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Jing Wang
University of Pennsylvania

Zongyuan Chen
University of Pennsylvania

Michael Mauk
University of Pennsylvania

Kuang-sheng Hong
University of Pennsylvania

Mengyan Li
Drexel University

See next page for additional authors

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Self-Actuated, Thermo-Responsive Hydrogel Valves for Lab on a Chip

Abstract

An easy to fabricate, thermally-actuated, self-regulated hydrogel valve for flow control in pneumatically driven, microfluidic systems is described. This microvalve takes advantage of the properties of the hydrogel, poly(N-isopropylacrylamide), as well as the aqueous fluid itself to realize flow control. The valve was designed for use in a diagnostic system fabricated with polycarbonate and aimed at the detection of pathogens in oral fluids at the location of the sample collection. The paper describes the construction and characterization of the hydrogel valves and their application for flow control, sample and reagent metering, sample distribution into multiple analysis paths, and the sealing of a polymerase chain reaction (PCR) reactor to suppress bubble formation. The hydrogel-based flow control is electronically addressable, does not require any moving parts, introduces minimal dead volume, is leakage and contaminant free, and is biocompatible.

Keywords

Microvalve, microfluidics, hydrogel, lab-on-a-chip, metering, distribution, PCR

Disciplines

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Author(s)

Jing Wang, Zongyuan Chen, Michael Mauk, Kuang-sheng Hong, Mengyan Li, Shu Yang, and Haim H. Bau

Self-Actuated, Thermo-Responsive Hydrogel Valves for Lab on a Chip

Jing Wang^a, Zongyuan Chen^a, Michael Mauk^a, Kuang-sheng Hong^b, Mengyan Li^c

Shu Yang^b, and Haim H. Bau^{a}*

^a Department of Mechanical Engineering and Applied Mechanics,

^b Materials Science and Engineering, Chemical and Biomolecular Engineering,

University of Pennsylvania, Philadelphia, Pennsylvania 19104

^c School of Biomedical Engineering, Science and Health Systems,

Drexel University, Philadelphia, Pennsylvania 19104

* To whom correspondence should be addressed. E-mail: bau@seas.upenn.edu

ABSTRACT

An easy to fabricate, thermally-actuated, self-regulated hydrogel valve for flow control in pneumatically driven, microfluidic systems is described. This microvalve takes advantage of the properties of the hydrogel, poly(N-isopropylacrylamide), as well as the aqueous fluid itself to realize flow control. The valve was designed for use in a diagnostic system fabricated with polycarbonate and aimed at the detection of pathogens in oral fluids at the location of the sample collection. The paper describes the construction and characterization of the hydrogel valves and their application for flow control, sample and reagent metering, sample distribution into multiple analysis paths, and the sealing of a polymerase chain reaction (PCR) reactor to suppress bubble formation. The hydrogel-based flow control is electronically addressable, does not require any moving parts, introduces minimal dead volume, is leakage and contaminant free, and is biocompatible.

Keywords

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1. INTRODUCTION

In recent years, there has been growing interest in developing microfluidic systems for biological and chemical applications [1-3]. Typically, a microfluidic device consists of pumps, conduits, connectors, actuators, and valves. A fully functional valve is a key component in many microfluidic systems.

Conventional microvalves utilize magnetic [4], electrostatic [5, 6], heat [7], mechanical [8] (spring), pneumatic [9], and piezoelectric [10] mechanisms to induce the motion of a membrane. Often, such microvalves are miniaturized versions of their conventional, macro counterparts and consist of actuating, moving, and sealing components. These microvalves often require three-dimensional structures and complex assembly that may limit their use in emerging microfluidic systems. New concepts for simpler designs, easy assembly, and inexpensive components are needed to realize the potential benefits of microfluidic systems such as miniature size, multi-function integration, and low cost (particularly in the case of disposable units). Several novel approaches that have been utilized in microfluidic actuators/valves include hydraulically-actuated valves [11]; phase change valves operating with paraffin [12] and ice [13,14]; passive valves that take advantage of variations in surfaces' wetting properties [15]; check valves [16]; and viscosity-controlled valves. The latter types of valves operate with specialized fluids that change their viscosity in response to external stimuli such as temperature-responsive pluronic solution [17] and a ferrofluid that responds to magnetic-field variations [18].

