluid is stopped and the collecting reservoir is changed. Alternatively, hydrodynamic focusing has been used to direct a specific fraction of a sample towards another collection site [24]. These techniques have however severe limitations on their usability on a larger scale.

3.3. Acoustic particle trapping using flow focusing

As an example application within the field of acoustophoresis, non-contact trapping of 4 μm polystyrene particles was performed without and with flow focusing using a capillary interface. The result, as seen in figure 7, shows particles evading the acoustic trap by flowing along the edges of the capillary when no flow focusing is applied. By using 50/50 flow focusing, the particles can be pre-focused and aimed at the centre of the standing wave, thus increasing the trapping efficiency.

4. Concluding remarks

We show the design, fabrication and application of simple PMMA interfaces to increase the complexity of the microfluidics that can be performed in glass capillaries. Here, we have designed the interfaces to fit with 2 mm wide, rectangular glass capillaries but the design can easily be adjusted to fit glass capillaries of other cross-sections and sizes. We show two different designs of the PMMA interfaces, allowing for the injection of two different liquids in straightforward and highly controlled manner and the collection of aliquots of sample into target/waste reservoirs. The use of these capillary interfaces increases the number of applications that can be performed in single-use, commercially available capillaries and opens up for new combinations of acoustics and microfluidics that has previously been hard to accomplish.

Acknowledgments

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