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Stimulus-responsive polymers and other functional polymer surfaces as components in glass
microfluidic channels

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Microfluidics, polymer, stimulus responsive, gating, valve, pumping, actuation

Abstract

The integration of smart, stimulus-responsive polymers as functional elements within microfluidic devices has greatly improved the performance capabilities of controlled fluid delivery. For their use as actuators in microfluidic systems, reversible expansion and shrinking is a unique mechanism which can be utilized as both, passive and active fluid control elements to establish gate and valve functions (passive) and pumping elements (active). Various constituents in microfluidic glass channels based on stimulus-responsive elements have been reported, based on pH-responsive, thermoresponsive and photoresponsive coatings. Fluid control and robust performance have been demonstrated in microfluidic devices in a number of studies. Here we give a brief overview of selected examples from the literature reporting on the use of stimulus-response polymers as active or passive elements for fluid control in microfluidic devices, with specific emphasis on glass-based devices. The remaining challenges include improving switching times and achieving local addressability of the responsive constituent. We envisage tackling these challenges by utilizing redox-

responsive polymers which offer fast and reversible switching, and local addressability in combination with nanofabricated electrodes.

Introduction

Controlled manipulation and delivery of small amounts of liquids down to attoliters requires sophisticated fluidic devices that offer smart actuation volume with full external fluid flow control. The availability of such microfluidic systems would enhance biomolecular separation, controlled transport of drugs, micro- and nanoparticles and biomolecules at the micrometer or sub-micrometer length scales. Applications can also include power sources such as devices allowing for multi-stream laminar flow in fuel cells¹. Despite great progress in development of nanofluidic devices^{2,3}, numerous challenges remain regarding addressable switches and actuators for fluid control. In this respect, smart materials composed of stimulus-responsive polymers are particularly promising candidates to tackle these challenges. Here we give a short account on the current status of this field and sketch some opportunities for future development.

Stimulus-responsive polymers undergo large and abrupt changes in their chemical and physical properties in response to small variations in various stimuli in their environment.⁴ Conventional stimuli include changes in temperature^{5,6,7,8,9,10}, pH^{11,12,13,14,15,16}, ionic strength¹⁷, electric^{18,19} and magnetic²⁰ field, or a combination of these.^{21,22} For instance, the degree of stretching in polyelectrolyte brushes is strongly affected by changes in pH²³, ionic strength and the nature of the electrolyte in solution.²⁴ Stimulus-responsive polymers have been intensively researched^{25,26} and a plethora of applications for such polymers has been identified and developed^{27,28,29,30,31} ranging from switching surface wetting and adhesion, to protective coatings that adapt to the environment, artificial muscles, sensors and drug delivery.^{32,33,34} In the case of microfluidics, the responsiveness of polymers is especially apparent when they are densely grafted to the substrate, as shown schematically in Figure 1. Highly controlled

preparation of polymer brushes in-situ is possible via controlled radical polymerization using, for example, by employing surface-attached initiators.³⁵ For their use as actuators in microfluidic systems, the reversible expansion and shrinking of such brushes offer great opportunities, which can be utilized as both passive and active fluid control elements to establish gate and valve functions and pumping elements. Active systems are defined as systems where the polymer reacts to a certain stimulus, e.g. irradiation with light or a change in pH, inducing, for example, a change in flow rate. Passive systems are defined as systems in which the polymer does not actively contribute to the flow mechanism; the desired effect is static, a polymer coating that lowers the electro-osmotic flow constitutes a typical example.

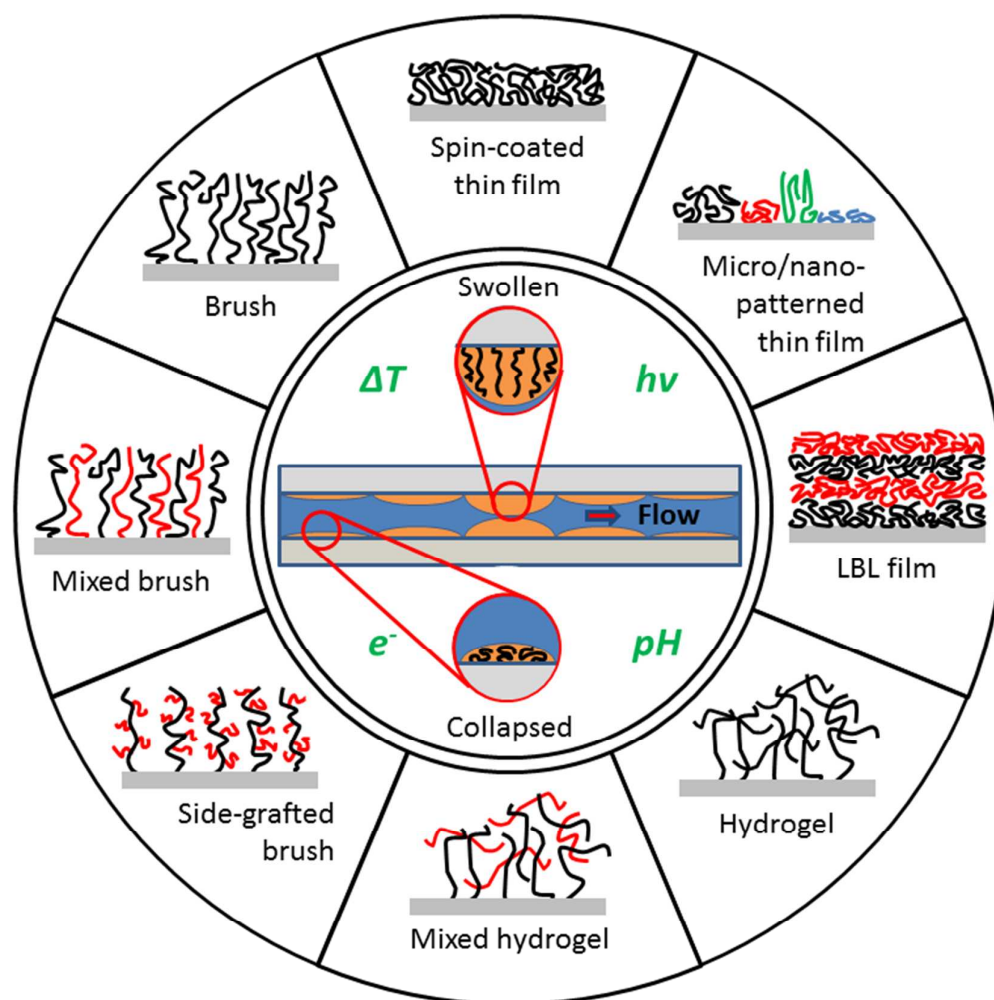


Figure 1: Various stimulus-responsive polymer systems can be placed in a channel to control microfluidic flow, by, for example, swelling and collapsing a brush or hydrogel coatings. Stimuli that are commonly used in fluidic devices encompass irradiation with light and changes in temperature, pH and of the redox state.