Hydrogels were first reported to exhibit reversible volume changes modulated by salt concentrations in the 1950s [19]. Since then, hydrogels have been extensively used in medicine [20], pharmaceuticals [21, 22] and tissue engineering [23]. The potential ability of hydrogels to control the transport of fluids was also recognized. However, because of their slow response,

which is proportional to the square of the gel's characteristic dimension [24-26], hydrogels were not used for valve construction until the appearance of microfluidic systems.

Earlier functional hydrogel-based microvalves were controlled by varying the solution's salt concentration and were fabricated by *in situ* photo patterning [27]. The need to alter the solution's composition in order to activate the valve limited the applicability of the salt-responsive valves. This problem was alleviated by the use of hybrid, hydrogel- membrane valves in which a membrane isolates the regulated flow stream from the hydrogel, and a separate control liquid is used to actuate the hydrogel [28]. Unfortunately, this arrangement increases the valve's complexity. Fortunately, there are hydrogels that respond to external stimuli other than the solution's salt concentration and retain the feature of self-regulation without a need for separate actuating fluids. Among the hydrogels that respond to glucose [29], antigens [30], electric field [31], magnetic field [32], and temperature [33], we selected the temperature responsive poly(N-isopropylacrylamide), Poly(NIPAM), or PNIPAAm, for the construction of our valves. An unconstrained hydrogel, saturated with aqueous solution, swells by as much as a factor of 10 when the temperature decreases from above to below the gel's lower critical solution temperature (LCST). The LCST of 32°C [35] facilitates actuation with low energy consumption.

Previous workers have reported on the fabrication of temperature-responsive hydrogel valves [36-39]. For example, Yu *et al.* prepared monolithic plugs of hydrogel by photo-initiated polymerization within the conduits of a microfluidic device and demonstrated over 120 open-close cycles [37]. Richter *et al.* reported on a microvalve, which can be electronically controlled by a thermal-electronic interface [38, 39]. In this paper, we focus on the integration of thermo-responsive, hydrogel microvalves into various components of a microfluidic system used for liquid distribution, metering, and the sealing of a PCR reactor to suppress bubble formation.

These various components are part of a disposable cassette for the diagnosis of pathogens in oral samples at the point of sample collection. The cassette is fabricated with polycarbonate and consists of multiple analysis paths for the processing and identification of antibodies, antigens, and genetic materials [40].

The hydrogel valve has many advantages such as simple fabrication and operation, perfect sealing, and the ability to withstand relatively high pressures. Moreover, in some cases, the valve may operate in a self-actuated mode, open-loop control mode, eliminating the need for a sensor to control the appropriate actuation time. For example, in a pneumatically driven system, the valve would allow the free passage of air prior to the arrival of the liquid slug and would seal at the desired time when the liquid slug arrives at the valve's location.

The paper is organized as follows. Section 2 describes the fabrication, operation, and characteristics of the hydrogel valves and points out how their performance can be improved. Section 3 describes the use of the valves in various components of the microfluidic system for sample metering and distribution and the sealing of the PCR chamber. Section 4 concludes.

2. HYDROGEL SYNTHESIS

N-Isopropylacrylamide (NIPAM) is the major monomer in our thermo-sensitive hydrogel. The quantities of the various chemical reagents blended to form the hydrogel are listed in Table 1. All the reagents were obtained from Sigma-Aldrich Chemical Corp. The crosslinking agent N, N'-methylenebisacrylamide (BIS) and NIPAM were first dissolved in de-ionized water (DI water). The initiator, ammonium persulfate (APS), and the accelerator, N,N,N',N'-tetramethylethylenediamine (TEMED), were then added to the oxygen-free (bubbled with N₂) solution. After crosslinking overnight (12h) at room temperature, the Poly(NIPAM) gel

was immersed in DI water for over 12 hours to leach out non-reacted reagents. Since NIPAM has an intense odor, it was easy to detect whether any uncross-linked residues were left.

