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Monolithic valves for microfluidic chips based on thermoresponsive polymer gels

The direct preparation of thermoresponsive monolithic copolymers by photopatterning of a liquid phase consisting of an aqueous solution of *N*-isopropylacrylamide, *N*-ethylacrylamide, *N,N*'-methylenebisacrylamide, and 4,4'-azobis(4-cyanovaleric acid) has been studied and the products used as valves within the channels of microfluidic devices. The volume change associated with the polymer phase transition at its lower critical solution temperature (LCST) leads to the rapid swelling and the deswelling of the 2.5% cross-linked monolithic gel thus enabling the polymer to close or open the channel and to function as a nonmechanically actuated valve. The LCST at which the valve switches was easily adjusted within a range of 35°C–74°C by varying the proportions of the monovinyl monomers in the polymerization mixture. The closed valve holds pressures of up to 18 MPa without noticeable dislocation, structural damage, or leakage. In contrast, following deswelling by raising the temperature above LCST the valve offers no appreciable flow resistance since its large, micrometer-size pores are open. Laser-triggered photobleaching of a fluorescent dye contained in the liquid phase enabled monitoring of flow through the device and determination of the times required to open and close the valve. The valves are characterized by very fast actuation times in a range of 1–4 s depending on the type of device. No changes in performance were observed even after repeated open-close cycling of the valves.

Keywords: Lower critical solution temperature / Microfluidic device / Miniaturization / Monolith / Thermoresponsive polymer / Valve
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1 Introduction

Progress in the design and fabrication of micrototal analytical systems (μTAS) [1–4] requires further development of building blocks enabling a variety of functions such as pumping, sample preparation, preconcentration, separation, mixing, *in situ* reaction, flow direction, and detection [5–12]. As a result of their integration in small-size devices, sophisticated setup, and possible multiplexing, microfluidic analytical systems have the potential to significantly improve throughput at both small sample and reagent consumption thereby reducing operating cost [13,14]. Valves incorporated in the microchannels constitute one of the most important elements since they provide for the directional control of flow in complex systems. Hasselbrink *et al*. [15] equals valves in microfluidic systems to transistors in electrical circuits thus underscoring their central role in the large-scale integration of individual building blocks on a microfluidic chip. The key requirements for a microfluidic valve are (i) endurance to high pressure in the close position, (ii) minimum leakage, (iii) low flow resistance in the open position, (iv) fast response, and (v) ease of fabrication.

The current literature presents numerous approaches to both mechanical [7, 10, 16–25] and chemical microvalves [8, 9, 26–31]. The latter family of valves also includes those based on stimuli-responsive polymers [8, 9, 26, 27, 29, 32–35]. These materials change considerably their properties in response to small changes in their environment such as pH, temperature, and electrical field [36–46]. Poly(*N*-isopropylacrylamide) (poly(NIPAAm)) is perhaps the best-known material of a class of temperature-sensitive polymers characterized by a lower critical solution temperature (LCST) of 32°C. At a temperature above the LCST, poly(NIPAAm) chains undergo a rapid and reversible entropy-driven phase transition from extended hydrated chains to collapsed hydrophobic coils that precipitate in water [46].

In the mid 1990s, we designed and demonstrated highly effective thermally actuated valves based on the changes in volume that accompany the swelling and deswelling of

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Abbreviations: LCST, lower critical solution temperature; **NEAAm**, *N*-ethylacrylamide; **poly(NIPAAm)**, poly(*N*-isopropylacrylamide); **SEM**, scanning electron microscopy

poly(NIPAAm) in response to changes in temperature [33]. In our initial work, we prepared the valves by grafting of poly(NIPAAm) chains from the pore surface of rigid porous polymer monoliths. We have also confirmed that proteins do not adsorb irreversibly on the swollen poly(NIPAAm) surface at temperatures below LCST. Following our early work, several groups used this concept and fabricated valves from stimuli-responsive polymers [8, 26–29, 31, 34, 35]. For example, irregular 0.5 mm particles prepared by milling cross-linked NIPAAm copolymers were used for the fabrication of large 5 mm chemomechanical valves [8, 26]. Beebe *et al*. [27–29, 31, 34, 35] developed an interesting approach to valves with pH-sensitive hydrogels obtained by copolymerizing acrylic acid, 2-hydroxyethyl methacrylate, and ethylene dimethacrylate.

