Single-use thermoplastic microfluidic burst valves enabling on-chip reagent storage

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Abstract A simple and reliable method for fabricating single-use normally closed burst valves in thermoplastic microfluidic devices is presented, using a process flow that is readily integrated into established workflows for the fabrication of thermoplastic microfluidics. An experimental study of valve performance reveals the relationships between valve geometry and burst pressure. The technology is demonstrated in a device employing multiple valves engineered to actuate at different inlet pressures that can be generated using integrated screw pumps. On-chip storage and reconstitution of fluorescein salt sealed within defined reagent chambers are demonstrated. By taking advantage of the low gas and water permeability of cyclic olefin copolymer, the robust burst valves allow on-chip hermetic storage of reagents, making the technology well suited for the development of integrated and disposable assays for use at the point of care.

Keywords Reagent storage · Thermoplastic fabrication · Orogenic fabrication · Point-of-care detection · Microfluidic valves

1 Introduction

Microfluidic systems aimed for use at the point of care offer great promise for integrating and miniaturizing

multiple discrete functional assay steps within inexpensive and disposable packages. While significant advances have been made toward realizing functional microfluidic diagnostics for use in point-of-care settings, the miniaturization of integrated instrumentation for introducing assay reagents remains a challenging issue and is often a bottleneck in achieving true portable and disposable platforms. As a potential solution to this challenge, single-use normally closed valves integrated directly into the microfluidic system can enable on-chip storage of assay reagents within hermetically sealed compartments, supporting on-demand release without the need for external fluidic interfacing. This capability is of particular importance for point-of-care diagnostics and other analytical applications in low resource settings (Aguilera-Herrador et al. 2010; Gervais et al. 2011).

Although a wide range of microfluidic valves actuated through a variety of transduction mechanisms have been reported (Oh and Ahn 2006), the development of normally closed valves that can be easily integrated into disposable devices remains a challenge. For example, while normally closed elastomeric-based valves have been widely explored (Hosokawa and Maeda 2000; Grover et al. 2006; Blazej et al. 2006; Yang et al. 2009; Kuo et al. 2009; Mosadegh et al. 2011), these components either require external pressure sources and pneumatic valves for their operation, or rely on internal pressure-induced deformation of an elastic substrate above an initially closed, but unbonded, valve interface. In each of these cases, valve operation depends on the use of an elastomeric substrate, and thus, these approaches cannot be readily implemented using thermoplastic materials that are ideally suited for low-cost and robust disposable devices. Hydrophobic valves, commonly used in lab-on-a-chip platforms (Madou et al. 2006; Gorkin et al. 2010), rely on the presence of an air/water interface and thus cannot

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provide hermetic sealing of fluids. Several different types of active valves employing materials including hydrogels (Beebe et al. 2000; Luo et al. 2003), polymers monoliths (Luo et al. 2003; Chen et al. 2008), waxes (Liu et al. 2004; Park et al. 2007), and ink (Garcia-Cordero et al. 2010) have been described, but these materials require external stimuli such as pH shift, light, or heat to actuate, often employ complex fabrication processes that are not amenable to low-cost diagnostics, and can suffer from issues with biocompatibility, contamination, and unwanted interaction with bioactive materials.

Here we demonstrate a new approach to the integration of single-use normally closed burst valves in thermoplastic microfluidic devices that are directly compatible with thermoplastic fabrication of cyclic olefin copolymer (COC) devices. The integration of burst valves into thermoplastic microfluidics presents a particular challenge, since established thermoplastic bonding methods have been developed with the goal of providing a permanent seal between the microfluidic substrates. The approach presented here takes advantage of a selective solvent swelling technique (Rahmanian et al. 2012; Rahmanian and DeVoe 2013) that allows small open pockets to be created within a surrounding substrate sealed by solvent bonding. By fabricating two discontinuous microchannels in the substrate prior to solvent bonding and positioning the unbonded pocket between the proximal ends of these channels, a continuous bridge supporting fluid flow between the channels is formed. Subsequent thermocompression bonding of the device closes this pocket, which is fabricated with a nominal height of several micrometers, generating an interfacial bond that is weaker than the surrounding solvent bonded substrate. The thermally bonded region acts as a robust single-use pressure-controlled valve, preventing fluid flow in its initial state, but permanently opening upon application of a pre-defined pressure within one of the discontinuous channels. This approach is conceptually similar to the successful realization of normally closed valves in elastomeric polydimethylsiloxane (PDMS) substrates through the selective patterning of unbonded valve regions (Mosadegh et al. 2011), but with the ability to tune the burst pressure with the addition of a thermal bonding step, and to achieve a permanently open channel following valve actuation, while taking advantage of the favorable properties of thermoplastic materials for microfluidic applications. In this paper, we detail the valve fabrication process and pressure-controlled actuation mechanism and investigate the relationships between valve design parameters and the resulting burst pressure and operational back pressure for the fabricated valves. Fabricated valves with actuation pressures in the range of 1–2 MPa and negligible back pressure are realized. Furthermore, by combining burst valves with integrated screw pumps, we explore the packaging of different