Fluid control and robust performance have been demonstrated in microfluidic devices by several groups^{36,37,38}. However, the use of external stimuli which addresses the grafts at the ensemble level as opposed to local trigger, are significantly limited in precise applications at small length scales, in particular if complex or synchronized actuation is needed. Temperature and pH variations are among such stimuli, but even the use of light has spatial limitations due to diffraction limits. Additionally, switching times are in the order of tens of seconds in the case of thermoresponsive polymers³⁹, up to even minutes in the case of photoresponsive polymers⁴⁰. These characteristics obviously pose limitations in applications for micro/nanofluidic actuation. Finally, pH or ionic strength as stimuli can only be applied directly via delivery of sample liquids; i.e. no controlled local response is possible by using these systems. In short, an independent external switching mechanism at small scale represents a severe bottleneck for microfluidic actuators and needs to be overcome if further progress is to be made in micro and nanofluidics.

It is generally considered, that an ideal microfluidic component has several important characteristics. Firstly, the response time of the component should be short, namely in the microsecond range. Secondly, it should be at least as chemically resistant as the channels of the device. This is a property that is unlikely to be realized in the case of organic materials, hence such systems will not be considered in this review. Thirdly it should be straightforward to fabricate the device including the treatment with stimulus responsive polymers, for

example, under ambient conditions. Finally, the device should be able to operate using a broad range of different liquids.

These challenges could be overcome if an electrical potential could be employed as the stimulus, using redox active polymers such as poly(ferrocenylsilane)s, for instance. One option is offered by organometallic polymers, including poly(ferrocenylsilane)s (PFS). PFSs are redox-active polymers with alternating silane and ferrocene moieties in the main chain^{41,42,43}, that can be covalently attached to a surface³¹ or used as cationic or anionic coating.⁴⁴ Electrochemical stimuli induce reversible changes of individual PFS chains by adjusting the redox state of PFS in an electrolyte solution. At surface grafted PFS brushes the reversible height change due swelling upon electrochemical oxidation was observed.^{45,46} In addition adhesive properties were switched electrochemically.⁴⁷ Hence PFS polymer films represent promising candidates as active and passive elements that can be switched electrochemically within designed microfluidic devices. In general, the potential of redox polymers as active elements in microfluidic devices has yet to be explored, despite promising results that have been published on the computational design of mixers and pumps for microfluidic systems.⁴⁸ A key advantage of electrochemically applied redox stimuli over classic stimuli (changes in temperature, pH, ionic strength) would be their utility for fast and reversible switching between the oxidized and reduced states. Critically, redox stimuli can be applied locally and, due to the wide availability of nanofabricated electrodes^{49,50} with reported widths down to 2 nm⁵¹, integration into microfluidic devices becomes feasible, thereby allowing the development of sophisticated nanoscale actuators.

In this paper we give an overview of selected examples from the literature reporting on the use of stimulus-responsive polymers as active or passive components for fluid control in glass microfluidic devices. However, in microfluidics, poly(dimethylsiloxane) (PDMS) is widely

used as the main structural material due to easy processing and good material characteristics^{52,53,54}, but polymer coatings have also been applied to other microchannel materials, e.g. poly(methyl methacrylate)^{55,56}, silicon⁵⁷, poly(ethylene terephthalate)⁵⁸, etc. Polymer-coated PDMS is used in electro-osmotic flow experiments^{59,60,61,62,63}, and in pH-responsive⁶⁴, thermoresponsive^{65,66} and photoresponsive⁶⁷ devices. For a more in-depth review about polymers coated to PDMS the reader is directed to reviews by Zhou, et al.^{68,69} and Wong and Ho⁷⁰.

However versatile, PDMS has several drawbacks compared to glass, mainly in terms of solvent compatibility⁷¹ and wettability. Also leeching of uncured PDMS into the fluid is reported and influences biological compatibility^{72,73}. Furthermore, the elasticity of PDMS restricts the amount of pressure that can be used in these devices.

Because of these drawbacks, this review focuses mainly on polymers “grafted to” or “grafted from” microchannels in glass or fused silica.

pH-responsive polymers in fluidics

One of the first applications of hydrogels as valves in microfluidic channels was described by Beebe, et al.⁷⁴, who used the swelling and shrinking properties of a pH-responsive hydrogel to create a valve.⁷⁵ 2-Hydroxyethyl methacrylate in acrylic acid (4:1 ratio) and ethylene glycol dimethacrylate (1 wt%) were photopolymerized using an inkjet-printed mask and Irgacure 651 as photoinitiator (3 wt%). The hydrogel valve was placed on a membrane such that the swelling gel sealed the fluidic channel. A second design implemented the swelling gel as a throttle valve by using an array of gel columns⁷⁴. Already Beebe’s design showed the versatility required for the fabrication of microfluidic devices: in-situ fabrication of valves using photolithography gives the option for rapid prototyping, as the authors have shown in their work. Their fastest switching time was found to be 16 s for a full cycle and required a pH change in the channel from below 4 to above 9. In later work⁷⁵, this requirement was

eliminated by separating the fluidic pathway from the pH regulation. The gel used in this study was separated from the fluidics by a poly(dimethylsilane) (PDMS) membrane. A response time of 24 s (under the assumption that the opening time was as fast as the closing time) was realized, which effectively removed the need for the pneumatics normally used in membrane valves by using a pH-responsive gel and a solution of a certain pH. However, the use of a PDMS membrane severely limits the choice of organic solvents. Furthermore, the response time should be further improved in order to qualify for high-throughput microfluidics.