Recently, we have prepared monolithic valves of poly (NIPAAm) gel cross-linked with 5% methylenebisacrylamide *via* photoinitiated polymerization within the channel of a microfluidic chip and demonstrated their valving function actuated by temperature [32]. These robust valves with a transition temperature slightly above 30°C responded very quickly to the external stimulus and kept their performance in repeated "open-close" cycles. However, their transition temperature was too low for some application such as the control of flow through microfabricated PCR chambers that must remain closed at much higher temperatures. Therefore, in the present work, we describe a new family of thermally actuated valves prepared from photocopolymerized cross-linked NIPAAm and *N*-ethylacrylamide (NEAAm) gels for which the LCST can be adjusted within a broad range to meet the temperature requirements specific for some applications. We also demonstrate their valving function in microfluidic devices.

2 Materials and methods

2.1 Materials

NIPAAm, *N,N*'-methylenebisacrylamide, and 4,4'-azobis(4-cyanovaleric acid), were purchased from Aldrich (Milwaukee, WI, USA). NEAAm was obtained from Monomer-Polymer & Dajac Labs (Feasterville, PA, USA) and coumarin 519 from Exciton (Dayton, OH, USA). NIPAAm was purified by recrystallization with hexane. All other reagents were used as received. Microfluidic chips with channels 100 μm wide and 40 μm deep were fabricated according to the procedures reported previously [11]. Figure 1 shows a schematic design of the chips. Teflon-coated 50 and 100 μm ID fused-silica capillaries were purchased from Polymicro Technologies (Phoenix, AZ, USA).

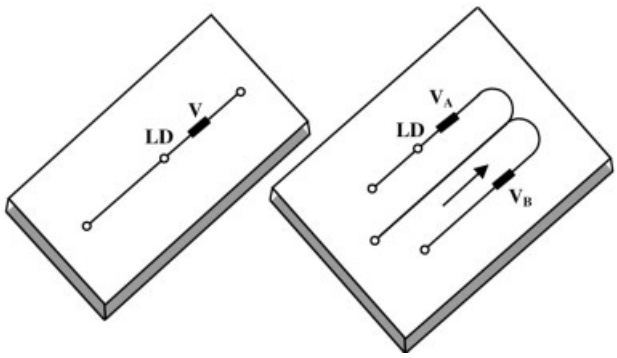

Figure 1. Designs of microfluidic chips used in this work. The black rectangles show the location of the valves V, V_A, and V_B; LD is the laser detection/photobleaching point.

2.2 Instrumentation

An Oriel deep UV-illumination system series 8700 (Stratford, CT, USA) fitted with a 500 W Hg-Xe lamp was used for UV-initiated polymerization reactions [12]. A He-Cd laser with a wavelength of 442 nm and an output power of 38 mW (OmNichrome Series 56; Melles Griot, Carlsbad, CA, USA) was used for the detection of fluorescence within the microchip channel. The laser beam passes an adjustable iris, a neutral density filter, then is directed by a set of mirrors, two additional adjustable irises, and reflected by a dichroic mirror into a 40× microscope lens (New Focus, Santa Clara, CA, USA), which focuses the laser beam onto the microchip channel. The fluorescence is collected by the same lens, detected by a photomultiplier (Model HC-120–05; Hamamatsu, Bridgewater, NJ, USA), and the signal is processed by a computer. Photobleaching experiments were carried out using a method published elsewhere [32, 47].

2.3 Preparation of valves

The walls of both microchannels and capillaries were vinylized to enable covalent attachment of the polymerized valves. They were washed first 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 a temperature of 120°C for 1 h. They were then filled with a 30% solution of 3-(trimethoxysilyl)propyl methacrylate in acetone and allowed to react at room temperature in the dark for 24 h. The vinylized surfaces were then washed with acetone and dried using a stream of nitrogen. To a mixture of NIPAAM and NEAAm (total = 2.92 mmol) was added 3.0 mg 4,4'-azobis(4-cyanovaleric acid), 9.375 mg *N,N*'-methylenebisacrylamide (2.5 mol% with respect to the monofunctional monomers), and 4 mL water. A small

amount of acetone may be added to facilitate dissolution of the initiator. This mixture was first purged with nitrogen for 10 min to remove dissolved oxygen. The channel was then completely filled with the polymerization mixture using a pipette and sealed with a tape. The surface of the chip was covered with a mask that had an open window allowing only a specific section of the channel to be exposed to the UV light, and affixed to a temperature-controlled metallic plate. After the polymerization process was completed, the monolith was washed with water at 60°C using a programmable micropump (Micro-Tech Scientific, Sunnyvale, CA, USA) to remove all unreacted components. While the capillary containing the valves can be attached to the pump directly, the microchip requires attachment of a 100 μm ID fused-silica capillary to its access holes using epoxy glue. A larger volume mold consisting of a circular Teflon base plate and a 10 cm quartz window separated by a 700 μm thick polysiloxane gasket was used in parallel bulk polymerizations. This assembly was placed between an aluminum plate and an aluminum ring held together with screws. The mold was then filled with the polymerization mixtures previously purged with nitrogen and irradiated under conditions similar to those used for the preparation of the valves in capillaries and microfluidic chips. Once the polymerization was completed, the mold was disassembled, the layer of cross-linked polymer gel was recovered and washed with cold water.

