

Flow Control Valves for Analytical Microfluidic Chips without Mechanical Parts Based on Thermally Responsive Monolithic Polymers

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Monolithic plugs of poly(*N*-isopropylacrylamide) cross-linked with 5% methylenebisacrylamide have been prepared by photoinitiated polymerization within the channel of a microfluidic device. The volume change associated with the polymer phase transition at its lower critical solution temperature of 32 °C allows both the rapid swelling and the deswelling of the monoliths enabling the polymer to close or open the channel as it functions as a nonmechanical valve. Thermoelectric elements capable of changing the temperature of the system between 17 and 57 °C were used to actuate the valve. Flow through the device was monitored by fluorescence measurements via the laser-triggered photobleaching of a dye contained in the liquid phase. Photobleaching occurs quickly once the flow is stopped, and the time required to open and close the valve was 3.5 and 5.0 s, respectively. No changes in function were observed even after 120 open–close cycles. Although the 2-mm-long valve was prepared from a polymerization mixture consisting of only a 5% aqueous solution of monomers, it resists pressures of up to 1.38 MPa (200 psi) without observable structural damage.

The field of microfluidics is evolving rapidly, and a number of applications in areas such as analytical devices, sensors, and chemical as well as biochemical reactors have already been demonstrated.¹ As a result of their small size, sophisticated setup, and possible multiplexing, microfluidic systems have the potential to significantly improve the throughput and efficiency of analytical systems. In particular, their use dramatically reduces the consumption of solvents and reagents since only very small sample volumes are required, thus reducing the total cost of the processes

and facilitating field applications.² The fabrication of microfluidic systems requires the integration of various building blocks including mixers, separation units, preconcentration elements, reactors, and detectors within a single microfluidic chip, and new materials are required for each of these functions.

Valves incorporated in the microchannels constitute an important element since they provide for the directional control of flow. The current literature presents a variety of designs that involve both mechanical³ and chemical actuation.⁴ The latter category also includes stimuli-responsive polymers. These polymers can undergo considerable changes in their properties in response to often small changes in their environment.⁵ Poly(*N*-isopropylacrylamide) (poly(NIPAAm)) is perhaps the best-known material of a class of temperature-sensitive polymers. At its lower critical solution temperature (LCST), 32 °C, poly(NIPAAm) chains undergo a rapid and reversible phase transition from extended hydrated chains below the LCST to collapsed hydrophobic coils above the LCST.^{5a} Using the volume transition exhibited by this

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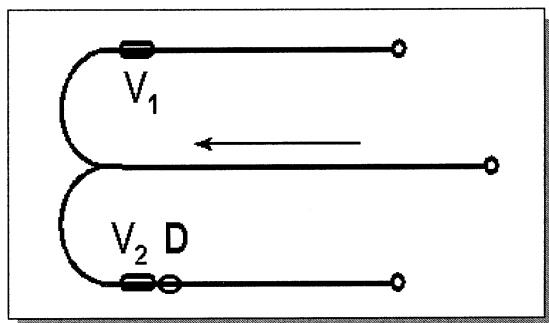


Figure 1. Scheme of the chip design and experimental layout. Elements V_1 and V_2 are monolithic valves; D point of the focus of the laser-induced fluorescence detector.

polymer in response to changes in temperature, we have designed highly effective thermally actuated valves and gates based on porous polymer monoliths with poly(NIPAAm) chains *grafted to the pore surface*.⁶ In this preliminary large-scale demonstration of the thermally actuated valving function, which was first of its kind, we used an external bath to effect the temperature changes. A similar concept involving irregular particles was later used for the fabrication of chemomechanical valves.^{4d} Building on our seminal work,⁶ Beebe et al. have developed similar valves from pH-sensitive hydrogels that have been prepared by copolymerization of acrylic acid, 2-hydroxyethyl methacrylate, and ethylene dimethacrylate.^{4a,b} However, their actuation required a very large change in pH—from pH 2 to pH 11—thus mandating a very significant change in the chemical composition of the liquid within the channel, a feature that is seldom desirable.

Herein, we describe a novel and more practical approach to polymer-based thermally actuated microfluidic valves. In contrast to our original multistep procedure involving monolith formation and subsequent modification by grafting, the monolithic valve is now prepared in a single step, *in situ*, using a procedure akin to photolithography.