A different pH-responsive system was devised by Salim, et al.⁷⁶ In order to investigate the influence of a pH responsive polymer on the electro-osmotic flow (EOF), they plasma-polymerized three different polymers, namely poly(tetraglyme), poly(acrylic acid) (PAA) and poly(allylamine), and coated the molecules to a microchannel wall. Polymerization was done *in-situ*.⁷⁷ When performing EOF measurements, they discovered that the magnitude and the sign of the electro-osmotic mobility depends on the pH, enabling optimization of capillary electrophoresis when working with solutions of various pH. In a follow-up experiment, the effect of protein adsorption on the electro-osmotic mobility was also investigated and the mobility was found to be dependent on the concentration of the proteins used, namely fibrinogen and lysozyme, except for the poly(tetraglyme) coated channels, indicating this is a suitable coating for protein-separation experiments. Covalently binding coatings to the channel greatly improves the number of times that the device can be used without the need for re-coating, compared to physically adsorbed layers. Using these coatings and tuning the pH the electro-osmotic mobility can be controlled. However, the chip used was a PTFE/glass device, clamped together by the set-up, which can become problematic in applications where high fluidic pressure is required.

Photoresponsive components

pH-responsive devices lack high speed and require addressability through a channel. By using light as actuator, the in-channel requirement can be removed. Walsh, et al.⁷⁸ synthesized poly(spiropyran-co-divinylbenzene) in a poly(tetrafluoroethylene)-coated fused silica capillary with a diameter of 100 μm . When irradiated with ultraviolet (UV) light, the C-O bond in the spiropyran moiety breaks, converting the colorless spiropyran to merocyanine. Irradiation with visible light converts the merocyanine back into the spiropyran.⁷⁹

The electro-osmotic flow was controlled using this property. Irradiating with UV light for 120 seconds reduced the electro-osmotic flow by 50%. After irradiating with visible light, the flow rate increased again. The increase in flow rate is from 75 nl/min to 150 nl/min. This is too low a flow rate for many uses, since flow rates in microfluidics are usually in the $\mu\text{l}/\text{min}$ range. Combined with the low switching time of at least 2 minutes, using this system as a high-throughput, fast microfluidic component will be challenging.

Thermoresponsive constituents

Arora, et al.⁸⁰ were inspired by an earthworm to fabricate a thermoresponsive device to displace a glass bead. Their active element was a poly(N-isopropylacrylamide) (PNIPAM) hydrogel. This gel has a lower critical solution temperature (LCST) of 32 $^{\circ}\text{C}$ in aqueous environment, meaning it shrinks above this temperature. The collapse of the polymer results in a change in various properties, like friction, adhesion and solvability.^{81,82} This change can be exploited in microfluidic systems both as a valve and as a pump.

Using Peltier elements, the gel was locally heated and cooled, and the accompanying expanding/shrinking cycle resulted in the directed motion of the gel through a microchannel, the process is schematically displayed in Figure 2. However, one cycle takes 40 minutes to complete. Wall slippage of the gel was also investigated and a rough channel wall was found to be beneficial for gel motion. In later work, the gel was used to transport a glass bead in a

channel with a radius of 500 μm , which attained speeds up to 15 $\mu\text{m/s}$.⁸³ The drawback with most temperature responsive systems, namely the speed of actuation, is relevant here as well, as a full heating cooling cycle that was reported took 14 minutes. On the other hand, the low voltage used for the heating/cooling Peltier elements (1.5V) means that the device can be powered by a battery.

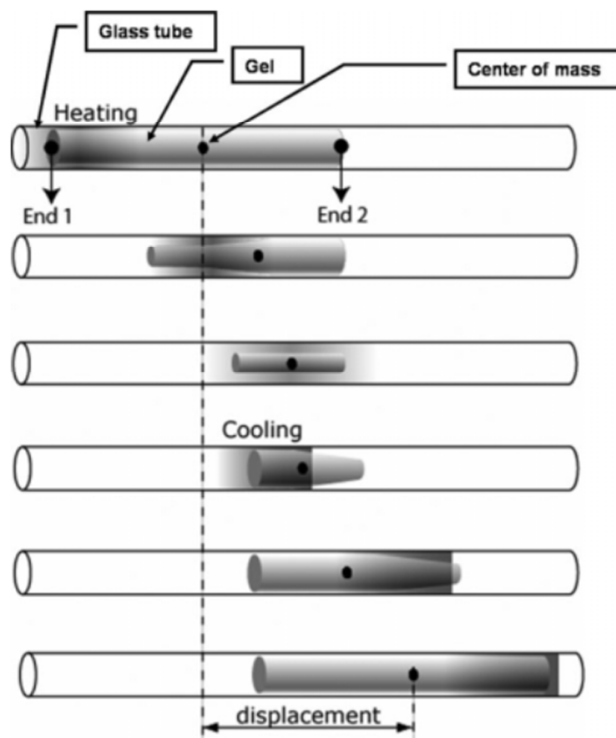


Figure 2: Schematic of motion of PNIPAM hydrogel by controlled heating and cooling, by Arora, et al.⁸⁰ By heating the channel at end 1, the polymer shrinks, contracting away from the heat source towards end 2. By subsequent cooling at end 2, the polymer swells again, away from both ‘ends’. When the swelling is complete, the polymer is displaced. Copyright (c) 2009 Wiley Periodicals, Inc.

Richter, et al.⁸⁴ used a PNIPAM hydrogel as a ‘normally closed’ valve. To place the gel inside the channel, they photopolymerized PNIPAM in-situ. Locally heating the hydrogel using platinum resistors, open/close cycles of around 14 seconds could be realized. However, the maximum pressure that the valve could sustain was not investigated. In later work, they

used PDMS instead of Pyrex glass as the cover layer.⁸⁵ This was done to create an elastic membrane that could be either displaced by the PNIPAM gel in a separate area, or as a counter-pressure mechanism. The first design used the gel as an actuator to close a channel by pressure generated by the swelling of the gel, pressing on the PDMS membrane, effectively blocking the flow, as shown in Figure 3. The second design used the swelling and shrinking effects to pump the liquid through the hydrogel itself (since swelling adsorbs liquid) and by shrinking the liquid is expelled. The PDMS cover slip ensured directed motion due to elastic deformation. Using peristaltic pumping, a flow rate of $0.54 \mu\text{L}/\text{min}$ was obtained, furthermore, in pulsatile operation this could be increased to $2.8 \mu\text{L}/\text{min}$. However, pumping ‘through’ a hydrogel greatly depends on the type of liquid used, and these flow rates are only valid for deionized water.