solutions on chip, followed by selective valve actuation and flow control using a simple manual pumping technique. Lastly, on-chip packaging, sealing, reconstitution, and release of dehydrated fluorescein salt are investigated to demonstrate the utility of the burst valves for on-chip storage of assay reagents within thermoplastic devices.

2 Experimental

2.1 Materials and reagents

Thermoplastic 1020R COC plaques (2 mm thick) were purchased from Zeon chemicals (Louisville, KY). Reagent grade cyclohexane was obtained from Sigma (St. Louis, MO) Wafer dicing tape was acquired from Semiconductor Equipment Corporation (Moorpark, CA). Needle tubing segments (gauge 22 s, 710 μm o.d. 150 μm i.d.) were purchased from Hamilton Syringe (Reno, NV) Stainless steel machine screws (3 mm diameter, 350 μm pitch) were purchased from Small Parts (Miami Lakes, FL).

2.2 Microfluidic chip fabrication

Microchannels were directly milled into a COC plate using a computer numerical control (CNC) milling machine (MDX-650A; Roland, Lake Forest, CA). Inlet/outlet ports and on-chip reagent reservoirs were milled using the same CNC tool using 650 μm and 2-mm-diameter drill bits, respectively. The inlet reservoirs were then self-tapped to form threaded ports using a stainless steel screw. The machined chips were sonicated in deionized (DI) water for approximately 10 min to clear debris and sequentially cleaned by methanol, isopropanol, and DI water, followed by drying in nitrogen gas and overnight degassing at 75 $^{\circ}\text{C}$ under vacuum.

2.3 Burst valve fabrication and bonding

The burst valve integration process is fully compatible with conventional thermoplastic chip fabrication based on solvent bonding. As described in Fig. 1, the surface of a COC chip containing microchannels is covered with a sheet of dicing tape patterned with holes aligned to the desired burst valve regions. The chip is placed in a UV-ozone (UVO) system (PDS-UV, Novascan Technologies, Ames, IA) for 20 min (Fig. 1b). After removing the blue tape, vapor phase solvent bonding (Chen et al. 2009b; Rahmanian et al. 2012; Rahmanian and DeVoe 2013) is used to bond the chip to a mating COC plaque (Fig. 1c, d). Briefly, a glass dish containing 200 ml of cyclohexane was heated to 30 $^{\circ}\text{C}$ in an oven. The UVO-exposed COC chip was positioned face down at the top of the glass dish, with

Fig. 1 Burst valve fabrication process. **a** Discontinuous microchannels are formed in a COC substrate which is, **b** selectively treated with UVO, yielding an oxidized chemical masking layer in the treated valve area. **c** Exposure of the substrate to solvent vapor induces orogenic growth in the unmasked areas, controllably raising the surface height outside of the valve region. During this process, solvent is prevented from entering the substrate within the oxidized UVO-treated valve region. **d** Aligned solvent bonding with a mating COC chip containing pre-milled access ports creates a gap connecting the discontinuous channels across the solvent-free valve region. **e** Thermal bonding of the chip collapses the valve gap, resulting in a bond in the valve region that is weaker than the solvent bond within the field of the chip

a sheet of solvent resistant tape to hold the chip in place, 5 cm away from the liquid cyclohexane surface. The fabrication process allows for either the chip containing microchannels or the COC capping layer to be exposed to solvent during bonding process. For cases where reagents are integrated into the microchannel substrate, exposure of the capping layer is preferred to prevent unwanted exposure of reagents to potentially harmful solvents. After exposure to solvent vapor for 5 min (Fig. 1c), the microchannel substrate was promptly removed from the solvent dish and brought into aligned contact with its mating COC lid

substrate containing inlet and outlet ports (Fig. 1d). The assembly was then placed in a hot press (AutoFour/15, Carver, Wabash, IN) at a pressure of 3,500 kPa for 1 min at room temperature to promote intimate substrate contact during the solvent bonding process. Following minimum 8-h incubation at room temperature to allow evaporation of solvent, the open-valve regions were thermally bonded at an elevated temperature of 95 °C and pressure of 4,800 kPa for 10 min (Fig. 1e).

