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Leveraging stimuli responsive hydrogels for on/off control of mixing

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ABSTRACT

Microfluidic mixers are an important component in microfluidic devices. This paper presents a micromixer which can control mixing with responsive hydrogel actuators to modulate mixing between two adjacent fluids dependant on the chemistries of the fluid. This is achieved by the responsive hydrogels swelling or contracting under different stimuli, which alters the mixing between the two fluids. We present this device using standard pH responsive hydrogels for two different device designs and demonstrate altered mixing profiles based on the pH of the fluid streams. For the T-shaped device an increase in mixing efficiency from 18.3% to 34.5% is observed between the contracted and expanded hydrogel states. For the modified T-shaped device mixing efficiency in the contracted state is 25.0% while in the expanded state efficiency increases to 72.9%. This can be used as a mixer that has on/off functionality of an active mixer, based on the pH of the mixing streams, with the advantages a passive mixer offers. Other responsive hydrogel chemistries could be substituted into the device to achieve many different triggers for mixing.

1. Introduction

Microfluidic technology allows for precise control over small volumes of fluid which have found applications in a broad array of disciplines ranging from diagnostics [1] to microbial biology [2]. Each microfluidic device is composed of some combination of microfluidic components including mixers, pumps, actuators, and microscale device geometries. In this paper, we combine a microfluidic mixer with stimuli responsive hydrogel actorators to demonstrate a new mixer component with on/off functionalities.

Microfluidic mixers have been demonstrated in a variety of geometries and are necessary for microfluidic bio-analysis and chemical reactions [3–5]. Generally, micromixers are categorized as either active or passive mixers [6]. Active micromixers use an external energy source such as acoustics [7] or pressure disturbances [8] to forcibly mix two components. Active mixers offer quick mixing and more control over mixing, but require external power. Alternatively, passive micromixers use geometries such as grooves [9], obstacles [10], flow focusing [11], or serial lamination [12] to increase lateral fluid mixing and/or shorten the path of diffusion. The advantage of passive micromixers is they do not require an external power source for mixing and are simple to fabricate.

Stimuli responsive hydrogels are another microfluidic tool that has been utilized in for multiple applications. These hydrogels can sense a change in a given stimulus and expand or contract based on that stimulus. Researchers have used hydrogels for microfluidic flow control as stimulus sensitive valves [13]. Hydrogel volumetric change has also been used to pump fluid from a microfluidic resivoir [14]. Detailed reviews of the use of stimuli responsive hydrogels in microfluidic devices can be found in the literature [15,16]. The combination of active sensing and actuation into a single element is an advantage of these hydrogels. Stimuli responsive hydrogels have been demonstrated for many stimuli such as pH [17], temperature [18], light [19], glucose [20], electric field [21], magnetic field [22], antigens [23], and protein [24].

In this paper we demonstrate the use of pH sensitive hydrogels in a microfluidic device for control over mixing. The mixing profiles in these devices are modulated by the pH sensitive expansion and contraction of the hydrogels within the microchannels. The devices allow a change in mixing based on the pH of the solution passing over the hydrogel array without external changes such as increased or decreased flow rate. Although these devices demonstrate mixing control with pH sensitive hydrogels, other stimuli responsive hydrogels could be integrated to achieve similar results for a different stimulus. Having this function built into a microfluidic device could be a useful tool for engineering lab on a chip diagnostics.

2. Materials and methods

2.1. Fabrication of PDMS microchannels

The microchannels were formed using common photolithography and soft lighography techniques. SU-8 photoresist (2015, Microchem Corp.) was photo patterned on 3″ silicon wafers