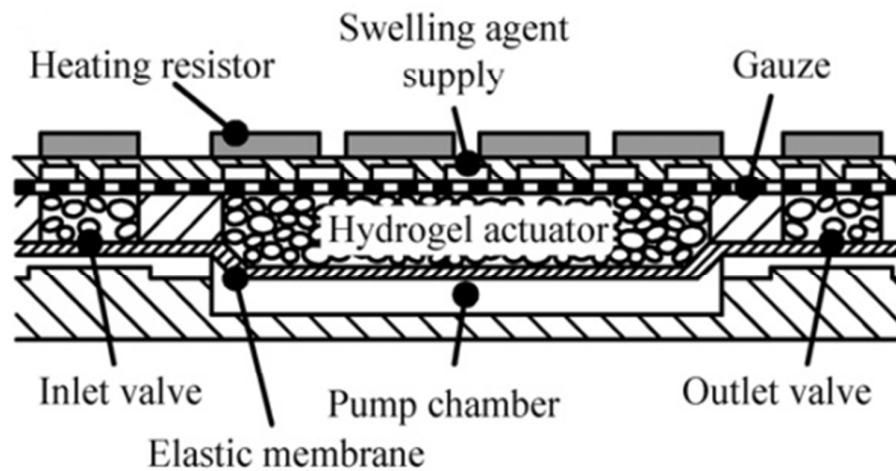


Figure 3: Displacement pumps as fabricated by Richter, et al.⁸⁵ By controlled swelling and shrinking of the hydrogel actuator, the elastic membrane presses down on the pump chamber and flow can be stopped. By tuning the heating cycles with the heating resistors this setup is also used as a pump. Reproduced from Ref.⁸⁵ with permission from The Royal Society of Chemistry.

Using Peltier elements to actuate PNIPAM was also done by Yu, et al.⁸⁶ A PNIPAM gel was also used as valve. Since glass is a poor thermal conductor, actuation of the gel was done by

integrated Peltier elements. Cavities were etched into the glass to incorporate the thermoelectrical elements, reducing the distance between the actuator and the gel. High-frequency experiments showed that one open/close cycle of the valve took 12 s. The closed valve maintained up to 1.38 MPa of fluid pressure without showing signs of damage or leakage.

In later work, by co-polymerizing the PNIPAM gel with N-ethylacrylamide (NEAM)⁸⁷ a tunable LCST between 32 and 72 °C was realized. A higher LCST means that only a heater is required, since the cooling cycle will happen once the gel releases its thermal energy to the surrounding environment. The optimum LCST was found to be 45 °C, at equal NIPAM and NEAM molar ratios. A cycle time of 3-4 seconds was observed. The closed valve maintained operations up to 18.0 MPa. By using the integrated Peltier elements, the response time is lower than the photoresponsive or pH-responsive devices discussed above, but still far from microseconds.

Instead of using the swelling/shrinking of the PNIPAM around the LCST, Londe, et al.⁸⁸⁻⁸⁹ used the hydrophobic/hydrophilic switch of the polymer to fabricate a valve. Above the LCST, PNIPAM is hydrophobic, whereas below it is hydrophilic. To coat the microchannel, 40 bilayers of poly(allylamine hydrochloride) (PAH) and silica nanoparticles were first deposited on the surface and annealed at 400 °C for 2 hours, thereby increasing the roughness of the surface. On top of this surface, two bilayers of PAH and PAA were deposited, with PAA as the topmost layer and used as an anchor for the PNIPAM initiator. After NIPAM polymerization, the switching effect was further increased by chemical vapor deposition of a (1H,1H,2H,2H-perfluorooctyl) silane on the polymer. The whole process results in a dramatic change in the water contact angle both above and below the LCST: from 14° at room temperature to 122° at 70 °C. Using a dye, the valve was studied qualitatively and found to be working as expected, as can be seen in Figure 4. Unfortunately, neither the cycle time nor the

time needed to heat and cool the sample was reported, therefore little can be said about the timescale on which this process operates. Furthermore the maximum pressure the valve can sustain was not characterized. However, the proof of principle works and shows promise of good chemical resistance due to the fluorinated silane as top layer.

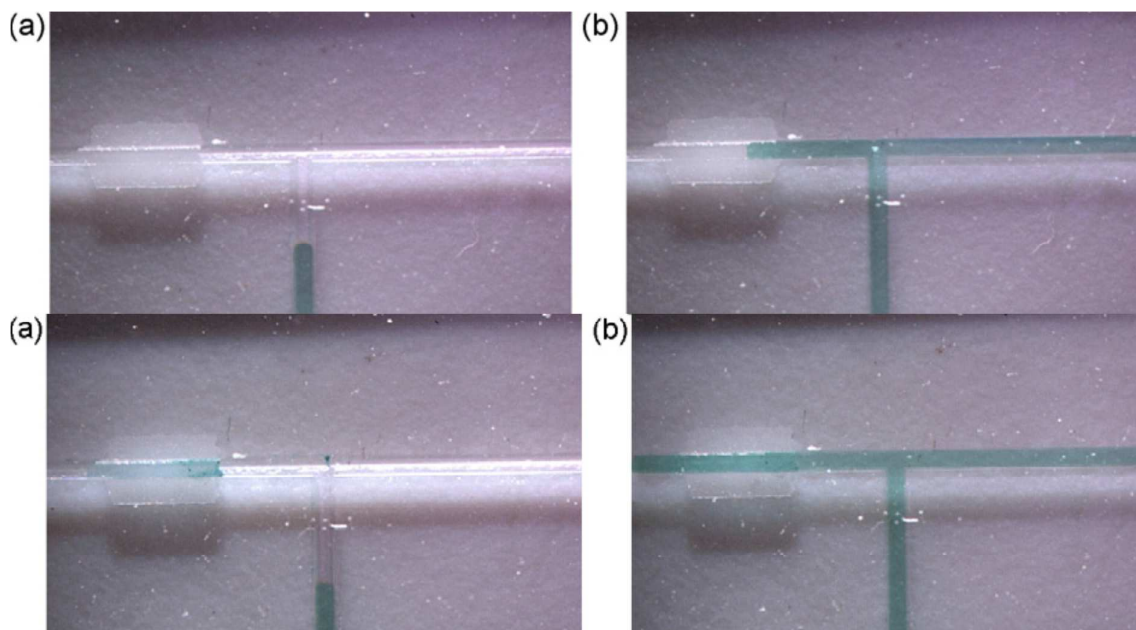


Figure 4: Closed (top) and open valve (bottom), by Londe, et al.⁸⁸ In the four different images the inlet is at the bottom of the image, in the left arm the valve is placed. The right column shows the fluid after reaching the valve. In the bottom-right image the valve is open and the liquid can pass, the top-right image shows the valve in the closed state and the liquid is blocked. Reprinted from⁸⁸, Copyright 2008, with permission from Elsevier.