2.4 Valve actuation and pressure measurements

High-pressure needle ports (Chen et al. 2009b) were used to interface the chip with an analytical liquid chromatography pump (PU-2089; Jasco, Easton, MD) providing precise volumetric flow rates and pressure measurements with 0.1 MPa resolution during device characterization. A constant flow rate was applied to the inlet, and the applied pressure was continually monitored using the pump. Valve actuation was readily determined by a large drop in back pressure and immediate visible release of liquid downstream of the valve. Valve actuation was also accompanied by a characteristic audible signal resulting from the sudden failure of the weak thermal bond at the valve interface. Back pressure was measured 1 min after initial opening of the valve while pumping DI water through the valve at 2 $\mu\text{L}/\text{min}$. Steady-state inlet pressure is typically observed following 20 s after valve actuation.

For manual valve actuation, small volumes of water spiked with food coloring were pipetted into the reagent reservoirs. Stainless steel screws were gently inserted to seal the reservoirs by turning approximately one half turn after seating each screw against its mating threads. Valve actuation was performed by manually rotating the screws with a small screwdriver.

For devices prepared with sealed fluorescein salt, syringe pumps (Harvard Apparatus) and glass syringes (Hamilton) were connected to chip inlets via capillary tubing (Cole-Palmer) and appropriate fittings (Upchurch Scientific) to provide the desired input pressure for valve actuation.

3 Results and discussion

Due to their low material cost and compatibility with a wide range of industrial-scale replication methods, thermoplastics are ideal materials for disposable point-of-care diagnostic assays. In particular, COC is an attractive material due to its high transparency and low autofluorescence, exceptional dimensional stability, low water absorption, and low gas permeability. In addition, COC offers excellent chemical compatibility, including good resistance to a range of common solvents. However, by selecting a solvent with

Fig. 2 Fabricated COC chip with multiple burst valves **a** after solvent bonding, and **b** following thermal bonding. The unbonded gaps at the channel discontinuity after solvent bonding are visible as colored regions in **a** due to light refraction at the *top* and *bottom* sur-

faces of the valve gap. Magnified images of the valve region for, **c** a closed burst valve after thermal bonding and **d** the same valve during flow of fluorescent dye confirm that the valve can be opened following application of sufficient pressure at the inlet

a solubility parameter similar to that of the COC itself, the solvent can be absorbed into the bulk substrate. Like most thermoplastics, COC is a moderately hydrophobic material with low surface energy. However, selective treatment of COC with UVO oxidizes the exposed surface, generating surface charges that can render it inert to solvent uptake and allowing regions with differential solvent absorption to be formed. We recently demonstrated that native COC surfaces exhibit permanent volume changes following cyclohexane uptake, resulting in surface height increases that can be controlled over a wide range by adjusting the solvent exposure conditions (Rahmanian et al. 2012; Rahmanian and DeVoe 2013). To form the thermoplastic burst valves, a short 5-min exposure to solvent vapor results in a 3- to 5- μm -height difference between the native and UVO-treated COC surfaces. By growing the entire surface of the microchannel substrate, except for a small UVO-treated region bridging two or more discontinuous channels, a shallow trench is formed between the deeper channel structures. After the initial solvent bonding step, this trench provides fluidic interconnection between the microchannels, as seen in Fig. 2a. The UVO-treated regions appear colored in this image due to the difference in refractive index within the shallow gap. After thermocompression bonding under sufficient pressure to collapse the shallow gap, the valve region appears clear due to elimination of the refractive index gradient (Fig. 2b). Since the bond strength at the solvent bonded interface is significantly stronger (Chen et al. 2009a; Tsao and DeVoe 2009) than thermally bonded valve region, the closed valve can be opened by applying a sufficient pressure within one of the microchannels. When the pressure reaches a critical point (Fig. 2d), the weakly bonded region fails, opening the seal and allowing fluid to flow between the newly interconnected channels. Because thermocompression bonding is performed at a relatively low temperature to prevent significant flow of polymer during the bonding step, significant residual stress within the