The gas formed during the crosslinking process rises to the surface, leaving pores behind. These pores are aligned and interconnected in the vertical direction. At room temperature, the naturally polymerized hydrogel has high water content, a low refractive index contrast relative to that of water, and low mechanical stiffness. When the temperature increases above the critical temperature, the gel volume reduces several folds, and the refractive index increases. Thus, combination of refractive index and porosity change causes the gel to appear opaque above and transparent below the critical temperature.

In order to increase the hydrogel's firmness, we compressed the hydrogel first. Compression parallel to the pore formation direction preserves the pore structure without significantly sacrificing the swelling rate of the hydrogel [41]. We cut the polymerized gel into cylindrical shapes with the cylinder axis aligned along the direction of pore formation. After the gel cylinder was inserted into a TeflonTM tube with inner diameter of 1.2mm (Daburn Electronics & Cable Corp.), it was compressed by two stainless steel rods that fit snugly into the Teflon tube, one on each side of the gel cylinder. See Fig. 1. The length of the hydrogel prior to (L_U) and after (L_0) the compression was recorded. The compression ratio is defined as the ratio of the slug's length after and before compression: $C=L_0/L_U$. We typically used hydrogels with a compression ratio of $C=0.6$. Once compressed, the gel retained its reduced length.

The mass of the dry gel (W_o) was determined after the gel was dried on a hot plate at 60°C for 48 h. The dried gel was then immersed in de-ionized water at 25°C until its swelling reached equilibrium. The mass of the uncompressed, wet gel (W_i) was determined after blotting

the excess water (outside the gel) with a filter paper. The water mass fraction, dubbed as the swelling ratio (SR), is defined as

$$SR = \frac{W_t - W_o}{W_o}. \quad (1)$$

The mass of the water-saturated, compressed hydrogel is denoted W_m , and the fraction of the water mass contained in the compressed hydrogel compared to the uncompressed one is

$$WC = \frac{W_m - W_o}{W_t - W_o} \times 100\% \quad (2)$$

The surface morphology of the hydrogel was examined with a FEI/Phillips XL30 Field Emission Environmental Scanning Electron Microscope (FEESEM, Philips, Netherlands) with an acceleration voltage of 15kV. The environmental capability of the microscope was not utilized in these measurements. The hydrogel samples were freeze-dried at -80°C . The freeze-dried specimens were coated with a Titanium metal layer to improve the surface's electrical conduction. Fig. 2 depicts FEESEM images of the hydrogel pore structure before (a) and after compression (b). The pore sizes of the uncompressed gel ranged from $30\mu\text{m}$ to $40\mu\text{m}$. The pore sizes of the compressed gel ranged from $10\mu\text{m}$ to $20\mu\text{m}$. Although the compression reduced the pore sizes, the pore structure was maintained after compression. The water carrying capacity of the compressed gel was measured as $WC = 52\%$, about half that of the uncompressed gel. The swelling ratios of the uncompressed and compressed gels were nearly identical. We measured $SR = 9.40$ and $SR = 9.42$ for the uncompressed and compressed gels, respectively.

3. VALVE CHARACTERIZATION

Two different experimental setups were used to characterize the hydrogel valves. In the first instance, the hydrogel was inserted into a 1.2mm diameter, Teflon tube as depicted in Fig. 1.

In the second instance, the hydrogel was embedded in a polycarbonate cavity (Fig. 6). The description of the polycarbonate-embedded valve will be given later in this section.

Two different methods were used to flow liquids through the hydrogel valves. In the first method, which we refer to as a controlled pressure mode, the water was supplied from an elevated reservoir. The inlet pressure was controlled by adjusting the reservoir's elevation, the water was discharged at atmospheric pressure, and the flow rate was measured at the device's exit. In the second method, which we refer to as a constant flow rate mode, the fluid was supplied with an adjustable syringe pump (KD Scientific Inc., Holliston, MA 01746), and the inlet pressure was measured.