A similar experiment to halt capillary flow was performed by Saitoh, et al.⁹⁰, their approach combined the use of Peltier elements with PNIPAM. Instead of optical inspection, spermine concentration measurements in saliva were used, using a two-junction method. At the first junction, the spermine was mixed with nickel(II) chloride for complex formation, whereas at the second junction a fluorescent marker, o-phthalaldehyde, was released, resulting in

fluorescence. However, in this work, the authors did not specify the time scale on which these processes take place. Nevertheless, the experiments show that the idea of using PNIPAM as valve has merit even when the PNIPAM is uncoated.

In order to control the flow rate in capillaries via temperature, Idota, et al.⁹¹ coated microcapillaries with radii between 50 μm and 500 μm with a PNIPAM brush. Capillary height measurements were performed, revealing a change in capillary height as function of temperature, from this the contact angle was calculated. The contact angle changed from 58° at 10°C to 81° at 40°C in the case of a 300 μm capillary. Changing the inner diameter of the channels led to no significant change in contact angle, if the temperature remained constant. To confirm control of flow by temperature, two capillaries were placed in parallel, one coated with PNIPAM, one uncoated. Water was pumped in at 10 MPa from a single inlet. Below the LCST, no flow was measured through the coated capillary. However, above the LCST the flow rate was nearly equivalent to the rate observed in the uncoated channel. The cycle time of this device was 30 seconds. The high pressure this valve can sustain indicates that it is an excellent candidate for thermoresponsive release in microchannels, perhaps combined with exothermic catalysis.

Most thermoresponsive systems use PNIPAM as active element. In an attempt to improve the response time of a thermoresponsive valve, Stoeber, et al.⁹² used the triblock copolymer Pluronic F127 (poly(ethylene oxide)₁₀₆-poly(propylene oxide)₇₀-poly(ethylene oxide)₁₀₆) with on-chip integrated heaters to exploit the gel formation characteristics of the polymer as a valve. The polymer was dissolved in water (15wt%) and transported through the channel. When locally heated with the integrated electrodes, fabricated in the channels and coated with a protective layer, the polymer formed a gel and blocked the channel. Results showed an open/close cycle of 33ms. (The cycle time could actually be less, but the time resolution during these experiments was limited by the optical setup used, i.e. the video capture rate.)

The fast response times were probably a result of the integrated electrode design. However, using this technique requires the polymer to be dissolved in the liquid, which limits the solvents that can be used. It also limits the use of other molecules dissolved in the liquid. Nevertheless, this is a good candidate if a pH-responsive device is not compatible with the system one wishes to employ.

In conclusion, from the properties of thermoresponsive polymers, swelling and shrinking are mostly used in microfluidic devices. Thermoresponsive polymers were reported both as valves and pumps. Characterization of said valves was often found lacking in terms of the timescale of operation and pressure limitations, other than the proof of principle of the device little characterization was performed. However, for many systems the response time of the device turned out to be the bottleneck. Integrated electrodes are promising candidates to speed up these devices.

Passive elements

Passive systems are defined as systems in which the polymer does not *actively* contribute to the flow mechanism; the desired effect is static, a polymer coating that lowers the electro-osmotic flow constitutes a typical example. Various types of devices have been reported, for example, pumps, valves, and coatings that modify the electro-osmotic flow for both flow-reduction as well as DNA separation.

For instance, in order to fabricate a field-free electro-osmotic pump, Joo, et al.⁹³ used poly(diallyldimethylammonium chloride) and poly(styrene sulfonate) as the cationic and anodic coatings, respectively. By coating two arms of a Y-shaped microfluidic channel with coatings of opposite polarity, a field-free flow could be created in the third arm, when the channel was filled with 10mM of phosphate buffer at pH 7.0, as shown schematically in Figure 5. Using this technique, field-free gated injection and on-chip field-free sorting was accomplished with sorting rates of up to 120 samples per minute. However, this is far below

other systems that go up to 100 samples per second, but it shows a proof of principle of the novel idea of using a momentum-driven electro-osmotic pump.

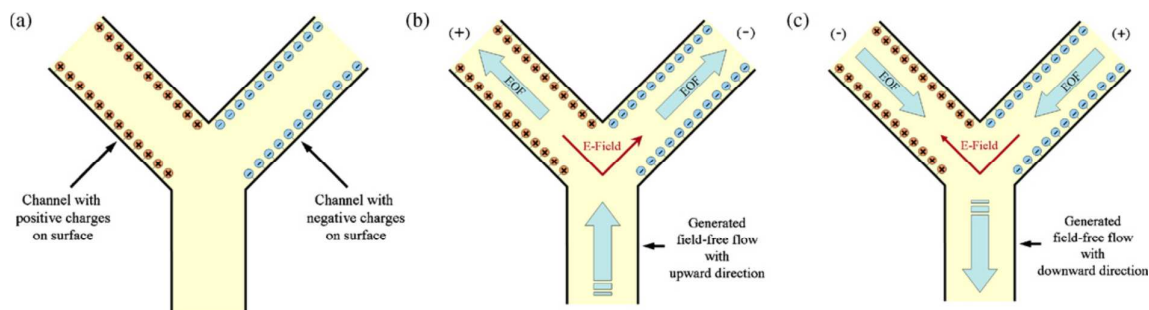


Figure 5: Field-free flow generation, by Joo, et al.⁹³ Two arms are coated with polymers with different charge. Depending on the polarity of the electric field from one arm to the other, flow is directed inward or outward (blue arrows) in the third channel. Reprinted from⁹³, Copyright 2007, with permission from Elsevier.

For fabricating a valve, Kirby, et al.⁹⁴ photopolymerized a polymer plug, that could be pressed, using pneumatics, against a valve seat in a channel. The plug blocked the channel, stopping the flow. The monomer was 2,2,3,4,4,4-hexafluorobutyl acrylate crosslinked with 1,3-butanediol diacrylate. Using this polymer monolith, they could close a 65 μm diameter cylindrical microchannel with pressures up to 7.0 MPa, limited only by the external materials or the glass substrate. The leakage was measured and found to be negligible. Further study showed the response time to be 33 ms.⁹⁵ Interestingly, this response time is similar to that reported by Stoeber, et al.⁹² giving the impression that this response time is perhaps also limited by the frame rate of the optical setup (30 frames per second). Using the polymer plug, a one-directional valve was fabricated by Hasselbrink, et al.⁹⁵, using the flow pressure instead of external pneumatics to close the valve in one direction, but leaving it open in the other. This type of component should show high chemical resistance due to presence of fluorinated alkanes and would prevent flow going towards the inlet. One drawback could be the sliding of the polymer plug: the lifetime of the plug has yet to be investigated.