Fig. 3 Sessile water contact angle on a COC surface, valve burst pressure (P_{burst}), and back pressure (P_{back}) of an opened valve as a function of UVO treatment time. Treatment times below 20 min do not generate sufficient surface functional groups to prevent solvent uptake within the valve region. The nominal dimensions of the valves used for this study are shown *inset*

valve region allows the gap to return to its original height after the valve is actuated, ensuring a well-defined fluid flow path for the open valve.

The critical burst pressure at which a valve will open and the final back pressure during steady-state flow of liquid through the opened valve are both affected by the UVO treatment time. Modifying COC surfaces by UVO treatment has been shown to enhance thermal bonding of COC by increasing wettability of the surfaces, enhancing mechanical interlocking and inter-diffusion of the polymer chains during bonding (Tsao et al. 2007; Tsao and DeVoe 2009). However, UVO treatment also impacts solvent absorption, with longer treatment times and higher levels of oxidation resulting in less solvent uptake. To estimate the extent of surface oxidation, sessile water contact angle was

used as an indirect measure of surface charge density, since hydrophobicity is inversely correlated with surface charge. As shown in Fig. 3, the contact angle decreased with longer UVO exposure time until reaching a lower limit of 20° at 20 min. Following solvent exposure of COC chips containing valve designs with nominal dimensions shown inset in Fig. 3, the chips were sealed and the valves were characterized to determine burst pressure and back pressure. For treatment times below 20 min, significant solvent uptake within the treated regions resulted in the valves remaining closed over the full range of inlet pressures up to 12 MPa, whereas for a 20-min UVO treatment, the valves opened at an average input pressure of 1.53 MPa. While no further decrease in contact angle occurred for longer UVO exposures, a significant reduction in burst pressure was observed when extending the exposure time from 20 to 30 min, suggesting that some amount of solvent is still absorbed by the COC at the 20-min time point, thereby strengthening the bond within the valve region. Increasing the exposure time to 30 min further inhibits solvent uptake, resulting in a primarily thermal bond at the valve interface that can be actuated at a lower input pressure. All further experiments were performed using a UVO exposure time of 20 min to enhance the difference between burst pressure and back pressure, making it easier to identify valve actuation events from pressure measurements alone.

In addition to UVO treatment time, the effects of valve geometry on burst pressure and back pressure were also studied, with results presented in Fig. 4. For all experiments, the nominal device dimensions were the same as those provided inset in Fig. 3. As shown in Fig. 4a, the burst pressure exhibits a slight drop as the width of the valve region is increased from 1 to 3 mm, but remains nearly constant as the width is further increased to 1 cm. The higher burst pressure observed for the 1-mm-wide valve is presumed to result from the low width:length ratio for this design, resulting in constrained lateral crack propagation relative to the crack length during valve opening. In contrast to the insensitivity of burst pressure to valve width, a significant drop in back pressure was found to occur with increased width, reflecting the larger cross-sectional flow path presented by the wider valve structure. In principle, smaller valve regions on the order of several tens of micrometers can be readily patterned using our previously demonstrated techniques (Rahmanian et al. 2012; Rahmanian and DeVoe 2013). However, since both burst pressure and back pressure rapidly increase as the valve area is reduced, the minimum practical valve geometry is dictated by the limits of these parameters that can be tolerated for a given design.

Valve performance was also evaluated as a function of inlet and outlet channel width, as shown in Fig. 4b. Significantly, neither burst pressure nor back pressure was

Fig. 4 Measured valve burst pressure and back pressure as a function **a** valve width, **b** channel width, and **c** channel gap. Inlet and outlet channels were positioned symmetrically about the center of the UVO-treated valve region for these experiments

found to be correlated with the channel width, even for designs where channel width is of the same length scale as the width of the valve itself. These data confirm that valve performance is determined by the properties of the valve region itself, indicating that burst valves may be designed independently from the microfluidic channels interfaced with the valves.