2.4 Valving operation

The temperature within the channels was controlled by 4×4 mm thermoelectrical elements (standard single-stage thermoelectric cooler; Marlow Industries, Dallas, TX, USA). These thermoelectrical elements were attached to the chip beneath the valves. 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.

2.5 Determination of LCST

The LCST of the polymer solution was determined using a DSC 6200 Differential Scanning Calorimeter (Seiko Instruments, Tokyo, Japan). Samples for the thermal analysis were prepared by placing the cross-linked NIPAAm and NEAAm copolymers first in distilled water. The swollen sample was transferred in a capsule that was then sealed in order to prevent evaporation and scanned at a rate of 5°C/min within the range of 90°C–20°C *versus* an empty reference pan. The phase separation temperature is assessed from the exothermic peak at the cooling scan of the thermogram.

2.6 Environmental scanning electron microscopy (SEM)

An environmental scanning electron microscope (Electroscan E3) was used for imaging the nonconducting hydrated gels without disruption their microstructures. The capillary containing the swollen gel was attached to the specimen stage at a temperature of 2°C. Pressure in the chamber was then decreased and the atmosphere rapidly replaced with water vapors. The equilibrium imaging pressure was first set to 530 Pa and the face side of the gel observed in the wet state. The pressure was then changed to 130 Pa to drive the water from gel thus enabling imaging of the polymer in the dry state without handling the sample.

3 Results and discussion

Poly(NIPAAm) undergoes a reversible phase transition at 32°C [38, 42, 44, 46, 48]. We have used this polymer for the initial demonstration of the concept of thermally actuated nonmechanical valves for microfluidic devices [32]. However, as a result of its low LCST, both active heating and cooling had to be applied to actuate the valve. Although our valve fabricated from cross-linked poly (NIPAAm) exhibited the desired function, its LCST is not suitable for applications that require actuation at a higher temperature. In contrast, the LCST values found in the current literature for poly(NEAAm) vary between 72°C and 82°C [49]. The higher LCST simplifies the valving system needed with this polymer as only an active heater is required to actuate the valve. The valve opens upon heating using a heating element while it closes by cooling *via* heat exchange with the surrounding environment without requiring a cooling device. Copolymerization of varying amounts of NEAAm and NIPAAm allows the adjustment of the phase transition temperature and the fabrication of valves operating at any temperature within the range afforded by each homopolymer.

3.1 LCST of copolymers

Differential scanning calorimetry (DSC) is the easiest method for the determination of the LCSTs of thermally responsive polymers swollen in water. A typical DSC thermogram recorded with 2.5% cross-linked poly(NIPAAm-*co*-NEAAm) with equimolar ratio of both comonomers is shown in Fig. 2. Using DSC, the LCSTs for both the cross-linked polymers were measured to be 34°C and 74°C, respectively. These values are slightly different from those reported for the soluble homopolymers [49–51]. This difference likely results from both the presence of the cross-linker and variations in the experimental conditions used

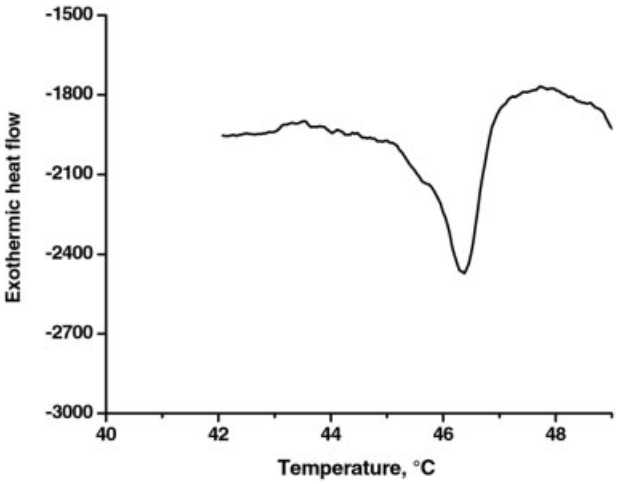

Figure 2. Differential scanning calorimetric trace for the determination of LCST for 2.5% cross-linked poly-(NIPAAm-*co*-NEAAm) (molar ratio 1:1). Scanning rate, 5°C/min; temperature range, 90–20°C.