EXPERIMENTAL SECTION

Fabrication of Microfluidic Chips. A simple chip with three microchannels 200 μm wide, 100 μm deep, and 6 cm long shown schematically in Figure 1 was used in this study. The microchips were fabricated in the microfabrication laboratory of the University of California at Berkeley. Briefly, Borofloat glass wafers (10 cm diameter and 1.1 mm thick) were cleaned in the piranha solution (3:1 mixture of concentrated sulfuric acid and 30% hydrogen peroxide) for 10 min and coated with a 1500-Å layer of amorphous silicon using low-pressure chemical vapor deposition. The wafers were then dehydrated at 120 $^{\circ}\text{C}$ for 30 min, primed with hexamethyldisilazane for 5 min, spin-coated with photoresist (Shipley 1818, Marlborough, MA), and soft-baked. The mask pattern of the desired channel network was transferred to the wafer by exposing the photoresist to UV irradiation in a Quintel UV contact mask aligner for a few seconds. The photoresist was developed in Microposit developer concentrate (Shipley). The exposed sacrificial amorphous silicon layer was removed by CF_4 plasma etching in a plasma-enhanced chemical vapor deposition

reactor (PIII-A, Technics West, San Jose, CA) and the glass substrate exposed. The wafers were then etched in a 47% aqueous HF solution to generate the microchannels. The remaining photoresist was removed with PRS-3000 photoresist stripper and the amorphous silicon removed by CF_4 plasma etching. Access holes (1.4 mm diameter) were drilled through the etched wafers using diamond-tipped drill bits (Crystalite, Westerville, OH). Finally, an unetched Borofloat wafer was thermally bonded to the etched wafer in a programmable vacuum furnace (Centurion VPM, J. M. Ney, Yucaipa, CA) heated at 623 $^{\circ}\text{C}$ for 3.5 h.

Preparation of Valves. The walls of the microchannels were vinylized to enable covalent attachment of the monolith.⁷ The channels were washed with acetone and water, filled with 0.2 mol/L NaOH for 30 min, washed with water, filled with 0.2 mol/L HCl for 30 min, washed again with water and acetone, and dried in an oven at 120 $^{\circ}\text{C}$ for 1 h. The channels were then filled with 30% 3-(trimethoxysilyl)propyl methacrylate in acetone and allowed to react at room temperature in the dark for 24 h. The vinylized channels were then washed with acetone and dried using a stream of nitrogen.

N-Isopropylacrylamide (187.5 mg) was mixed with 9.375 mg of *N,N*-methylenebisacrylamide, 0.9375 mg of 4, 4'-azobis(4-cyanovaleric acid), and 4 g of water and purged with nitrogen gas for 10 min to remove the dissolved oxygen. The mixture was injected into the microchip channel using a volumetric pipet through the access hole that was then sealed with tape; the chip covered with a mask, placed on an aluminum plate heated to 32 $^{\circ}\text{C}$ by thermostated water, and polymerized upon irradiation with a UV Oriel deep UV illumination system series 8700 (Stratford, CT) fitted with a 500-W HgXe lamp from a distance of 30 cm for 45 min. The resulting 2-mm-long poly(NIPAAm) monolith was tested for leaks in the closed mode by cooling it to room temperature, attaching to a syringe pump with a back pressure set to 0.45 MPa (65 psi). No drop of pressure was observed for a correctly operating valve. The chip was then submersed into a 42 $^{\circ}\text{C}$ water bath, the valve opened, pressure dropped, and the remaining monomers were washed out. For further experiments, the chip was connected to a micro-HPLC pump via a 100- μm -i.d. fused-silica capillary.

Valving Operation. The temperature within the channels was controlled by 4 \times 4 mm thermoelectrical elements (Standard single-stage thermoelectric cooler, Marlow Industries, Inc., Dallas TX). The thermoelectrical elements were attached to square-shaped cavities etched at the outside of the chip in the area located beneath the location of the valve, and the thickness of the glass wall was reduced to 45 μm . A specially designed holder with two embedded thermoelectrical elements was used to ensure good physical contact between the surface of the glass chip and the thermoelectrical elements.