In a further study of valve geometry, the distance between the tips of the inlet and outlet channels was varied between 500 and 1,500 μm . As illustrated in Fig. 4c, channel gap appears to have a strong influence on valve performance, with both burst pressure and back pressure directly correlated with the channel gap. With larger gaps, the burst pressure required to open the valve increases rapidly. At the same time, back pressure increases due to the longer flow path between the inlet and outlet channels, resulting in higher hydrodynamic resistance for larger

Fig. 5 Measured burst pressure as a function of the minimum radial distance between the inlet channel and center of the UVO-treated valve region reveals a linear relationship, regardless of the gap between inlet and outlet channels, which varies between 500 and 2,000 μm

gaps. However, because the valve area is held constant in these experiments, with inlet and outlet channels positioned symmetrically about the center of the valve region, variations in channel gap result in different lengths by which the channels intrude into the valve area. To evaluate whether the channel gap or the channel position relative to the valve region is responsible for the changes in burst pressure observed in Fig. 4c, an additional set of experiments was performed using square valves with constant 3 mm side lengths and different channel gaps, similar to the devices used for Fig. 4c, but with variations in positioning of the channels relative to the valve center. By allowing the channels to be asymmetric within the valve region, the influence of channel position could be assessed independent of channel gap. The resulting measurements are presented in Fig. 5, which displays the measured burst pressure as a function of the minimum radial distance between the inlet channel and the center of the valve region. A clear linear trend is observed for all data sets, regardless of the channel spacing. Although not explicitly displayed in this figure, the results also demonstrate that positioning of the outlet channel does not impact the burst pressure.

While the valve opening process is presumed to involve crack opening consistent with the principles of fracture mechanics applied to the bonded valve interface, the results presented in this work are based on experimental characterization of bond failure using internal pressures applied within laterally confined channels, similar to pressure burst tests used to characterize microchannel substrate bond strength.

While the complex loading state used in these experiments limits the ability to infer details of the bond interface physics, the results provide engineering insight into valve behavior and reveal several important aspects of the underlying valve opening mechanism. Following thermal bonding of the valve region, a high residual tensile stress is imposed at the bond interface, as the closed micrometer scale gap seeks to relax to significant role in defining the burst pressure than the total force exerted over the full width of the valve. As the separation force is moved closer to the center of the valve region, the maximum bending moment generated in the thermoplastic substrate rapidly increases, resulting in higher strain energy at the interface and thus a lower burst pressure. This hypothesis is consistent with the burst pressure versus channel position measurement presented in Fig. 5, as well as the data in Fig. 4c which show that as the channel gap widens, and the tip of the input channel is positioned further from the center of the valve region, the burst pressure increases. Within the limits of our optical measurements, with image capture performed at a rate of 60 frames/s, valve response time is essentially instantaneous. While the relationship between substrate thickness and burst pressure was not explored in this work, the expected reduction in bending stiffness associated with a thinner capping layer may provide an additional approach to controlling the burst pressure.

After investigating the fundamental relationships between valve design and actuation pressure, a microfluidic device employing multiple valves operating with different burst pressures and coupled with manual on-chip pumps was fabricated to evaluate the utility of the thermoplastic burst valves for microfluidic operations including reagent storage and fluid mixing. Individual liquid reagent storage wells were integrated upstream of four on-chip burst valves, as can be seen in Fig. 6a. Each storage reservoir was first tapped to create threaded a port, and solutions of water mixed with different colored dyes were introduced into each of the open wells by pipette. The ports were then capped using fine pitch stainless steel screws, sealing the fluid packets between the upstream screw and downstream burst valves. Actuation of each valve was realized by manual rotation of the corresponding screw (Fig. 6b).

In this demonstration device, the burst valves were configured to couple multiple inlets with single outlets, allowing for mixing of reagents by Taylor dispersion following successive actuation of each valve as seen in Fig. 6c, d. Opening of the valves using the screw pumps was found to be robust, with consistent actuation observed. By sealing different air volumes within the reservoirs, the number of turns required to reach the defined burst pressure could also be controlled. The valves themselves were highly resistant to accidental opening during chip handling, with no actuation observed after dropping chips multiple times onto a rigid floor from a standing position.