for the preparation and the measurements. Since the LCSTs for cross-linked poly(NIPAAm-*co*-NEAAm) have never been reported, we prepared a series of copolymers with various mole ratios of NEAAm in the monomer mixture and measured their LCSTs. The results are shown in Fig. 3. As expected, the LCST values for the copolymers increase with increasing NEAAm content. Since NEAAm is more hydrophilic than NIPAAm, the increased hydrophilicity of the polymer results in an increase in LCST. However, this function is not linear. Liu and Zhu [50] proposed Eq. (1) for the calculation of the LCST values of copolymers:

$$T_{\text{copol}} = \frac{\mu T_1 + K\mu_2 T_2}{\mu_1 + K\mu_2} \quad (1)$$

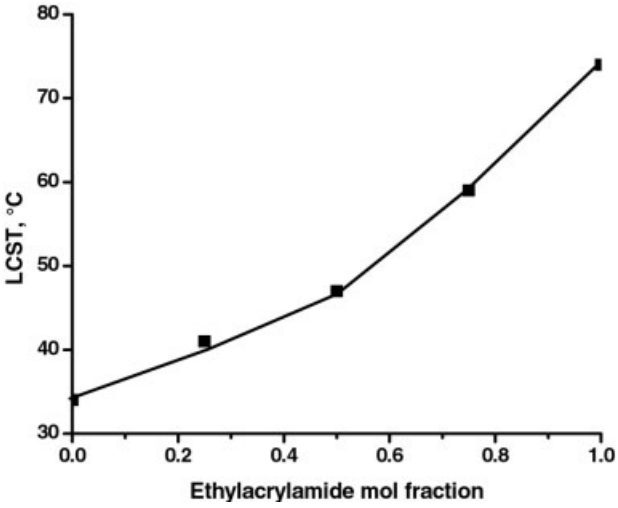

Figure 3. LCST of gels as a function of the mole fraction of NEAAm in the copolymer.

where T_{copol} is the LCST of the copolymer, μ_1 and μ_2 are the molar fractions of each monomer ($\mu_1 + \mu_2 = 1$), T_1 and T_2 are LCSTs of the respective homopolymers, and K is a weighting parameter obtained from fitting the experimental results. Our experimental data afford a K-value of 0.53 for poly(NIPAAm-*co*-NEAAm) gels.

While thermally sensitive gels are swollen in water below their LCST increasing their temperature above LCST leads to an entropy-driven collapse of the polymer chains and phase separation [52]. Observation of the valve by optical microscopy reveals that the polymer gel is transparent at room temperature since it is completely solvated with water. In contrast, following phase transition and expulsion of the water of hydration at 60°C, pores are formed and the valve appears opaque as the phase-separated chains have a refractive index different from that of water.

3.2 Pressure resistance

The actual valves were prepared by copolymerizing NIPAAm and NEAAm in the presence of 2.5% methylenebisacrylamide used to insolubilize the plug of polymer gel. Using masks with openings of varying length, valves with lengths of 500 μm–5 mm were easily prepared. Below the LCST, this gel is swollen with water and the valve is closed. Once heated to a temperature above LCST, the polymer chains quickly desolvate, shrinking the gel and opening the valve. One of the key requirements for the practical use of such valves is their resistance to the pressure that builds up in front of the valve when it is closed. This includes two parameters: resistance to (i) dislocation and (ii) mechanical fracture of the gel itself. The former is achieved through attachment of the gel plug to the wall as linking to the vinyl functionalities introduced by silanation is achieved during polymerization, while the latter is an intrinsic property of the gel. To investigate the behavior of the valve under pressure, we prepared a 5 mm long valve from cross-linked poly(NIPAAm-*co*-NEAAm) (1:1 molar ratio) in a capillary. The capillary was connected to a pump and the back pressure in the system was monitored. Almost no back pressure was observed at 57°C with the valve in the open position. In contrast, Fig. 4 shows the linear increase in pressure within the capillary when the valve is closed. No deviation from linearity is observed even at a back pressure as high as 18 MPa at which the pump was switched off since such extreme pressures are unlikely to ever be encountered in real microfluidic applications. For comparison, a similar experiment also shown in Fig. 4 was carried out with an empty capillary sealed at one end. Clearly, the results of both measurements are identical. This indicates that