We first examined the response time of the hydrogel plug when confined in the Teflon tube (Fig. 4). During the swelling process, the valve was videotaped with a digital video camera (SONY DCR-PC330) at a temporal resolution of 30 frames per second. Fig. 3 depicts the relative change in the hydrogel's plug length $\Delta L/L_0$ (solid squares) as a function of time when the length of the dry plug $L_0=700\mu\text{m}$. Since the plug's expansion is constrained in the radial direction, the ratio $\Delta L/L_0$ equals the relative volumetric change $\Delta V/V_0$. The data correlates well with the exponential law (solid line)

$$\frac{\Delta L(t)}{L_0} = A \cdot \left[1 - \exp\left(-\frac{t}{\tau}\right) \right], \quad (3)$$

where t is time, A is a constant, and τ is the time constant of the gel that depends on the plug's original length. Experiments similar to the one depicted in Fig. 3 were repeated with gel plugs of various lengths (not shown here), and the data was correlated using equation (3). The insert in Fig. 3 depicts the characteristic time τ (hollow circles) as a function of the plug's initial length L_0

on a log-log scale. The characteristic time correlates well with a power law of the form $\tau \propto L_o^{1.5}$ (solid line).

Equation (3) is similar to correlations reported by other researchers [24-26] for unconstrained expansion of the gel. In the case of a freely expanding gel, however, the characteristic time varies with the square of the initial volume, i.e., $\tau \propto V_o^2$. In contrast, in the case of the confined gel in our experiment, we find that the exponent is 1.5. This difference in the exponents indicates faster expansion in the constrained case than in the unconstrained case. A faster expansion of the constrained gel was also observed by other investigators who reported a scaling law with the exponent of 1.85 [33].

We next describe the hydrogel valve's performance when confined in the 1.2mm diameter Teflon tube (see the insert in Fig. 4). The experiment was carried out in the constant pressure mode. The response time from "OPEN to CLOSED" transition is defined as the time duration from the instant that the liquid front arrived at the upstream edge of the hydrogel slug to when the liquid flow completely stopped. To open the valve, the tube containing the water-saturated gel plug was placed on a heater. The temperatures of both the heater and the inner wall of the Teflon tube were continuously monitored as functions of time with K-type thermocouples (OMEGA Engineering, Inc., Stamford, Connecticut 06907). The "CLOSED to OPEN" transition time is defined as the time interval from the instant when the heater was turned on to the instant when the liquid flow resumed. The heat transfer time interval is defined as the time interval from the instant when the heater was turned on to the instant when the inner wall temperature reached 32°C. Fig. 4 depicts the valve's closing time (s) at the ambient temperature of 24°C (solid squares), the opening time (s) when the hot plate temperature was set to 58°C (solid circles), and the time constant (s) associated with the heating of the tube's wall (solid

upright triangles) as a function of the hydrogel plug's length (mm). The solid lines are the best-fit lines. The closing time of the hydrogel valve was about 4.5 seconds. The valve's closing is due to the local swelling of the upstream end of the gel's plug and is independent of the plug's length. The opening time was measured from the instant that the tube was exposed to the hot plate and it includes the heating time of the tube's wall. The opening time increased nearly linearly as the gel's length increased. In order to open the valve, the entire length of the gel slug must de-swell, and the excess water must be expelled through the gel's length. To obtain an estimate of the opening time that is independent of the tube's thermal inertia, we measured the time until the thermocouple attached to the inner wall of the tube registered a temperature reading of 32°C and subtracted this time from the hydrogel valve's opening time. Accordingly, the actual opening time of the hydrogel ranged from 5s when the gel plug was 300μm long to 12s when the gel plug was 1500μm long.

The pressure tolerance of the hydrogel valve confined in a Teflon tube was measured at room temperature. A fully saturated (swelled) hydrogel valve, which blocked the conduit, was subjected to air pressure. The air pressure was applied with a syringe pump. The pressure was estimated from the volume of the compressed air upstream of the gel plug using the ideal gas law. The upstream pressure was increased gradually until the breakdown pressure was reached. The breakdown pressure was defined as the pressure that either caused the gel to move or deform. Fig. 5 depicts the breakdown pressure (kPa) as a function of the gel's initial length before swelling. When the gel plug's length was above 500μm, failure occurred when the pressure forces exceeded frictional forces, and the entire plug was pushed downstream. Not surprisingly, when the gel's length was above 500μm, the breakdown pressure increased nearly linearly with the plug's length. This experiment can be used to estimate the friction force at the plug-tube

interface. When the plug's length was smaller than 500 μ m, the breakdown pressure decreased rapidly as the plug's length decreased. At breakdown, the plug deformed, allowing the compressed air to escape. The breakdown pressure can be readily increased through the addition of an appropriate support structure for the hydrogel.

