Voltage-addressable on/off microvalves for high-pressure microchip separations

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Abstract

We present a microchip-based, voltage-addressable on/off valve architecture that is fundamentally consistent with the pressures and solvents employed for high-pressure liquid chromatography. Laser photopatterning of polymer monoliths inside glass microchannels is used to fabricate mobile fluid control elements, which are opened and closed by electrokinetic pressures. The glass substrates and crosslinked polymer monoliths operate in water–acetonitrile mixtures and have been shown to hold off pressures as high as 350 bar (5000 p.s.i.). Open/closed flow ratios of 10^4 to 10^6 have been demonstrated over the pressure range 1.5–70 bar (20–1000 p.s.i.), and the pressure–leak relationship shows the potential for valving control of flow through packed or monolithic chromatography columns. We expect that this valve platform will enable multiplexing of multiple chromatographic separations on single microchips.
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1. Introduction

The functionality of microscale separation systems is fundamentally limited by the sophistication of the fluid control techniques used. Massively parallel, multidimensional separations and real-time decision-making on chip requires arbitrary control of fluid and current flow. The most fundamental fluid control element is an on/off valve, which can shut off flow in a channel using some external actuation. On/off valves play a role in fluidics analogous to the transistor in microelectronics, and successful implementation of robust, solvent-compatible, well-se-

aling on/off valves is expected to have similar impact. On/off valves can be used to route flow through different channels [1], perform peristaltic pumping [2], or seal reaction zones. In liquid chromatography systems, on/off valves can be used to parallelize chromatographic separations, or dynamically select a second separation dimension (e.g., affinity, ion-exchange) based on the output of a first separation dimension (e.g., reversed-phase). In a similar manner, solvents used for these separations may be chosen in real-time using on/off valving systems. Key requirements for practical on/off valving systems for high-pressure chromatography include the following: (a) the valves must be able to hold off high pressures used to drive fluid through chromatographic media, and allow leak rates that are small compared to the flow through the media; (b) the valves must operate in the wide range of solvents used for separations; and (c) the valves must be

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constructed using an inexpensive and process-compatible fabrication technique.

Although extensive effort has been placed in developing microscale valves, little success has been achieved in developing valves for microchip-based HPLC-type applications. For example, gradient HPLC through a packed or porous monolithic chromatography column poses a challenging microscale fluid control problem because high pressures and a variety of solvents are used. For successful operation, a microvalve must have a Darcy permeability substantially less than the HPLC column for all solvents within the gradient space. The permeability requirement demands extraordinary flow restriction. Compatability with a wide variety of solvents places extreme demands on the material choice, since many materials (e.g., many elastomers, non-crosslinked polymers, and polymer gels) swell or dissolve in solvents used for chromatographic separations.

A large body of research has been directed to developing microvalves in a variety of platforms, including silicon, elastomers, and hydrogels. Micromachined silicon valves [3] are limited in that they often dissipate substantial power [3], are often incompatible with chemical separation techniques that employ high voltages, and are generally unable to hold off HPLC-type pressures. Seeking in part to avoid these limitations, a number of microfluidic devices in polymeric materials have been presented [4]. For example, Unger et al. [2] have demonstrated pumps and valves created using multiple layers of intersecting channels in poly(dimethylsiloxane) (PDMS). The straightforward fabrication techniques employed to create these devices allows parallelization of flow-control devices on a single substrate. They have been used primarily in low-pressure water systems due to the limited solvent resistivity and pressure holdoff capacity of PDMS substrates. Hydrogel valves [1] are actuated by fluid properties and offer the intriguing possibility of autonomous flow-control devices; however, these have not been employed for HPLC separations since they only offer about an order of magnitude reduction in flow-rates and are limited by solvent effects and slow actuation times. Recently, the use of mobile polymer monolith elements for microfluidic control [5] has been proposed as a straightforward and inexpensive means to address, among other issues, the challenge of con-

trolling flow in HPLC-type situations on chip. Check valves and microchip-based pipetting functions have been demonstrated.