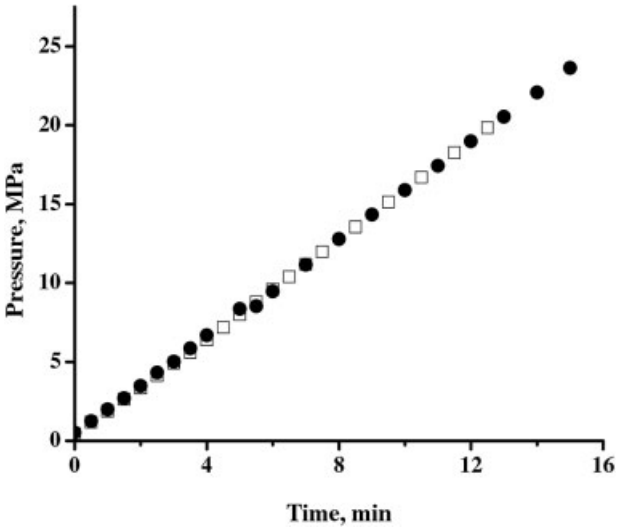

Figure 4. Pressure buildup (□) in an open capillary with a closed valve and (●) in an empty capillary sealed at one end.

the 5 mm long gel plug completely stops flow through the capillary with no leakage even at a very high pressure. Visual examination of the valve in an optical microscope following these experiments did not reveal any structural damage or dislocation of the valve. Similarly, a much shorter valve only 0.5 mm in length prepared within a 50 μm ID capillary can withstand pressures of up to 0.35 MPa without damage. Although the structural strength of this thin membrane-like plug is lower than that of the much longer valve, its pressure resistance is still sufficient for numerous microfluidic operations.

3.3 Effect of polymerization conditions

Polymerization conditions such as temperature and time are known to significantly affect the properties of the LCST gels [48,53]. Using the UV lamp described in the Section 2.2, a polymerization time of at least 10 min was required to obtain functional poly(NIPAAm-*co*-NEAAm) valves. Valves prepared using shorter polymerization times were not mechanically stable, most likely as a result of incomplete polymerization. Optical micrographs shown in Fig. 5 illustrate that the structure of the cross-linked poly(NIPAAm-co-NEAAm) gels swollen in water differs depending on the polymerization temperature at which the gel is prepared. Hoffman and Yan [53] have observed the formation of macroporous structures in polyNIPAAm gels prepared at a temperature above LCST. The polymer chains formed below LCST are completely hydrated during their polymerization and the gel remains transparent throughout the process. In contrast, above

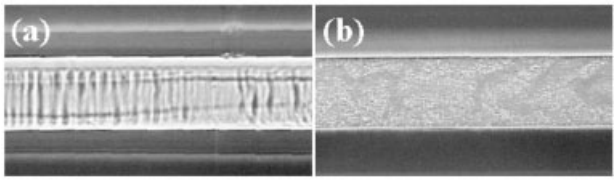

Figure 5. Microstructure of 2.5% cross-linked poly-(NIPAAm-*co*-NEAAm) monolithic gel valves prepared by polymerization at (a) 35°C and (b) 50°C.

LCST the poly(NIPAAm-*co*-NEAAm) chains precipitate from the aqueous solution forming aggregated mutually cross-linked bundles. The appearance of this polymer is opaque and it swells and deswells in water to a much lower extent than the gel prepared below LCST. Polymerizations at higher temperatures do not afford valves with the desired properties. The optimum temperature for the preparation of poly(NIPAAm-*co*-NEAAm) valves with good performance from mixtures containing equimolar proportion of both monomers was found to be 45°C.

3.4 Valving function in straight channel

The performance of the 5 mm long poly(NIPAAm-*co*-NEAAm) valve located in a capillary was monitored *via* photobleaching using a 100 nmol/L aqueous solution of coumarin 519 at a flow rate of 1 μL/min driven by a micro-HPLC pump. The repetitive opening and closing of the valve shown in Fig. 6 was achieved by submerging the capillary in a water bath heated to 60°C to open and removing it from the bath to close. Any leakage, which would result in flow through the valve, incomplete photobleaching, and detection of appreciable fluorescence, would readily be observed if it occurred but no leakage could be seen even at an extremely high pressure of 15 MPa. The inlay in Fig. 6 is an enlarged trace of one cycle. The actuation is rather fast with 1 and 2 s required for opening and closing, respectively. These results could be repeated with several valves prepared independently at different times thus confirming good reproducibility of the preparation process.