Several groups have done work on reducing the flow internally. For example, in a rectangular microchannel, the four walls were individually modified with poly(neopentylmethacrylamide-co-N-4-(trimethylsilyl)phenylmethacrylamide), by Sultana, et al.⁹⁶ This hydrophobic coating resulted in various shapes of the air/water interface in the channel for the four possibilities. A tapered shape was seen when a single wall was coated, and a saddle-like shape when opposite walls were coated. The flow rate was found to be dependent on the surface modification, but the flow rates dropped as more walls were coated. Since these authors used a three layer system, they could achieve all possible combinations of hydrophilic and hydrophobic walls. However, the substrates were bonded using adhesives, which limited the solvent compatibility of the system. A different approach was done by Lanotte, et al.⁹⁷ They showed that grafting a poly(hydroxyethylmethacrylate) brush from a fused silica surface reduced the flow rate, more than would have been expected of the reduced channel diameter. The maximum velocity in the channel of the pressure-driven flow decreased by 35%. The authors explain their results with the aid of molecular simulations and conclude that the decrease in velocity is due to the brushes stretching and recoiling, resulting in a net backflow near the channel wall. However, statically reducing the flow rate is of little use in microfluidic components and shall be used, as the authors point out, as a prelude to studying blood flow in the microvascular system.

To control the electro-osmotic mobility as function of surfactant concentration in their channels, Mora, et al.⁹⁸ coated fused silica capillaries with PDMS. Coated capillaries were used instead of PDMS microchannels to readily combine the channel with a regular capillary electrophoresis setup. Using sodium dodecyl sulfate, a non-linear increase in mobility was observed when increasing the concentration; this was ascribed to the change in zeta potential due to the electrolytes adsorbing to the PDMS surface. The result is shown in Figure 6.

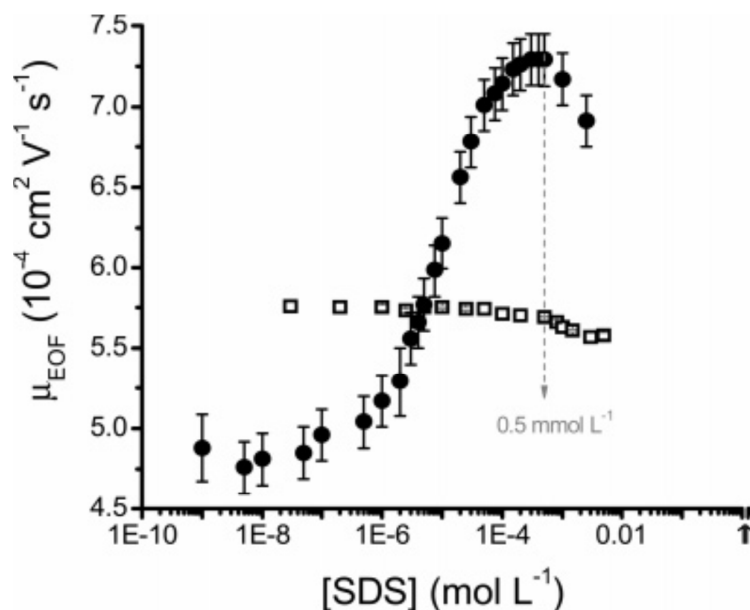


Figure 6: Effect of adding sodium dodecyl sulfate (SDS) to the electrolyte solution in bare (open rectangles) or PDMS-coated (closed circles) capillaries. From Mora, et al.⁹⁸ Coated capillaries show a clear effect of the surfactant concentration on the electroosmotic mobility. Reprinted with permission from⁹⁸. Copyright 2007 American Chemical Society.

To investigate the effect of gold nanoparticles on electrophoresis, Pumera, et al.⁹⁹ coated a glass capillary with poly(diallyldimethylammonium chloride) and flushed the channel with a citrate-stabilized gold nanoparticle solution for 30 minutes. When measuring electropherograms of the coated and uncoated channels, they found that, for aminophenols, the resolution increased dramatically. However, on the downside, the analysis time increased from 1.5 minutes to 2.5 minutes, due to the longer retention time of the analyte. As a control experiment, they also performed electrophoresis on channels without the gold nanoparticles, but found that no analytes reached the detector. However, only aminophenols were investigated, and it is not certain that this modification will increase the resolution of measurements on other molecules.

Kohlheyer, et al.¹⁰⁰ fabricated a free-flow electrophoresis device using poly(acrylamide) as an ion permeable membrane as a prelude for a device with a pH gradient. An *in-situ* photopolymerized membrane was used as an electrical connection between the electrodes and the sample channel. At the same time the membrane resisted hydrodynamic flow, ensuring the channel is leak-free. In later work, these authors realized a pH gradient across the channel using preprepared ampholytes.¹⁰¹ The linearity of the pH gradient was confirmed using IEF markers ranging from pH 4 to 10, and the theoretic separation limit $\Delta(pI)$ was 0.23. The in-channel separation as well as the experiment without applied potential are shown in Figure 7. The gradient can be used for microreactors in combination with pH-sensitive reactions.