RESULTS AND DISCUSSION

Preparation of Monolithic Valves. Photopolymerization through a mask directly affords an appropriately dimensioned and fully functional valve of porous cross-linked poly(NIPAAm) at the desired locations of the microfluidic channel. The optimized polymerization mixture consists of a 5% aqueous solution of

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monomers and initiator. The percentage of cross-linker in the monomer mixture (5%) is higher than that used in typical hydrogels to ensure that the monolithic material possesses sufficient mechanical strength. The channels of a model glass chip shown schematically in Figure 1 were first vinylized using primer silane reagent 3-(trimetoxysilyl)propyl methacrylate to enable covalent attachment of the monolithic polymer to the wall and then filled completely with the polymerization mixture. A mask was attached to the top of the chip leaving only two 2-mm-long openings through which the contents of the channel were illuminated with UV light to achieve photopolymerization. Despite the low concentration of initiator, the polymerization process is rather fast. At a temperature of 32 °C, the photopolymerization is completed in 45 min. A polymerization temperature slightly higher than room temperature was used since it favors the creation of macroporous gels with faster response. While even higher temperatures have been advocated for such gels,⁸ we found that their use led to a simultaneous thermally initiated polymerization that led to polymer plugs larger than the features of the masks with “fuzzy” ends consisting of looser gel.

Valving Function. The temperature within the channels is controlled by thermoelectrical elements. Since glass is a poor thermal conductor, square-shaped cavities able to accommodate thermoelectrical elements were etched at the outside of the chip in the area located beneath the location of the valve to reduce thickness of the glass wall. In addition, a specially designed holder with two embedded thermoelectrical elements was used to ensure good physical contact between the surface of the glass chip and the thermoelectrical elements, as required to obtain rapid changes in the temperature. The opening and closing functions were actuated by varying the temperature between 16 and 57 °C. Although the actuation itself can be achieved at temperatures much closer to the LCST of poly(NIPAAm) at 32 °C, this much broader range was used to rapidly cool or heat the bulk of material of the chip to achieve the desired rapid temperature change within the channel.

At 16 °C, the valve is in the closed mode as the poly(NIPAAm) monolithic valve is solvated with water and the polymer chains fill the channel completely, blocking the flow of the mobile phase. When viewed in an optical microscope, the material of the hydrated valve appears transparent. When heated to 57 °C, the valve switches to the open mode as desolvation of the polymer causes drastic shrinkage opening the channel and enabling flow through the valve. Simultaneously, the visual appearance of the polymer changes to opaque. The valving function of the porous poly(NIPAAm) monolith was detected via photobleaching using a 25 nmol/L aqueous Coumarin 519 solution at a flow rate of 2.5 μ L/min driven by a micro-HPLC pump. At the start of the experiment, both thermoelectrical elements are in the heating mode and therefore the solution flows simultaneously through both side channels since both valves V_1 and V_2 (Figure 1) are open. The laser-induced fluorescence detector focused at point D located behind valve V_2 monitors a constant fluorescence signal (Figure 2). Once the thermoelectrical element beneath V_2 is switched to the cooling mode, flow through V_2 is stopped. The laser beam bleaches the dye, and a rapid drop in the fluorescence intensity is observed. Switching the thermoelectrical element back

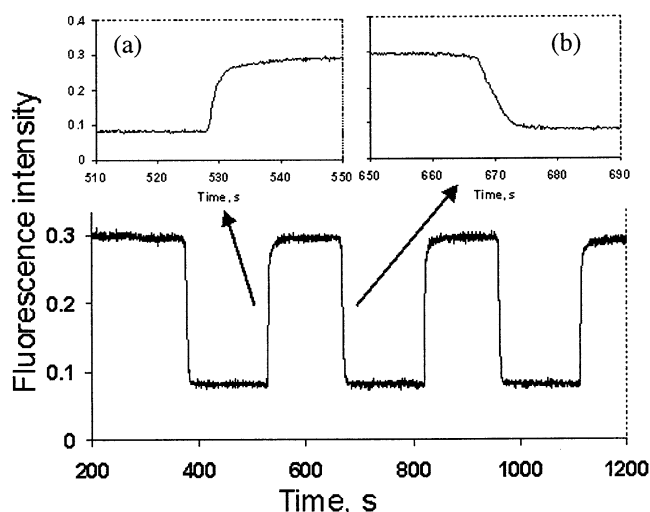


Figure 2. Monitoring of the valving function of the poly(NIPAAm) monolith using photobleaching of 25 nmol/L aqueous Coumarin 519 solution at a flow rate of 2.5 μ L/min. Insets: expanded parts of the opening (a) and closing (b) profiles.