In addition to the experiments performed in a capillary, the valve was also prepared in a glass chip as illustrated in Fig. 1a. The switch to “open” position was actuated by a thermoelectrical element attached to the chip beneath the valve. The rate of both the opening and closing steps equaled that measured for the valve in a capillary. However, to avoid the buildup of high pressure within the channel of the chip, the switching operation from the closed position to the open position must be initiated

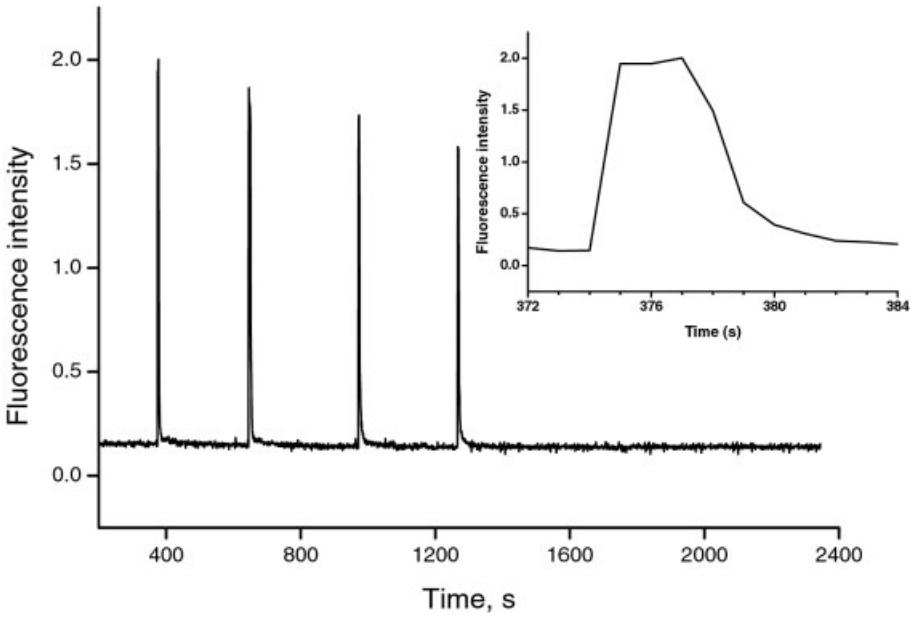

Figure 6. Monitoring of the valving function of the 2.5% cross-linked poly(NIPAAm-*co*-NEAAm) monolith in a straight channel using photobleaching of 100 nmol/L aqueous coumarin 519 solution at a flow rate of 1 μL/min for the measurement. Inlet: expanded part of the profile at the top of the "first" peak.

by heating earlier. In contrast to capillaries, chips are generally much less pressure-resistant and any high pressure could lead to their delamination and irreparable damage.

3.5 Direction of flow in a branched system

We also tested the microchip shown in Fig. 1b that contains a pair of valves for alternating flow through two channels [32]. Once again, photobleaching of the 100 nmol/L aqueous coumarin 519 solution was used to detect the closing of the channel. Typically, the thermoelectrical element beneath the first valve A was set to heat to a temperature of 57°C while the other serving valve B is set to cool to 16°C. These two temperatures were preset in our testing unit and used throughout. In this setup, valve A is open while B was closed and all the coumarin solution flows at a rate of 2 μL/min only through valve A. As a result, the detector focused at point LD located behind valve A monitors a constant fluorescence signal. When the functions of the two thermoelectrical elements are reversed, valve A switches to the closed mode with no flow through and the monitored fluorescence intensity decreases rapidly. All of the solution now flows through the opened valve B. This cycle was repeated several times at 300 s intervals. Figure 7 shows the intensity of fluorescence recorded during this cycling. Enlarged views of the peaks near the temperature inversion are shown in Fig. 7 indicating response times in the range of 3–4 s for both closing and opening. These response times are slightly longer than those observed with the single-valve capillary system shown in Fig. 6. In the single-valve implementation, the significant pressure of several MPa that builds up in front of the valve propels the liquid through the valve even before it is fully opened. In contrast, the branched system features no significant internal pressure since one channel is always open and flow is continuous. Therefore, the force driving the flow is smaller and the valve becomes permeable only when one channel is largely open and the other is completely closed.

The switching function of the poly(NIPAAm-*co*-NEAAm) valve was also tested with a higher rate of switching. The temperature of the thermoelectrical elements was switched between 16°C and 57°C each 10 s to actuate the valves and the flow was directed through the respective branches. Figure 8 shows the changes in fluorescence during a series of measurements. No change in performance was observed even after more than 100 cycles. Since it is unlikely that the valve would only operate in pure water, we also tested its performance in aqueous solutions of salts. No effect on the function was observed after repetitive cycling even when a 1.0 mol/L sodium chloride solution in 20 mmol/L phosphate buffer (pH 7.4) was used. This finding clearly demonstrates the versatility of our valve and its ability to perform well under conditions typical of numerous microfluidic processes.