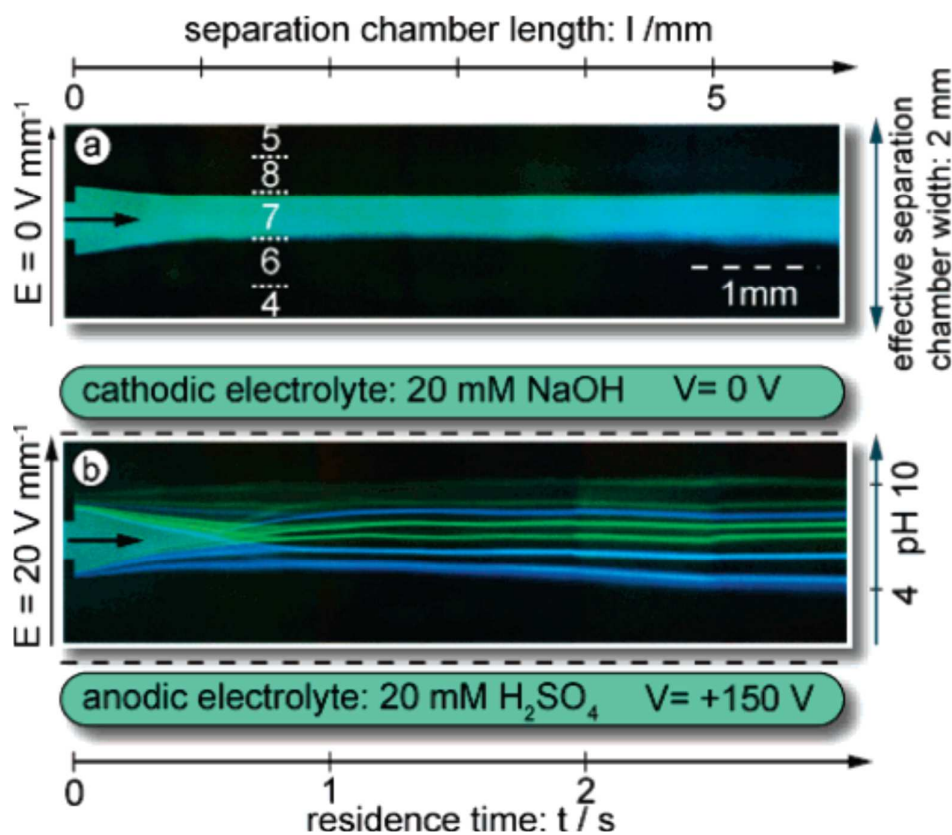


Figure 7: Free-flow isoelectric focusing; Kohlheyer, et al.¹⁰¹ Top: device with no potential applied. Bottom: device with 150V applied. The in-channel separation of the markers from pH 4 to pH 10 can be clearly seen. Reprinted with permission from¹⁰¹.

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Due to their diversity, polymer coatings are also used in biological applications. Wen, et al.¹⁰², photopolymerized 3-(trimethoxysilyl)propyl methacrylate *in situ* as a solid state filter for DNA purification. Using a protein catching step, they achieved an extraction efficiency of 69% when using whole blood. When using this device, a 2-propanol washing step when combining DNA capture with Polymerase Chain Reaction (PCR) to remove unwanted proteins could be omitted. However, the question of the long-term stability of the filtering device was not addressed.

A different polymer for PCR products was used by Toriello, et al.¹⁰³, who copolymerized linear poly(acrylamide) with a 5' acrydite modified oligonucleotide as the capture element for nanoliter amounts of PCR products. A capture efficiency of 100% was reported. The captured products were released in a capillary electrophoresis (CE) channel for subsequent size-based separation. The proposed injection scheme, displayed in Figure 8, improved the signal strength a hundredfold.

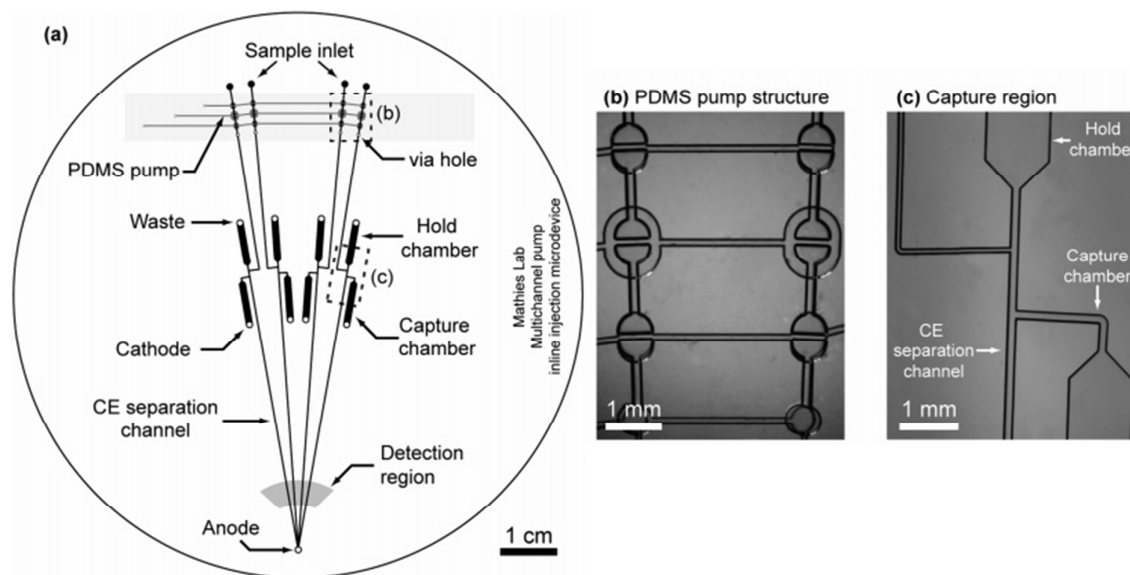


Figure 8: The capture, purification and direct CE separation device from Toriello, et al.¹⁰³ The four-layer glass-PDMS-glass-glass device contains a PDMS pump (b), a capture region (c), separation channel and detection region. Each cycle pumps 10nL of

liquid to the hold chamber. Reprinted with permission from ¹⁰³. Copyright 2007 American Chemical Society.

Several different coatings for DNA separation by electrophoresis in fused-silica capillaries were investigated by Doherty, et al. ¹⁰⁴. The polymer coatings tested were poly(acrylamide), poly(*N,N*-dimethylacrylamide), poly(*N,N*-diethylacrylamide) and *N,N*-diethylacrylamide/*N,N*-dimethylacrylamide random copolymers. The results showed that polymers of above 15000 monomer units reduced the electro-osmotic mobility significantly. Also, the coatings should be as hydrophilic as possible so that the electrical double layer is pierced.

Costantini, et al. ¹⁰⁵ grew a 2-hydroxyethylmethacrylate-tetraethylene glycol dimethacrylate gel on the channel wall to immobilize silver and palladium nanoparticles in a microreactor. This was done to enhance the catalytic activities of the metal nanoparticles due to the increased specific surface area of a microchannel compared to conventional reactors. After reaction with succinic anhydride in dry pyridine, the channels were flushed with a 0.05M silver or palladium nitrite solution in a 3:1 DMSO/water mixture, resulting in 20 ± 5 and 30 ± 3 nm particles for Ag and Pd, respectively. In other work, poly(methacrylic acid) brushes were grown to study the immobilization of lipase using EDC-NHS chemistry. ¹⁰⁶ When studying the activity of the enzyme using 4-nitrophenol acetate they found that the reactivity of the enzyme did not change after immobilization on the surface. In recent work, poly(2-hydroxyethyl methacrylate) brushes were used to link glucose-oxidase and horseradish peroxidase to the channel surface. Using an enzymatic cascade reaction, they could detect glucose in blood within 20 s, the limit of detection and quantification being 60 μ M and 200 μ M, respectively. ¹⁰⁷ The authors reported high stability and reusability of their enzymes. The papers discussed show that stimulus-responsive polymers in microfluidics are a promising technology for PCR separation, enzyme activity studies and biological applications in general. A summary of all discussed research, both active and passive, is given in Table 1.