In order to increase the hydrogel valve's pressure tolerance as well as use the valve as part of a microfluidic system, we integrated the hydrogel into a polycarbonate (Ensinger Ltd., Pontyclun, UK) substrate (Fig. 6). Conduits and cavities were fabricated in the polycarbonate by Computer Numerical Control (CNC) machining (Fadal VMC15XT milling machine, CA 91311 USA). The microfluidic devices consisted of two thermally bonded polycarbonate plates. The conduits and reaction chambers were machined in a 0.8mm thick polycarbonate plate. The conduits had square cross-sections with widths and depths ranging from 250 μ m to 500 μ m. The cavities to house the gel valves were located in a second 1.5mm thick polycarbonate plate. Subsequent to the milling and cleaning with isopropyl alcohol, the hydrogel plugs were inserted in the appropriate cavities. The gel tended to stick to the cavity's bottom. This adhesion was preserved during the thermal bonding process and subsequent use of the valve. The polycarbonate plates were bonded together with a hot press machine (No. 3850, Twelve Ton Press, CARVER INC., IN 46992-0544 USA) at a temperature of 140 °C and a force of 1500 Newton for 50 minutes. Next, the hot plates were cooled by heat exchange with the ambient while maintaining the polycarbonate pieces pressurized. Due to the relatively high bonding temperature, the pre-loaded hydrogel was completely dry. Finally, a type K thermocouple for temperature monitoring was inserted into the chamber with its tip protruding through the chamber wall. The thermocouple well was sealed with epoxy.

A thermoelectric (T/E) module (Melcor Corp., NJ 08648-4587, USA) was used for heating and cooling. A proportional-integral-differential (PID) controller was programmed with LabVIEW (National Instruments, Austin, TX) to facilitate temperature control.

Fig. 6 depicts the gel valve integrated into the polycarbonate structure and illustrates how the hydrogel valve works. The left and right columns depict, respectively, top and side views of the gel valve. The upper and bottom rows depict, respectively, the valve in open and closed positions. The dimensions of the cavity housing the hydrogel valve were larger than the dimensions of the conduit that the hydrogel valve was designed to seal. The conduits had a square cross-section with width a ranging from 250 μm to 500 μm . The valve chamber's width w and depth h varied from 700 μm to 1400 μm . The volume of the dry hydrogel inserted in the cavity was smaller than the cavity's volume. The volumetric ratio of the dry hydrogel plug and the valve's cavity is denoted r .

When the gel was dry or when the temperature of the saturated gel was increased above the critical temperature T_c , the hydrogel was in the de-swelled state and the conduit was open to liquid flow (top row in Fig. 6). When the temperature decreased below the critical temperature T_c in the presence of aqueous solution, the hydrogel swelled and the conduit was blocked (lower row in Fig. 6). Since the cavity improved the mechanical support of the hydrogel, the pressure tolerance of the valve was greatly increased in comparison with the case of the gel plug inserted in a Teflon tube (Fig. 1).

Fig. 7a depicts the normalized flow rate through the valve as a function of the valve's temperature when $r=0.2, 0.4, 0.6, 0.8$, and 1. The inlet pressure was 6kPa, and the flow rate was normalized with its maximum flow rate at each case. When $r < 0.4$, the gel's swelling was insufficient to completely block the flow passage and the valve could only modulate the flow

rate upon swelling. When $0.4 < r < 0.6$, the hydrogel plug completely blocked the liquid's passage at room temperature. When $r > 0.6$, the gel significantly blocked the flow passage even when in "open" position. Witness that the transition from the closed to the open position is gradual and not abrupt as the temperature increases from below to above the critical temperature. This gradual transition is most likely due to the plug not being maintained at a uniform temperature. In other words, the thermocouple reads the temperature at a particular point in space. When the thermocouple reads a transition temperature, the temperature at other locations in the plug may be different. One can take advantage of this behavior to design a valve that can regulate the flow.