Here, we present straightforward and inexpensive techniques for making mobile polymer monolith elements and demonstrate their application as high-pressure, voltage-addressable, on/off microvalves. These monolith elements can hold off pressures suitable for HPLC and, when actuated by pressure generated by an electrokinetic pump, can be controlled using voltage. In the following sections, we will present techniques for fabricating and testing valves, and discuss valve performance as compared to the requirements for microchip-based on/off valving for HPLC applications.

2. Experimental

Mobile monolithic polymer elements [5] can be used for a variety of valving functions on microchips. Porous polymer monoliths may be fabricated in situ and therefore conform to microchannel geometries, and can, through careful polymer formulation, be engineered to be mobile within microchannels. Through design of microchannel and polymer geometries, these mobile polymer monoliths can be cast in place such that, upon actuation with pressure, the motion of the polymer monolith performs a valving function. A picture and schematic of such a valve function is shown in Fig. 1, in which a mobile monolith can be actuated from left with positive or negative pressure; positive pressure moves the monolith into the top-bottom channel, shutting off flow; while negative pressure moves the monolith out of the channel, opening the channel to flow. A side view of the three-dimensional geometry is also shown. This on/off valving function is geometrically quite similar to one proposed in [2], except that the control action is in the same plane as the microchannels. Another key conceptual difference is the hardness of the material. In contrast to PDMS substrates which are quite soft and deform significantly at the pressures used, the polymers and glass substrates used here do not deform significantly when in use, allowing precisely defined fluidic boundary conditions. These hard polymer formulations would be brittle and useless at macroscales, but their me-
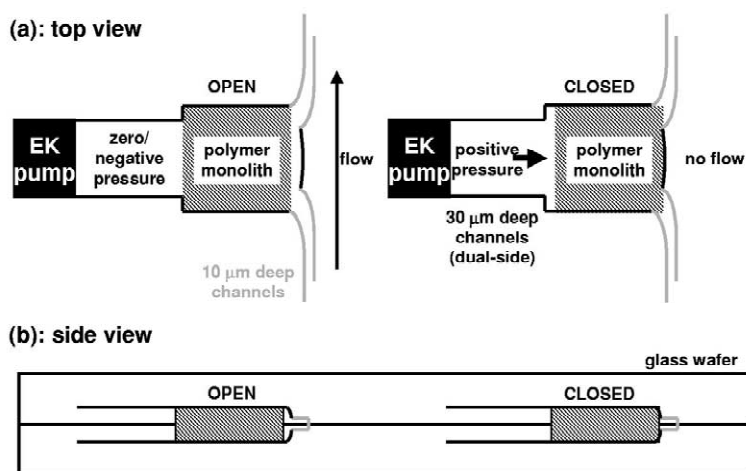
Fig. 1. Schematic of on/off microvalve operation using mobile polymer monolith elements. EK pump: electrokinetic micropump. (a) Top view: with no pressure or negative pressure applied at left, the monolith is positioned to the left, and the channel at right is open. When positive pressure is applied at left, the monolith is pushed into the channel at right, shutting off flow. (b) Side view: two layers of etch are used so that a complete valve seat is formed. Good leak rate performance at high pressure has required two-level etches.

chanical strength at microscales allows for high-pressure performance.

The valves discussed here are fabricated through in situ photopatterning of mobile polymer elements inside microchannels. The technique is relatively straightforward, and allows for rapid valve fabrication using a simple, tabletop setup outside of clean-room environments. Glass or fused-silica microchips (fabricated using standard photolithography, wet etch, and bonding techniques) are used. Typical chips have two separately etched levels, one deep (~30 μm) and one shallow (~10 μm). In contrast to typical microchip fabrication procedures, both glass substrates (base and cover) are etched. The base substrate contains the majority of the microfluidic channels. In the region where the polymer element is to be fabricated, the cover substrate is etched with a mirror image of the base wafer pattern. Base and cover wafers are aligned (typically to within 1–6 μm) and bonded. The three-dimensional structure generated by this etch process leads to a nearly cylindrical microchannel geometry (65-μm width, 60-μm depth) and a nearly cylindrical polymer element, which seats against a shallow channel (25-μm width, 20-μm depth) generated by the shallow etch.