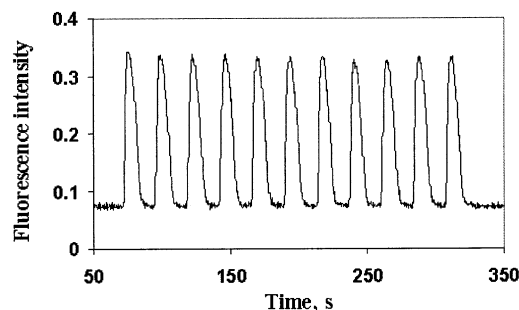


Figure 3. High-frequency valving operation of the poly(NIPAAm) monolith actuated every 12 s monitored via photobleaching of 25 nmol/L aqueous Coumarin 519 solution at a flow rate of 2.5 μ L/min.

to the heating mode opens valve V_2 , and the flow through the channel is restored as demonstrated in Figure 2 by the concurrent increase in fluorescence intensity. The opening/closing cycle was repeated multiple times with the same result obtained. The changes in fluorescence intensity noted using the expanded time scale shown in the top panels of Figure 2 establish that the time required for closing the valve is 5.7 s, while the valve opens in only 3.5 s. Since the photobleaching itself requires a time of ~ 0.7 s as determined in an independent experiment, the closing rate of the valve is actually ~ 5.0 s. These rates are much faster than those observed for the single-feature pH-actuated valves.^{4a,b}

Actuation of the valve under higher frequency conditions is demonstrated in Figure 3. The temperature of the thermoelectrical element was switched every 12 s between 16 and 57 °C causing valve V_2 to respond appropriately and flow to be switched on and off with no change in performance during repeated use.

The alternating function of the two-valve system was also tested, in this case, setting the thermoelectrical element beneath valve V_1 to cool while that serving valve V_2 was set to heat. Since valve V_1 is closed while V_2 is open, the Coumarin 519 solution flows only through valve V_2 . Thus, the detector focused at point D located behind valve V_2 monitors a constant fluorescence signal. When the functions of both thermoelectrical elements are reversed, valve V_2 switches to the closed mode and the fluorescence intensity decreases rapidly since all of the solution now flows

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through valve V_1 . This cycle can be repeated several times and affords a graph similar to that of Figure 2. However, the response time is different since the valve closes in 6.8 s but opens in less than 1 s. Compared with the single valve operation, the opening time is shorter while the closing time is longer. This likely results from the simultaneous action of both valves that at a certain point restricts flow. This leads to a transient increase in the internal pressure within the inlet channel; therefore, liquid is forced through the closing valve for a slightly longer period of time, leading to the observed slightly longer closing time. In contrast, this transient pressure forces the liquid through the partly opened valve leading to a decrease in the opening time. This hypothesis is supported by the observations of negative peaks when the laser detection point during this experiment is set at the center channel, which indicates that the flow is slowed for a very short period of time during which partial bleaching is observed. This effect could be eliminated by appropriate adjustments in the timing of the electrically controlled switching operations.

It is worth noting that the monolithic polymer valve in the "closed" position can tolerate pressures of at least up to 1.38 MPa (200 psi) without any observable leakage, structural damage, or dislocation. This demonstrates the excellent mechanical stability of the material and shows that it can resist extreme conditions unlikely to be encountered in real microfluidic applications. In contrast, the system exhibits a very low back pressure of less than 0.3 MPa (5 psi) in the "open" mode even at a very high flow rate of 50 $\mu\text{L}/\text{min}$ that represents a flow velocity of 5 cm/s. This suggests that the pores created after shrinkage of the monoliths are rather large. Indeed, these pores are large enough to enable the passage of large, 2- μm monodisperse beads labeled with a fluorescent marker to permit their tracking as they flow through the monolith. While multiple experiments have been carried out under a number of different conditions, our valves easily survived 120 successive "open–close" cycles.

CONCLUSION

The short macroporous poly(NIPAAm) plugs lithographically prepared in a single step within the channels of a microfluidic chip can be used as temperature-actuated valves. These robust valves respond very quickly to the external stimulus and keep their performance in numerous "open–close" cycles. Our current research is now focused on the study of the effects of the valve length that can be easily controlled via the opening in the photomask during the preparation on the pressure resistance and the achievement of even faster response times. We envision that this can be achieved using a resistor heater built in the channel and polymers with higher LCST. In the current implementation, the valve is closed at room temperature and can be, for example, used in dosage units driven by pressurized flow. Although our approach to the thermally actuated valves was demonstrated using poly(NIPAAm) with a lower critical solution temperature of 32 $^{\circ}\text{C}$, it can be extended to other polymers and the LCST can easily be adjusted to the desired switching temperature both below and above room temperature.

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