Fig. 7b depicts the maximum flow rate through the valve (at 60°C) as a function of r at an inlet pressure of 6kPa. The case of $r=0$ corresponds to a conduit without a hydrogel plug. The presence of the hydrogel valve introduces significant resistance to the flow. For example, when $r=0.4$, the maximum flow rate in the presence of the valve is about 25% of the flow rate in the absence of the valve.

We subjected the valve to repetitive cycles of heating and cooling, and measured the flow rate during each cycle. Fig. 8 depicts the valve's temperature and the flow rate through the valve as functions of time. The conduits' dimensions were $a = b = 500\mu m$, the chamber for the hydrogel was $1mm \times 1mm \times 1mm$, and the volume ratio $r=0.5$. The thermoelectric unit for the cycling of the temperature was placed under the valve chamber. The opening and closing times were, respectively, 6s and 5s. In the open state, a flow rate of $83.8\mu L /min$ was measured when the pressure drop across the valve conduit was 6kPa. In the absence of the gel valve and under similar pressure head, the flow rate of the conduit was $1.9mL/min$. The closed valve did not exhibit any visible leakage up to a pressure of 200kPa. We could not test the valve at higher

pressures due to the de-bonding of the polycarbonate substrate. Witness that the valve exhibited a reproducible, predictable behavior for a large number of cycles.

4. APPLICATIONS OF HYDROGEL VALVES

In this section, we describe a few components of a microfluidic system in which we utilized the hydrogel valves. These include a distribution and metering component and a PCR reactor.

Metering/distributor

In certain analytical devices, predetermined volumes of a fluid sample need to be allocated to several analysis paths. Fig. 9 depicts schematically a distribution component that divides a sample into four metering chambers. The device was fabricated with polycarbonate. The metering chambers may have either identical or different volumes. The conduits' segments downstream of the metering chambers are equipped with hydrogel valves. The sample enters through the inlet conduit and fills the metering chambers. The meniscus at the front end of the liquid slug acts as a piston that displaces the air from the metering chamber through the outlet conduits (and through the dry hydrogel). The slug's velocity and the chamber's dimensions need to be designed with some care to avoid breaking the liquid slug. Slug breakage typically does not occur when the chamber walls are dry prior to the slug's introduction. The chamber that offers the smallest hydraulic resistance fills first. When the liquid reaches the hydrogel plug, the hydrogel swells and blocks the passage in this particular branch. Subsequently, one of the remaining chambers fills up, and so on until all the metering chambers have been filled. This application demonstrates the self-actuated operation of the hydrogel valve without a need for a

sensor to detect the position of the liquid slug. Consistent with our needs, the valve allows free passage of air and blocks the passage of the liquid. Once all the metering chambers have been filled, a valve upstream of the manifold (not shown) closes, the hydrogel valves are heated to open positions, and the aliquots are propelled into the four different analysis paths. In our application, these analysis paths consist of antibody, antigen, DNA and RNA detections [40].

Another variant of a metering chamber is depicted in Fig. 10. The device is equipped with two chambers with hydrogel valves positioned downstream of each chamber. Chamber 1 serves as a metering chamber and chamber 2 is designed to accommodate the excess liquid. The valves downstream of chambers 1 and 2 are denoted, respectively, as valves A and B. The passage to chamber 1 is designed to have a deeper conduit, thus lower hydraulic resistance, and this chamber fills first. As before, prior to the sample introduction, the hydrogel valves are dry and maintained at temperatures below the transition temperature. Once the liquid arrives at the hydrogel valve located downstream of chamber 1, the valve saturates, swells, and seals. The excess liquid then flows into chamber 2. Subsequently, valve A is opened, and the sample is propelled into the analytical device.

PCR amplification

When attempting to detect and identify the presence of genetic materials, one often needs to carry out PCR amplification of the DNA. One method of achieving chip-based PCR is to hold the reagents in a chamber while cycling the chamber's temperature (stationary PCR). A problem that is often experienced with stationary PCR micro-reactors is bubble formation. The bubbles may expel the reagents from the PCR chamber, adversely affect temperature uniformity, and reduce the amplification efficiency. One way to minimize or eliminate the bubble formation is to

pressurize the PCR chamber by sealing it. Here, we describe the use of hydrogel valves for this purpose.