External fluidic elements (e.g., capillaries, syringes) are integrated with the microchip with in-house-designed fittings. Female fittings may be bonded to the microchip with epoxy for a semi-permanent connection that operates up to 5000 p.s.i., or may be press-fit with an O-ring seal for a quickly changed connection that operates up to approximately 1000 p.s.i. (1 p.s.i.=6894.76 Pa). Male fittings are then inserted, forming a screw-on compression-seal with an inserted capillary.

Monomers are obtained from Aldrich, and chosen generally from the family of acrylates and methacrylates (e.g., 2,2,3,4,4,4-hexafluorobutyl acrylate crosslinked with 1,3-butanediol diacrylate); choice of monomers affects pore size, surface energy, and mechanical strength. Solvents (e.g., 2-methoxyethanol) are obtained from Aldrich and used as received, chosen to control the phase separation properties of the mixture and hence polymerization times and pore sizes. Aromatic solvents are avoided in order to minimize the effects of trace aromatics on fluorescence diagnostic systems. The monomer mixture and solvent mixture are added in a nominal 60:40 ratio. Suitable photoinitiators (e.g., 2,2′-azobisisobutyronitrile) are obtained from Aldrich and used as received; as needed, the level of polymerization inhibition (due to added or naturally occurring inhibitors such as dissolved oxygen, hydroquinone, or 4-methoxyphenol) is reduced through sonication under vacuum and/or purification by

filtering through activated alumina particles; alternately it can be increased by addition of inhibiting elements. Following off-chip mixing and preparation, monomer/solvent mixtures of volumes 100 μl–1 ml are injected using pressure. The chip ports are then closed off from the environment, and the fluid is allowed to become quiescent. Close attention to the quiescence of the fluid is required to optimize the resolution of the photopatterning.

Phase-separation polymerization is induced in situ by locally exciting the photoinitiator using a shaped laser beam from a frequency-tripled Nd:YAG laser (Continuum Mini-lite) operating at 10 Hz with nominal local beam fluence of 4 mJ/cm^2. The region defined by the intersection of the open channel area and the laser beam shape defines the eventual geometry of the polymer monolith. Polymerization typically occurs in 20–90 s. The shape of the laser beam is controlled by focusing the beam using cylindrical or spherical optics and, for complicated shapes, imaging onto the microchip the diffraction pattern caused by focusing the laser through a chrome-on-glass mask. Previous work [1,5] has reported use of contact lithographic masks to define the laser excitation region; this works well for the relatively large (>300 μm) monoliths reported therein, but leads to a number of experimental challenges as the monolith size is decreased to be incorporated into small microchannels and to minimize valve dead volume. On typical glass microchips, which use 0.5–1.5-mm thick wafers, the lithographic mask is far enough away from the region of polymerization for diffraction to play a prominent role, and the resolution of a sharp edge is limited as follows [6]:

$$2b_{min} = 3\sqrt{\lambda(s + z/2)} \qquad (1)$$

where $2b_{min}$ is the resolution limit, λ is the wavelength (355 nm), s is the distance between mask and surface (500–1500 μm), and z is the thickness of the polymerized region (20–60 μm). This leads to resolution limits ranging approximately from 40 to 70 μm, which strongly limits the ability to pattern elements below 100 μm in size. By shaping the beam directly, the polymerization process is improved in two ways: (a) resolution is improved, since diffraction around mask edges is not present, and (b) polymerization is more uniform, since edges can be sharply and uniformly defined. A schematic is shown in Fig. 2. Here, collimated 355 nm light is spatially filtered, then focused onto the microchip in one of two ways: (a) the light is focused directly using a cylindrical optic, forming a thin sheet of light approximately 5-mm long and limited in width by the focal length of the cylindrical optic and the size of the spatial filter; (b) the light is focused with a spherical optic onto a lithographically defined mask; the diffraction pattern from this image is collected and focused by one or more spherical optics, generating an image of the mask at the microchip. The latter is of course a standard projection lithography configuration. In either case, the polymerized region is the intersection of the beam shape (as defined by the optics) and the microchannel geometry (as defined by the microfabrication).