Fig. 6 Images of a fabricated COC chip containing multiple burst valves with on-chip reagent storage and manual screw valves. **a** Colored solutions pipetted into storage reservoirs are later capped with fine pitch stainless steel screws. **b** Manual rotation of each screw pressurizes the reagent pouches, until, **c** accumulated pressure overcomes the thermal bonding at the valve interface, pumping the liquid through the device. **d** Actuation of multiple reservoirs results in mixing within the downstream serpentine channel. As seen in **d**, each coupled valve is designed with a different burst pressure, preventing unwanted actuation of multiple valves

Long-term on-chip reagent storage is an essential requirement for point-of-care analytics and diagnostics. Currently, the majority of platforms designed for near-care use lack on-chip storage, and rely on the end user to manually pipette or pump reagents into the device prior to assay execution (van Oordt et al. 2013). Various approaches to on-chip reagent storage have been explored, including storage of liquid reagents packaged in glass ampoules (Hoffmann et al. 2010), and both liquid and dry reagents in foil pouches (van Oordt et al. 2013), with controlled release in a lab-on-a-cd platform. Similarly, long-term storage of horseradish peroxidase conjugated antibodies in paper-based microfluidic devices has been explored (Ramachandran et al. 2014).

One goal of this work was to demonstrate the integration of dried reagents within reagent storage reservoirs on thermoplastic chips without the need for additional components such as paper, glass ampoules, or foil packaging as used in prior work. To demonstrate on-chip reagent storage, a saturated 0.5 μL fluorescein salt solution was pipetted into open reagent reservoirs located between two burst valves prior to sealing the device, i.e., after the first step in Fig. 1. The fluorescein was air dried, and the resulting substrate containing dehydrated reagent was then bonded to a capping layer as described in Fig. 1b–e. DI water was injected in the micro-channel using a syringe pump, allowing pressure to build up until reaching the critical level for valve actuation. Upon actuation of the first valve, with a designed burst pressure of 1.2 MPa, reconstitution of the dried fluorescein salt is initiated. Since the second valve was engineered to open above

2 MPa pressure, a brief period of pressure accumulation is observed, during which fluorescein salt becomes fully dissolved in water that has entered the reagent reservoir. As the pressure within the reagent chamber increases, the second downstream valve opens, allowing fluorescent solution to pass through downstream channels. A sequence of images showing each stage of this process is depicted in Fig. 7. It is notable that employing valves with different burst pressures in this configuration has two advantages. First, valve actuation can be automated, with no interference from the end user required once the syringe pump is set at predefined volumetric flowrate. Second, the differential burst pressure can be engineered to define the desired time for dry-stored reagents to fully dissolve prior to opening the second valve.

A potential concern regarding the packaging of reagents using the presented burst valve technology is that the temperature employed during valve bonding may affect thermally sensitive reagents. We note that for a wide range of reagents, such as polymerase, nucleotides, and surfactants used in polymerase chain reaction (PCR), and many proteins which can resist denaturation at 95 $^{\circ}\text{C}$, thermal degradation under the current process conditions is not an issue. Furthermore, thermal stability of proteins including antibodies required for immunoassays can be significantly enhanced by lyophilization in the presence of sugar-based cryoprotectants (Cleland et al. 2001; Meyer et al. 2009; Chang and Pikal 2009). For other reagents where thermal stability remains an issue, local control over chip temperature during bonding may provide an alternative solution to this challenge.

Fig. 7 Image sequence revealing **a** on-chip packaging of dehydrated fluorescein salt within an integrated storage chamber, **b** pressure-induced valve actuation upstream (at 1.2 MPa) and downstream (at 2 MPa), thereby rehydrating the dye and releasing it into the outlet channel, and **c** complete emptying of the reagent storage chamber

4 Conclusion

A single-use normally closed valve technology suitable for integration into a wide range of thermoplastic microfluidic systems has been developed. The fabrication process requires minimal infrastructure and is fully compatible with conventional processing methods employed for microfluidic device development using COC substrates. Parametric studies revealed the key relationships between valve design parameters and performance, enabling both burst pressure and final back pressure of the valves to be independently tuned for specific applications, and allowing multiple valves with different actuation pressures to be achieved within a single device. Fabricated devices are realized that require low burst pressures of only 1–2 MPa, making the valves compatible with a simple manual actuation method leveraging integrated screw pumps. Effective on-chip packaging, reconstitution, and release of dehydrated reagents were demonstrated, with an engineered burst pressure differential between upstream and downstream valves used to control the reagent release process. The fabrication of multiple valves into a device supporting on-chip liquid storage and selective reagent delivery and mixing using manual screw pumps was successfully demonstrated. We anticipate that the normally closed valves will find utility for sealing and long-term on-chip storage of bioactive reagents in liquid or dried format, enabling reconstitution, mixing, and delivery of stored reagents for a range of disposable point-of-care assays that can take advantage of the low material cost and attractive chemical and physical properties of thermoplastic microfluidic devices.