Fig. 11a is a photograph of a polycarbonate PCR reactor equipped with two hydrogel valves. The PCR mixture is driven into the reaction chamber through the inlet hydrogel valve. During this process, the inlet valve is maintained well above the transition temperature and it allows unhindered passage of the liquid through it. The liquid fills the PCR chamber, displacing the air through the exit valve. At this stage, the exit valve is dry and allows free passage of the displaced air. Once the air has been displaced out of the chamber and the liquid arrives at the exit valve's location, the hydrogel swells and blocks the passage. Subsequently, the inlet hydrogel valve is cooled below the transition temperature and closes. Since, at this instant, the sample is stationary, the precise closing time of the upstream valve is not critical, and the valve's thermal actuator can be programmed in an open control loop mode. Alternatively, the intake valve can be replaced with a check valve that allows flow passage only in the downstream direction. Once both the upstream and downstream valves are closed, the temperature of the PCR reactor is cycled according to standard protocols. The valve and chamber materials provide sufficient flexibility to keep the pressure in the chamber from exceeding the structural limits of the substrate material.

To test the compatibility of the PCR process with the hydrogel, we carried out a sequence of tests. In one set of experiments, the PCR reactants were flown through the hydrogel valve and subsequently subjected to a PCR process in a bench top device. In another set of tests, a hydrogel plug was submerged in a vial in a bench top PCR machine, and the reaction was carried out in the presence of the gel. These experiments were motivated by our concern that the DNA molecules and enzymes may adsorb to the hydrogel and the solution may get depleted of some of

its essential ingredients. Finally, PCR reaction was carried out in the chip-based PCR chamber equipped with the hydrogel valves.

We amplified 305-bp fragments from 5,224,283 bp *Bacillus cereus* genomic DNA [41]. To avoid carry-over contamination, we used a virgin polycarbonate-based chip. The inlet and outlet conduits were both 250 μ m $\times$ 250 μ m (width $\times$ height). The PCR chamber's volume was 8 μ L. In parallel, control runs were carried out in a standard bench-top PCR thermocycler (Techne Incorporated, Princeton, NJ). Both the control and on-chip PCR were carried out with the same reagents: 50 mM Tris-HCl (pH 9.0), 20 mM (NH₄)₂SO₄, 1.5-3.5 mM MgCl₂, 200 μ M dNTP, and 0.1 μ g/ μ L BSA. Primers for the amplification of the 305-bp fragments of the *B. cereus* genomic DNA were 1.5 μ M (5'-TCT CGC TTC ACT ATT CCC AAG T-3', 5'-AAG GTT CAA AAG ATG GTA TTC AGG-3'). The concentration of the DNA template was 4ng/ μ L and the concentration of Taq was 0.1 unit/ μ L. Both the PCR protocols for the benchtop and the microfluidic chip consisted of initial denaturation at 95 °C for 120 sec, 25 cycles (95 °C, 20s; 55 °C, 20s; 72 °C, 20s), and a final extension at 72 °C for another 120s. The PCR products were analyzed by agarose gel (1%) electrophoresis and stained with ethidium bromide.

The electrophoresis results are shown in Fig. 11b. The left lane is the marker. Lane 1 is the PCR positive control carried out in the bench top apparatus with a target DNA volume of 20 μ L. Lane 2 is the negative control carried out in the PCR reactor equipped with hydrogel valves, where the PCR solution contains all the necessary ingredients except for the DNA template. Lane 3 corresponds to results obtained with the PCR reactants first being transmitted through the hydrogel valve and then reacted in a bench top apparatus. Lane 4 describes results from similar conditions as lane 3. In this case, however, the hydrogel valve was washed repeatedly to remove any uncross-linked material. The results of lanes 3 and 4 indicate that the

hydrogel does not significantly adsorb the PCR reactants. Lane 5 corresponds to the amplification experiment in a bench top apparatus with a hydrogel plug being submerged in the PCR mixture. Since lane 5 appears to be nearly identical to lane 1, we conclude the hydrogel does not inhibit PCR. Lane 6 corresponds to the PCR products obtained from the on-chip device (Fig. 11a). The on-chip device exhibited successful amplification.