While photoinitiation can be used with a variety of UV sources, including a host of UV lamps, we employ a nanosecond-pulse laser for polymerization for several reasons: (a) the intense UV light during the pulse leads to high radical populations and relative insensitivity to inhibitors. This is particularly important for dissolved oxygen, since a polymerization system that is sensitive to dissolved oxygen must pay close attention to gas handling; by removing this parameter we are able to repeatably polymerize monolithic elements despite exposing the monomer solution to air. (b) By increasing the local fluence, the time required for polymerization is reduced, leading to shorter diffusion lengths and better resolution. Compact and very inexpensive lasers may be used since the required fluences to achieve this goal are small.

Following polymerization, the channels are flushed thoroughly with acetonitrile and filled with the working fluid. Once the monoliths are polymerized and the working fluid has been injected, pressure is applied using an HPLC pump, syringe pump, or electrokinetic pump to mobilize the monoliths and achieve a valving operation. Electrokinetic pumps are preferred, as they allow the position (open/closed) of the valve to be controlled using voltage and provide the clearest path to automation and computer control. Images of a chip through polymerization and actuation are shown in Fig. 3. This chip is designed to rout fluid from the port at left to either the top or bottom port. Two control lines (each
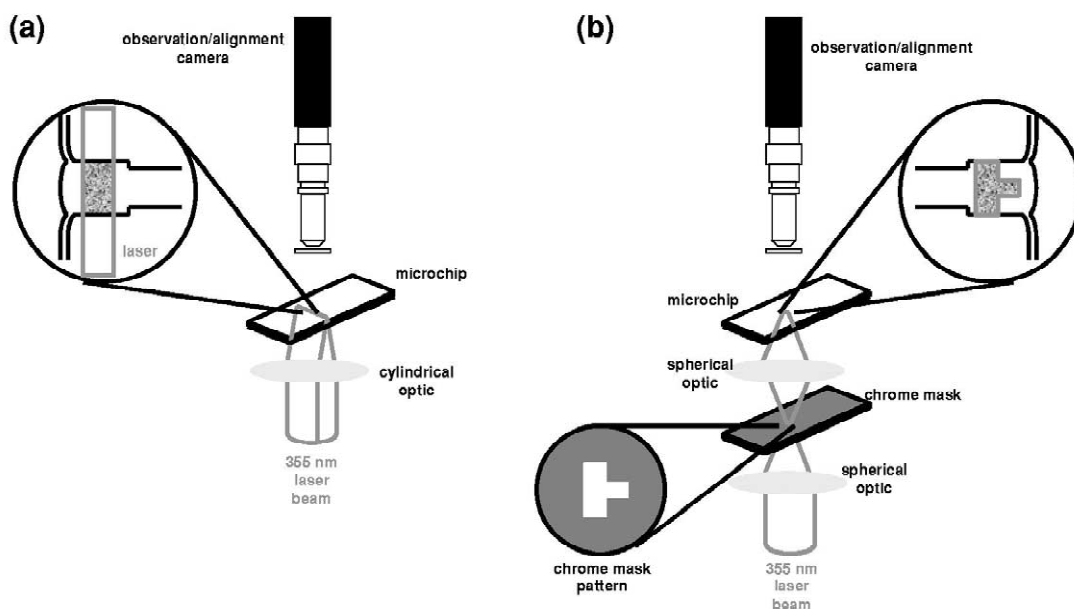
Fig. 2. Optical setups for photopatterning mobile polymer monoliths. (a) Cylindrical optics focus the laser beam into a 60-μm wide sheet, whose thickness constrains one dimension (length) of the monolith. The diameter of the monolith cylinder is specified by the microchannel dimensions. (b) Standard projection printing configuration. Spherical optics image a chrome mask onto the microchip. Here, the two-dimensional shape of the monolith can be dictated entirely by the shape of the excitation beam. The technique in (a) is straightforward and suitable for most monoliths; the technique in (b) can be used to photopattern more complicated shapes.
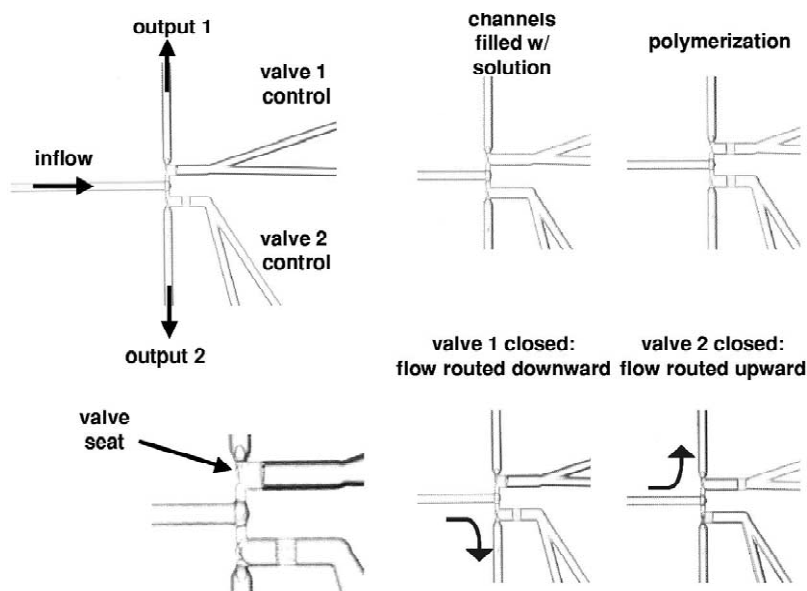