Table 1: Summary of polymer systems

Monomer	Polymer State	Initiator	Type of polymerization	Deposition	Response	Ref
2-hydroxyethyl methacrylate/acrylic acid	gel	Irgacure	photopolymerization	photomask	pH	74
tetraglyme, acrylic acid, allylamine	film	n/a	plasmapolymerization	vapor phase	pH	76
spiropyran- <i>co</i> -divinylbenzene	copolymer film	2,2-azobisisobutyronitrile	60°C, 5 days	adsorption from liquid	light	78
<i>N</i> -isopropylacrylamide	gel	potassium persulfate and TEMED ¹	radical polymerization	liquid	thermal	80
<i>N</i> -isopropylacrylamide	gel	2-hydroxy-4'-(2-hydroxyethoxy)-2-methylpropiophenone	photopolymerization	photomask	thermal	85
<i>N</i> -isopropylacrylamide	gel	4,4'-azobis(4-cyanovaleric acid)	photopolymerization	3-(trimethoxysilyl)propyl methacrylate	thermal	86
<i>N</i> -isopropylacrylamide/ <i>N</i> -ethylacrylamide	gel copolymer	4,4'-azobis(4-cyanovaleric acid)	photopolymerization	3-(trimethoxysilyl)propyl methacrylate	thermal	87
<i>N</i> -isopropylacrylamide	film	2,2'-azobis(2-methylpropionamide) dichloride	65°C	adsorption from liquid	thermal	88
<i>N</i> -isopropylacrylamide	film	ammonium persulfate and TEMED	radical polymerization	allyltrimethoxysilane	thermal	90
<i>N</i> -isopropylacrylamide	brush	4,4'-azobis(4-cyanovaleric acid)	surface-immobilized radical polymerization and electron-beam irradiated polymerization	3-aminopropyltriethoxysilane	thermal	91
Pluronic F127	triblock copolymer	n/a	n/a	in-situ gelation	thermal	92
diallyldimethylammonium chloride styrene sulfonate	film	n/a	n/a	layer-by-layer	passive	93
2,2,3,4,4,4-hexafluorobutyl acrylate	solid	2,2-azobisisobutyronitrile	photopolymerization	photomask	passive	94
neopentylmethacrylamide- <i>co</i> - <i>N</i> -4-(trimethylsilyl)phenylmethacrylamide	copolymer	2,2-azobisisobutyronitrile	free radical polymerization	Langmuir-Blodgett	passive	96
2-hydroxyethylmethacrylate	brush	2-bromo-2-methylpropionyl bromide	atom transfer radical polymerization	3-aminopropyltriethoxysilane	passive	97
dimethylsiloxane	film	n/a	thermal crosslinking	adsorption from liquid	passive	98
diallyldimethylammonium chloride	film	n/a	n/a	adsorption from liquid	passive	99

acrylamide	membrane	2,2- dimethoxy-2-phenylacetophenone	photopolymerization	3-(trimethoxysilyl)propyl methacrylate	passive	100
3-(trimethoxysilyl)propyl methacrylate	solid	Irgacure	photopolymerization	photomask	passive	102
acrylamide- <i>co</i> -acrydite modified oligonucleotide	gel	ammonium persulfate and TEMED	-	-	passive	103
acrylamide, <i>N,N</i> -dimethylacrylamide, <i>N,N</i> -diethylacrylamide	(co)-polymer	ammonium persulfate and TEMED	50°C	adsorption from liquid	passive	104
<i>N,N</i> -diethylacrylamide/ <i>N,N</i> -dimethylacrylamide						
2-hydroxyethyl methacrylate-tetraethylene glycol dimethacrylate	gel	2,2'-bipyridyl	atom transfer radical polymerization	3-(trichlorosilylpropyl) 2-bromo-2-methylpropionate	passive	105
methacrylic acid	brush	n/a	photopolymerization	EDC-NHS	passive	106
2-hydroxyethyl methacrylate	brush	2,2'-bipyridyl	atom transfer radical polymerization	3-(trichlorosilylpropyl) 2-bromo-2-methylpropionate	passive	107

Conclusions

A variety of stimulus responsive polymers have been used as active or passive elements for fluid control in glass- and PDMS based microfluidic devices. Usually, response of these polymers are triggered by external stimuli at the medium (ensemble) level, including variations in temperature, solvent composition, pH or illumination with light. Although fluid control has been demonstrated in microfluidic devices in a number of publications, a severe drawback of external stimuli consists of locally controlling responsive elements of functional structures at small scales, ideally at the nanometer length scale. In addition, switching times of seconds to several minutes severely hampers usability for highly controlled and advanced fluid delivery. The latter is especially so for thermally controlled systems. Nevertheless, these systems show that they can withstand high fluidic pressures meaning that robust components can be fabricated from thermoresponsive polymers, mainly when time scales are not of importance. However, for biological applications the operating temperature window is small, and mostly passive system were reported in this regard, where covalent binding of the polymer coating to the channel wall has preference over physical adsorption to prevent solvent contamination. Applying stimulus through the solvent, as in the case of pH-responsive systems, should be avoided to be able to use a broad range of solvents and solutes. However, if the solvent is compatible, pH-responsive systems are a strong candidate for microfluidic components. We propose to tackling these challenges, namely fast switching, solvent compatibility and biological applicability, by utilizing redox-responsive polymers which offer fast, reversible switching and local addressability in combination with nanofabricated electrodes. For example, a poly(ferrocenylsilane) based hydrogel crosslinked with PNIPAM can be reduced and oxidized isothermally, collapsing and swelling the gel accordingly²². Microfluidic devices have not been constructed from poly(ferrocenylsilane)s. When applied to a microchannel wall a peristaltic pump could be created. Alternatively, a redox-active

polymer could be used to change the hydrophobicity in the channel¹⁰⁸, thereby creating a capillary valve.

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