3.6 Pore size

Our attempts to use conventional SEM to image the gel valve were unsuccessful since the measurement required drying of the sample, a process that always damaged

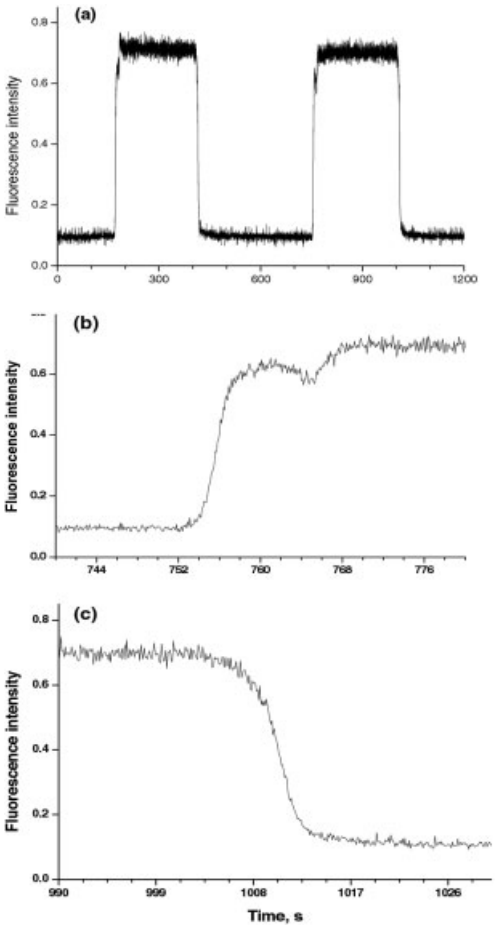

Figure 7. Monitoring of the valving function of the 2.5% cross-linked poly(NIPAAm-*co*-NEAAm) monolith in two-valve M-shaped microfluidic system of Fig. 1b. Photobleaching of 100 nmol/L aqueous coumarin 519 solution at a flow rate of 1 μL/min is used for the measurement. (a) Original trace; (b), (c) expanded parts of the opening and closing profiles.

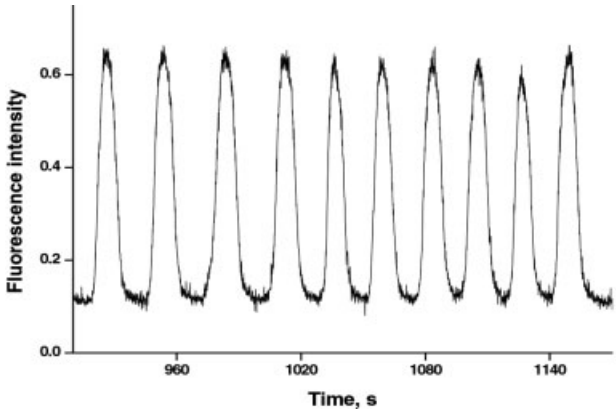

Figure 8. High-frequency valving operation of the 2.5% cross-linked poly(NIPAAm-*co*-NEAAm) monolithic gels in the M-shaped two-valve microfluidic system actuated every 10 s for an extended period of time. For experimental conditions see Fig. 7.

the original polymer. In contrast, environmental SEM is capable of imaging both wet and dry specimens of conductive or nonconductive materials at magnifications typical of SEM. Dehydration of the sample can be inhibited by a specialized pump-down procedure involving the replacement of air normally located in the chamber by an imaging gas: water vapors. This enables wet samples to be observed in their "natural state". Figure 9 show the environmental SEMs of the poly(NIPAAm-*co*-NEAAm) valve in both wet and dry state. No features are seen in the image of our hydrophilic gel swollen with water. The valve is "closed" and no pores can be observed in the homogeneous mass of gel at the magnification used in this imaging. Upon decreasing pressure in the chamber, water evaporates from the gel and the polymer dries. Partial shrinkage and displacement of the polymer from the cleaved capillary is observed under the conditions of this extreme treatment. However, Fig. 9b clearly demonstrates that upon removal water of solvation the originally homogeneous gel becomes a lace-like material with a distinct porous structure.