Fig. 3. Images of a chip during polymerization and valve actuation. Microchannels are 65 μm in diameter, 60 μm deep. Control lines are doubly redundant; the extra port is not used for control but facilitates rapid flushing after polymerization. Monolithic elements were polymerized from a 65:35 mixture of monomer (2,2,2-trifluoroethyl acrylate–1,3-butanediol diacrylate, 50:50) and solvent (2-methoxyethanol) initiated with 0.5% (w/w) AIBN.

doubly redundant) are used to actuate an on–off valve, opening one and closing the other. Fluid is routed in the direction of the open valve. Pressures near 0.7 bar (10 p.s.i.) were used to actuate the valves in this configuration.

Actuation pressure requirements were evaluated inside capillaries by polymerizing elements for different lengths of time and measuring the pressure at which the polymer monolith moved (Fig. 4). Experiments were repeated to remove any ambiguity caused by stiction effects.

Valve sealing performance was evaluated by measuring flow-rates versus pressure with the valves open and closed. While the sealing characteristics of polymer monoliths seated against glass stops have been evaluated at pressures as high as 350 bar (5000 p.s.i.) in capillary and 200 bar (3000 p.s.i.) on-chip [5], the pressure handling system (microchip, housing, and fittings) used for these measurements were only capable of handling pressures up to 70 bar (1000 p.s.i.), limited primarily by O-ring seals of the chip housings or (in some chip designs) failure of the glass substrate; thus pressures up to 70 bar (1000 p.s.i.) were explored. Flow rates were inferred from motion of a meniscus in an open channel or by measuring the fluorescence signal from a bulk solvent flow mixed with the tracer-seeded leak flow.

Valves were actuated using electrokinetic pumps. Electrokinetic pumps have no moving parts, and are ideal for controlling the low flow-rates and high pressures needed for microchip-based HPLC [7,8]. Here, the electrokinetic pumps are used to apply positive or negative pressure to a mobile polymer monolith element and apply that pressure through a volume change on the order of 200 pl. Electrokinetic pumps are fabricated by packing a silica capillary with non-porous silica microspheres using a slurry packing technique. A 10-cm length of 100-μm I.D. silica capillary was packed with 3-μm non-porous silica beads using slurry packing at 500 bar (7000 p.s.i.). The column length was chosen for convenience; at the same voltage, shorter lengths lead to improved time response until Joule heating causes a decrease in performance. Longer lengths facilitate extremely high-pressure operation, which was not needed for this application. The bead size also impacts pressure performance and time response; 3-μm beads were found to give acceptable flow-rates (100 nl/min) at pressures required to actuate the valves. Silica was chosen as a convenient material that has a relatively high zeta potential with aqueous solutions at neutral pH.