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References

- Aguilera-Herrador EE, Cruz-Vera MM, Valcárcel MM (2010) Analytical connotations of point-of-care testing. *Analyst* 135:2220–2232. doi:[10.1039/c0an00307g](https://doi.org/10.1039/c0an00307g)
- Beebe DJ, Moore JS, Bauer JM et al (2000) Functional hydrogel structures for autonomous flow control inside microfluidic channels. *Nature* 404:588–590. doi:[10.1038/35007047](https://doi.org/10.1038/35007047)
- Blazej RG, Kumaresan P, Mathies RA (2006) Microfabricated bio-processor for integrated nanoliter-scale Sanger DNA sequencing. *Proc Natl Acad Sci USA* 103:7240–7245. doi:[10.1073/pnas.0602476103](https://doi.org/10.1073/pnas.0602476103)
- Chang LL, Pikal MJ (2009) Mechanisms of protein stabilization in the solid state. *J Pharm Sci* 98:2886–2908. doi:[10.1002/jps.21825](https://doi.org/10.1002/jps.21825)
- Chen G, Svec F, Knapp DR (2008) Light-actuated high pressure-resisting microvalve for on-chip flow control based on thermoresponsive nanostructured polymer. *Lab Chip* 8:1198–1204. doi:[10.1039/b803293a](https://doi.org/10.1039/b803293a)
- Chen C-F, Liu J, Chang C-C, DeVoe DL (2009a) High-pressure on-chip mechanical valves for thermoplastic microfluidic devices. *Lab Chip* 9:3511. doi:[10.1039/b912014a](https://doi.org/10.1039/b912014a)
- Chen CF, Liu J, Hromada LP et al (2009b) High-pressure needle interface for thermoplastic microfluidics. *Lab Chip* 9:50. doi:[10.1039/b812812j](https://doi.org/10.1039/b812812j)
- Cleland JL, Lam X, Kendrick B, Yang J (2001) A specific molar ratio of stabilizer to protein is required for storage stability of a lyophilized monoclonal antibody. *J Pharm Sci* 90(3):310–321
- Garcia-Cordero JL, Kurzbuch D, Benito-Lopez F et al (2010) Optically addressable single-use microfluidic valves by laser printer lithography. *Lab Chip* 10:2680. doi:[10.1039/c004980h](https://doi.org/10.1039/c004980h)