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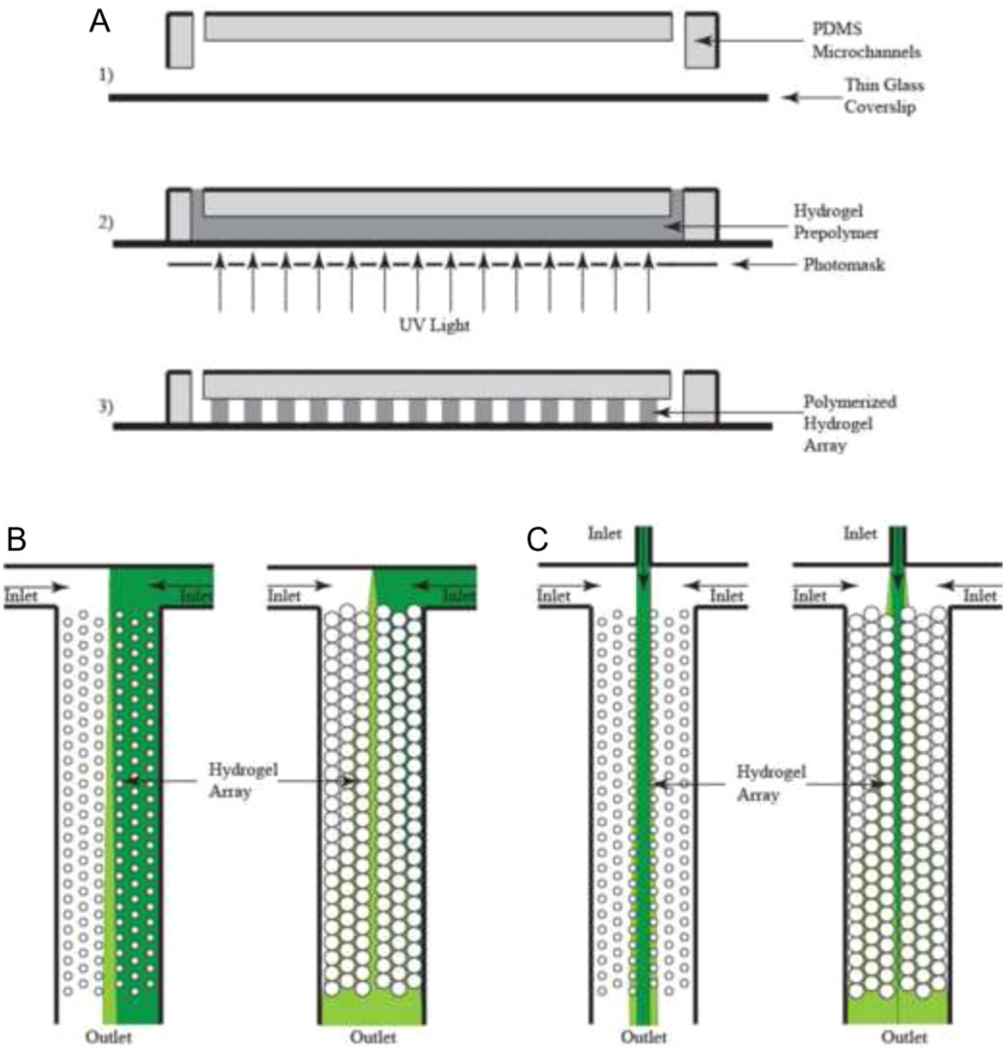

Fig. 1. Illustration of device fabrication and design. (A) A diagram describing device fabrication steps. First, the PDMS microchannels are bonded to a thin glass coverslip. The device is then filled with hydrogel prepolymer and photomask is aligned to desired position. Finally, the hydrogels are formed by exposure to UV light and excess prepolymer is washed from the device. (B) Illustration of the T shaped device design. (C) Illustration of T shaped device design with additional center fluid stream.

(Silicon Sense Inc.) in the desired microchannel design. Microchannels were molded from masters using PDMS (Sylgard 184 Kit, Dow Corning) mixed in a 10:1 weight ratio and cured on a hot plate at 75 °C for 2.5 h. Microchannels were cut out and peeled off masters and bonded to 0.17 mm thick glass coverslips using oxygen plasma treatment (Handheld Corona Treater BD-20, Electro-Technic Products Inc.). Final dimensions for the main channel of the device are 1 mm width, 30 μm height, and 5 cm in length. Left and right inlet and outlet channels were 200 μm while the center inlet and outlet channel was 100 μm for the modified T-shaped device.