3. Results/discussion

3.1. Valve actuation

Sample results of actuation pressure measurements are shown as a function of integrated exposure in Fig. 4. The sharp rise in actuation pressure at 0.3 J is indicative of the point at which the monolith is uniformly polymerized throughout the channel. At times previous to 0.3 J, a weakly cross-linked polymer exists that does not effectively fill the channel. Additional exposure beyond 0.3 J increases the cross-linking and mechanical strength of the monolith, simultaneously leading to increased pressure actuation requirements.

Experiments measuring the dependence of actuation pressure on monolith diameter [5] and length (data not shown) are consistent with simple tribological models that describe the friction force on the monolith as a function of a phenomenological fric-
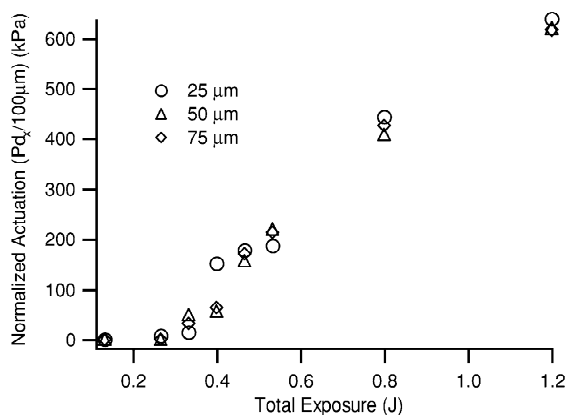

Fig. 4. Required monolith actuation pressures as a function of exposure time. Actuation pressures are normalized by a diameter ratio to collapse results with several diameters to one curve. Monolithic elements were polymerized from a 60:40 mixture of monomer (2,2,2-trifluoroethyl acrylate:1,3-butanediol diacrylate, 50:50) and solvent (4:4:1 2-methoxyethanol:1,3 dioxolane:5 m*M* Tris) initiated with 0.5% (w/w) AIBN.

tion coefficient and the integrated force on the monolith–substrate interface:

$$F_f = c_f F_n$$

where F_f is the friction force, c_f is a phenomenological friction coefficient based on the polymer formula, substrate material, and degree of polymerization, and F_n is the normal force between the monolith and the substrate. These experiments indicate further that these polymers exhibit a low Poisson's ratio, so the pressure at the monolith–substrate interface is constant and the friction force is proportional only to the friction coefficient and the surface area of the monolith–substrate interface. From these relations, we can straightforwardly infer that the pressure required to actuate a cylindrical monolith is a function of two parameters: fabrication technique (P_{act} is proportional to the friction coefficient, affected by polymer formulation and polymerization time) and geometry (P_{act} is also proportional to the length/diameter ratio of the monolith). Optimized valve designs minimize the friction coefficient through careful optimization of polymer formulation, strict attention to using the minimum polymerization time required to form a channel-filling monolith, and using monoliths with length/diameter ratios from 1.0 to 1.5.

The time required to actuate these microvalves is equal to the time required to move the valve through its dead volume (roughly equal to the volume of the monolith) at the actuation pressure. For syringe pumps this is typically on the order of 1 ms. With the electrokinetic pump used in this work, valves were opened and closed at 1 Hz.