5. CONCLUSIONS

The temperature responsive hydrogel, poly(N-isopropylacrylamide), saturated with an aqueous solution undergoes a significant, reversible volumetric change when its temperature is increased from room temperature to above the phase transition temperature of 32°C. When confined in two space directions, the gel exhibited a relaxation time $\tau \sim L^{1.5}$, where L is the length of the gel. This should be contrasted with the unconfined gel that exhibits a relaxation time $\tau \sim V^2$.

We took advantage of this dramatic volumetric change to construct hydrogel-based, thermally actuated valves for microfluidic systems. These valves are attractive since they do not require any mechanical components, they do not involve any moving parts, and they are easy to integrate within various substrate materials. For example, we embedded the hydrogel in polycarbonate plates prior to the thermal bonding of the plates. The exposure of the hydrogel to the thermal bonding temperatures did not have any apparent adverse effect on the gel.

One important advantage of the hydrogel valve is that when dry, it allows free passage of gases. In pneumatic systems, the dry hydrogel valve will allow the displacement of air from cavities and conduits upstream of an advancing liquid slug. Once the aqueous liquid arrives at the hydrogel's location, it will saturate and swell the gel, blocking the flow passage. We refer to

this mode of operation as self-actuated in the sense that the valve closes (by itself) just at the right time without any need for external actuation (which typically would require a sensor and a controller). The valve can be opened and the blockage can be removed when desired by heating the hydrogel to above its transition temperature. Some of the valve's disadvantages are relatively slow response time, high hydraulic resistance when in open position, and relatively large manufacturing tolerances.

We constructed two types of valves. In one case, the valve consisted of a cylindrical plug inserted in a Teflon tube. In the second case, the valve was inserted in a cavity embedded in a polycarbonate structure. In the latter case, the hydrogel occupied about half of the cavity's volume. In both cases, the valve's closing (swelling) time was about 4.5s. The deswelling (opening) time depended on the hydrogel's volume. The hydrogel valves were operated for many opening and closing cycles with good reproducibility and without any visible deterioration in performance. The valves could withstand pressures higher than 200kPa without any noticeable leakage. The valves' response times were less than 6 seconds, and the valves operated reversibly and reproducibly over many cycles.

The utility of the hydrogel valves for flow switching, distribution and metering, and the sealing of a PCR reactor chamber was demonstrated. The hydrogel proved to be biocompatible and not to hinder PCR. Moreover, the hydrogel valves did not appear to absorb significant quantities of DNA and enzymes suspended in PCR buffer.

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Table 1: Chemical composition of the PolyN-Isopropylacrylamide hydrogel

Chemicals	Name	Function	Amount
BIS	N,N'-Methylenebisacrylamide	crosslinker	0.02g
NIPAAM	N-Isopropylacrylamide		1.56g
TEMED	N,N,N',N'-tetramethylethylenediamine	accelerator	200ul
APS	Ammonium Persulfate	initiator	0.075g
	DI Water		50mL

Figure 1: A hydrogel plug confined in a Teflon tube. L_u and L_0 denote, respectively, the free and compressed lengths of the hydrogel plug.

(a)

(b)

Figure 2: Environmental Scanning Electron images of naturally formed (a) and compressed hydrogel (b).

Figure 3: The relative change in the length of a gel plug when confined in a Teflon tube as a function of time (s). The symbols and the solid line in the main part of the figure correspond, respectively, to experimental data and to a fitting curve of the form $\Delta L/L_0 \sim (1 - \exp[-t/\tau])$. The insert depicts the relaxation time τ (s) as a function of the gel's length (L_0 , μm). The solid line in the insert corresponds to $\tau \sim L_0^{1.5}$, where L_0 is the dry gel's length.

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(a)

(b)

Figure 7: (a) The normalized flow rate as a function of the temperature for various relative volumes of the hydrogel $r = 0.2, 0.4, 0.6$, and 0.8 , where r denotes the ratio of the volume of the dry gel and the volume of the cavity. The flow rate was normalized with the maximum flow rate when the valve is fully “open.” (b) The maximum flow rate as a function of r . A pressure difference of 6 kPa is applied across the gel valve.

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Figure 10: Photograph of metering and overflow chambers.

(a)

(b)

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