- Gervais L, de Rooij N, Delamarche E (2011) Microfluidic diagnostic devices: microfluidic chips for point-of-care immunodiagnostics (Adv. Mater. 24/2011). *Adv Mater* 23:H208–H208. doi:[10.1002/adma.201190098](https://doi.org/10.1002/adma.201190098)
- Gorkin R, Park J, Siegrist J et al (2010) Centrifugal microfluidics for biomedical applications. *Lab Chip* 10:1758. doi:[10.1039/b924109d](https://doi.org/10.1039/b924109d)
- Grover WH, Ivester RHC, Jensen EC, Mathies RA (2006) Development and multiplexed control of latching pneumatic valves using microfluidic logical structures. *Lab Chip* 6:623–631. doi:[10.1039/b518362f](https://doi.org/10.1039/b518362f)
- Hoffmann J, Mark D, Lutz S et al (2010) Pre-storage of liquid reagents in glass ampoules for DNA extraction on a fully integrated lab-on-a-chip cartridge. *Lab Chip* 10:1480. doi:[10.1039/b926139g](https://doi.org/10.1039/b926139g)
- Hosokawa K, Maeda R (2000) A pneumatically-actuated three-way microvalve fabricated with polydimethylsiloxane using the membrane transfer technique. *J Micromech Microeng* 10:415–420. doi:[10.1088/0960-1317/10/3/317](https://doi.org/10.1088/0960-1317/10/3/317)
- Kuo C-H, Wang J-H, Lee G-B (2009) A microfabricated CE chip for DNA pre-concentration and separation utilizing a normally closed valve. *Electrophoresis* 30:3228–3235. doi:[10.1002/elps.200900112](https://doi.org/10.1002/elps.200900112)
- Liu RH, Bonanno J, Yang J, Lenigk R (2004) Single-use, thermally actuated paraffin valves for microfluidic applications. *JMEMS*. doi:[10.1016/j.snb.2003.09.037](https://doi.org/10.1016/j.snb.2003.09.037)
- Luo Q, Mutlu S, Gianchandani YB et al (2003) Monolithic valves for microfluidic chips based on thermoresponsive polymer gels. *Electrophoresis* 24:3694–3702. doi:[10.1002/elps.200305577](https://doi.org/10.1002/elps.200305577)
- Madou M, Zoval J, Jia G et al (2006) Lab on a CD. *Annu Rev Biomed Eng* 8:601–628. doi:[10.1146/annurev.bioeng.8.061505.095758](https://doi.org/10.1146/annurev.bioeng.8.061505.095758)
- Meyer JD, Nayar R, Manning MC (2009) Impact of bulking agents on the stability of a lyophilized monoclonal antibody. *Eur J Pharm Sci* 38:29–38. doi:[10.1016/j.ejps.2009.05.008](https://doi.org/10.1016/j.ejps.2009.05.008)
- Mosadegh B, Mosadegh B, Tavana H et al (2011) High-density fabrication of normally closed microfluidic valves by patterned deactivation of oxidized polydimethylsiloxane. *Lab Chip* 11:738–742. doi:[10.1039/C0LC00112K](https://doi.org/10.1039/C0LC00112K)
- Oh KW, Ahn CH (2006) A review of microvalves. *J Micromech Microeng* 16:R13–R39. doi:[10.1088/0960-1317/16/5/R01](https://doi.org/10.1088/0960-1317/16/5/R01)
- Park J-M, Cho Y-K, Lee BS et al (2007) Multifunctional microvalves control by optical illumination on nanoheaters and its application in centrifugal microfluidic devices. *Lab Chip* 7:557–564. doi:[10.1039/b616112j](https://doi.org/10.1039/b616112j)
- Rahmanian O, DeVoe DL (2013) Pen microfluidics: rapid desktop manufacturing of sealed thermoplastic microchannels. *Lab Chip* 13:1102–1108. doi:[10.1039/C2LC41057E](https://doi.org/10.1039/C2LC41057E)
- Rahmanian O, Chen C-F, DeVoe DL (2012) Microscale patterning of thermoplastic polymer surfaces by selective solvent swelling. *Langmuir* 28:12923–12929. doi:[10.1021/la302704t](https://doi.org/10.1021/la302704t)
- Ramachandran S, Fu E, Lutz B, Yager P (2014) Long-term dry storage of an enzyme-based reagent system for ELISA in point-of-care devices. *Analyst* 139:1456–1462. doi:[10.1039/c3an02296j](https://doi.org/10.1039/c3an02296j)
- Tsao C-W, DeVoe D (2009) Bonding of thermoplastic polymer microfluidics. *Microfluid Nanofluidics* 6:1–16. doi:[10.1007/s10404-008-0361-x](https://doi.org/10.1007/s10404-008-0361-x)
- Tsao CW, Hromada L, Liu J et al (2007) Low temperature bonding of PMMA and COC microfluidic substrates using UV/ozone surface treatment. *Lab Chip* 7:499–505. doi:[10.1039/b618901f](https://doi.org/10.1039/b618901f)
- van Oordt T, Barb Y, Smetana J et al (2013) Miniature stick-packaging—an industrial technology for pre-storage and release of reagents in lab-on-a-chip systems. *Lab Chip* 13:2888. doi:[10.1039/c3lc50404b](https://doi.org/10.1039/c3lc50404b)
- Yang Y-N, Hsiung S-K, Lee G-B (2009) A pneumatic micropump incorporated with a normally closed valve capable of generating a high pumping rate and a high back pressure. *Microfluid Nanofluidics* 6:823–833. doi:[10.1007/s10404-008-0356-7](https://doi.org/10.1007/s10404-008-0356-7)