The environmental SEM micrographs of Fig. 9 illustrate the significant differences in pore structure that exist between the dry and wet states of the thermosensitive copolymer. While the image of gel in the wet state at low temperature (valve in the closed position) is probably representative of normal operating condition, the structure of the gel in water above LCST is certainly different from that seen in the SEM micrograph of the dry sample being extruded from the capillary by the extreme forces generated under high vacuum. Since it is difficult to directly observe the valve under normal "open" conditions, we have investigated the pore size of the open valve using fluorescently labeled monodisperse beads. A similar method has been used recently for imaging flow in microfluidic bubble-based actuators [17]. Obviously, since liquid does not flow through the valve below LCST, none of the particles with diameters in a range of 0.020–7.20 μm

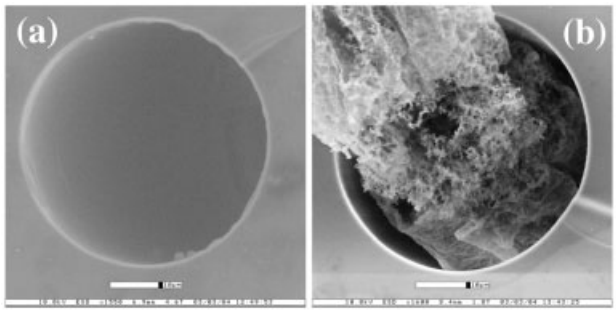

Figure 9. Environmental scanning electron micrographs of the cross-linked poly(NIPAAm-*co*-NEAAm) valve (a) in wet state showing a featureless gel and (b) in the dry state showing a porous morphology being pulled from the capillary by the evacuation.

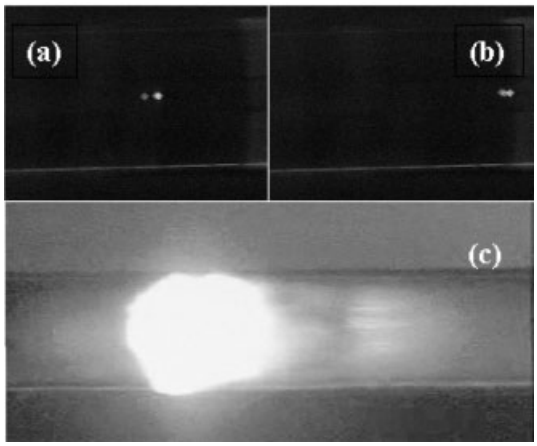

Figure 10. Optical microscopic monitoring of pore size of poly(NIPAAm-*co*-NEAAm) valve in the open position using fluorescently labeled monodisperse beads. (a) and (b) are taken at the same point at different times and illustrate the flow of labeled 2 μm polystyrene microspheres through the valve; (c) shows the accumulation of monodisperse FITC-labeled 7.2 μm poly(glycidyl methacrylate-*co*-ethylene dimethacrylate) beads in front of the valve since its pores are not permeable for beads of this large size.

can be seen flowing through the valve. However, upon increasing the temperature of the valve flow is restored and particles with a size of up to 3.69 μm can permeate the valve with virtually no interference from polymer matrix. For example, Fig. 10 shows two optical micrographs of the 2 μm beads as they move through the open valve. In contrast, the valve is not permeable to monodisperse beads with a size of 7.20 μm. As a result, all these beads accumulate in front of the valve as it operates as an efficient filter (Fig. 10c). To confirm these findings, we also collected the liquid flowed through the valve to observe any beads that passed through the open valve. As expected, using optical microscopy, a large number of fluorescent beads were found in the test fluid prepared with 3.69 μm beads while no beads were found in the effluent of a test dispersion of 7.20 μm beads. These measurements indicate that the actual pore size of the valve while in its open position is within the range of 3.69–7.20 μm. The presence of such large pores in the valve is not surprising given our earlier observation of extremely low resistance to flow and lack of back pressure in the system.

4 Concluding remarks

The cross-linked poly(NIPAAm-*co*-NEAAm) gel plugs, 0.5–5 mm in length, prepared in a single polymerization step within the channels of a microfluidic chip using photolithographic techniques can be used as valves actuated at temperatures well above the room temperature. These robust valves respond very quickly to the external stimulus – temperature – and keep their performance even after numerous "open-close" cycles. Despite the current unsophisticated implementation temperature control from outside of the device, the response of our valves is rather fast with both closing and opening achieved in 1–4 s depending of the type of device. However, it is likely that much faster actuation times could be achieved using resistor heaters built directly into the channel. This approach requiring the design of completely new type of chips is currently under investigation. While our thermally actuated valves were previously demonstrated with poly(NIPAAm) [32, 33] and the concept was extended in this work to copolymers with NEAAm enabling a broader temperature range, it is clear that further extension of LCST range can be found enabling new applications.

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