3.2. Leak rate performance

Pressure-leak rate performance of a mobile monolith on/off microvalve is shown in Fig. 5, in which flow-rates are shown for on/off valves in both on and off positions. Flow is reduced by a factor ranging approximately from 10^4 to 10^6 within this range. These ratios compare favorably with other microfluidic valve architectures. In the on (open) position, flow-rate is linear with applied pressure (although for this microchip geometry, a Reynolds number near 3000 and hence transitional flow should occur in certain regions of the chip at 0.1 ml/s). In
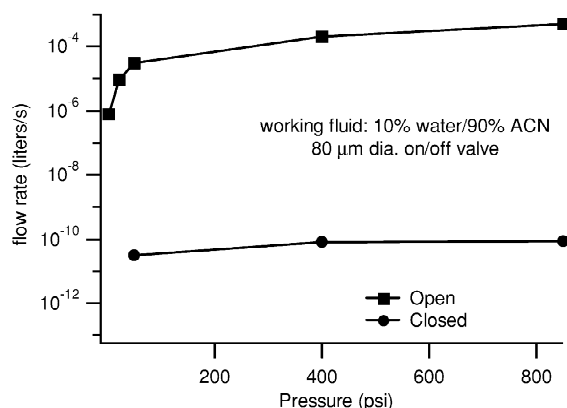

Fig. 5. Flow rates as a function of pressure for mobile polymer monolith on/off valves in open and closed positions. Open/closed flow ratios are as high as 10^6. Monolithic elements were polymerized from a 70:30 mixture of monomer (2,2,3,4,4,4-hexafluorobutyl acrylate:1,3-butanediol diacrylate, 50:50) and solvent (2-methoxyethanol) initiated with 0.5% (w/w) AIBN.

the off (closed) position, a nonlinear relationship is found, indicating that the Darcy permeability of these monoliths decreases with increasing pressure, as a function of the monolith's ability to conform and seal to the glass. This can be a nonlinear function and is dependent on chip-to-chip fabrication variability. Characteristic pore sizes are near 30 nm, based on scanning electron microscopy (SEM), Hg porosimetry, and BET measurements (data not shown); these pore sizes are much smaller than those typical of chromatographic media. The scale separation between pore size of these monoliths as compared to those of packed or monolithic chromatographic media allow these valves to effectively shut off flow at the pressures and flow-rates in use for chromatography. The Darcy permeability of one of these valves at 70 bar is comparable to that calculated (using standard Kozeny–Carman relations) for a 20-cm length of reversed-phase chromatography column in use in our laboratories [9,10]. This is indicative of the usefulness of these valves in routing fluids in high-pressure chromatography systems.

The leak rate data presented here (as was the case for Ref. [5]) is for a working fluid consisting of an acetonitrile–water mixture (90:10). This solvent is chosen to improve the performance of weakly polymerized monoliths by matching the swollen state

present when the monoliths are polymerized to the state present when leak rate data is collected. Recent polymer formulation developments have led to robust leak rate performance in all water–acetonitrile systems; those results, though, are beyond the scope of this work and will be presented in a later paper.

4. Conclusions

Component-level performance and fabrication techniques have been presented for a voltage-addressable on/off valve architecture with properties consistent with fluid control for multiplexed microchip-based HPLC. Cylindrical phase-separated porous polymer monoliths with diameters and lengths near 60 μm have been photopatterned in glass microchannels and mobilized with pressure generated by an electrokinetic pump. The motion of these monoliths has been used to effect on/off valve function by pressing the monolith into a stop and closing off a fluid path. Results with one particular formulation have been shown to actuate at modest pressures (5–10 p.s.i.). Valves using this formulation show a ratio of open/closed flow-rates ranging from 10^4 to 10^6 over the pressure range 1.5–70 bar (20–1000 p.s.i.) with a working fluid of acetonitrile–water (90:10). The Darcy permeability of one of these valves at 70 bar is comparable to that calculated for a 20-cm length of reversed-phase chromatography column in use in our labs, indicating that fluid routing in microchip-based HPLC systems is quite feasible. The applicability of this polymer formulation is limited by the tendency of these monoliths to change size in response to solvent changes, effectively reducing valve quality; however, recent changes in polymer formulation have eliminated this problem and will be presented in a future paper. Other future work includes optimization and characterization of the protein adsorption properties of these monoliths, as well as application for fluid routing in microchip-based HPLC systems.

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