2.2. Polymerization of hydrogels

Hydrogel prepolymer solution and polymerization of hydrogel post array was preformed in a method previously reported [14]. Fig. 1a illustrates these steps. Briefly, the prepolymer consisted of 2-hydroxyethyl methacrylate (Sigma) and acrylic acid (Fluka) in a 4:1 weight ratio in addition to ethylene glycol dimethacrylate (1 wt%, Sigma), and Irgacure 651 (3 wt%, Ciba Specialty Chemicals Inc.). Hydrogel prepolymer solution was selectively photopolymerized within the main microfluidic channel by the use of a photolithography mask. An array of 1956, 75 μm diameter hydrogel posts were formed by aligning the mask over the channel with the aid of a dissection microscope and injecting the hydrogel prepolymer solution into the device. The device was allowed to sit for 30 min for flow to stop before hydrogels were photopolymerized *in situ*. Hydrogel posts were formed by exposing the solution to 15 mW/cm^2, 365 nm UV light (Omnicure Series 1000 Spot Curing System, EXFO) for 210 s. Unexposed prepolymer solution was washed out of the microchannels with methanol leaving the newly formed hydrogel post array. Illustrations of the T-Channel and modified T-channel designs are shown in Fig. 1b and c, respectively.

2.3. Characterization of devices

The devices ability to change the diffusion and mixing profile was characterized by measuring the mixing profile of concentrated green food dye (Green Shade, ESCO Foods) 200 μm from the outlet channels. Initially, FITC was used, but this dye reacted with the pH buffers. Hydrogel posts were expanded and contracted by the use of standard pH buffers (pH 1.68 and pH 10.0 Oakton pH buffer solutions, Oakton Instruments). As this is an anionic hydrogel, the expansion occurs above the isoelectric point of pH 5.3. Therefore, buffers of pH 10 and pH 1.68 were used for these studies. For the T shaped device, green dye was added to the buffer solutions for one inlet while the other inlet was only buffer solution. In the modified T shaped device green dye was mixed with water and used for the center inlet of the device. The right and left inlets were buffer only.

Fluid was injected into the device using 3 mL syringes and syringe pumps (NE-300 Single Syringe Pump, New Era Pump Systems). Inlet flow rates were set at 5 μL/min for all inlet streams.

Images were taken of the mixing profile of the green dye using an Olympus LX51 microscope at 4X with a CCD camera (QImaging Retiga-SRV). A line scan of the mixing profile at 200 μm from the outlet of the main channel was used to analyze the intensities. The measured transmittance of light was used to calculate an absorbance by applying Beer's Law. The calculated absorbance values were then normalized from 0 to 1 to produce a final profile. The normalized results were used to calculate the mixing efficiency of each device in the contracted and expanded hydrogel state using a method described in the literature [10]. A total of three devices were tested independently for each condition and averaged to determine a final profile with standard deviations indicated by error bars. Mixing profiles at the inlet of each device as well as mixing profiles for devices without hydrogels are included for comparison.

2.4. *Characterization of hydrogel post expansion/contraction*

In order to illustrate the on/off functionality of the mixing devices the expansion and contraction characteristics of the hydrogel posts were measured. Buffer solution was injected into the device at 5 μL/min. The injected buffer solution pH was changed, at $t = 0$, from 1.68 to 10.0 for the expansion experiment. For the contraction experiment the solution pH was switched from 10.0 to 1.68. Images of the hydrogel post array were taken every 10 s using an Olympus LX51 microscope at 4× with a CCD camera (QImaging Retiga-SRV). The diameter of the hydrogel posts were then measured to create a plot that illustrates the hydrogel volume change over time.

The effect of solution pH on the size of the hydrogel posts was also characterized. The hydrogel posts were exposed to buffer solution pH between 1.68 and 10.0. At each buffer solution pH an image of the hydrogel posts was taken using an Olympus LX51 microscope at 4× with a CCD camera (QImaging Retiga-SRV). The hydrogel post diameter was then measured and plotted against the buffer solution pH.

3. Results

In order to demonstrate the on/off functionality of the hydrogel mixing devices the change in the hydrogel diameter during expansion/contraction was measured. In this experiment the expansion of hydrogel posts was measured after changing the injected buffer solution from low pH to high pH. The contraction of the hydrogel posts was measured in the opposite manner, changing the solution pH from high to low. Fig. 2 illustrates the results of this experiment. When the buffer pH is changed the posts are able to expand/contract quickly due to the changing stimulus. In less than 2 min the hydrogel posts can be cycled between the two states, which in turn changes the output mixing profile and mixing efficiency of the device.

The effect solution pH has on hydrogel diameter was also characterized and the results are contained in Fig. 3. When the pH is less than 4 and greater than 9 we can see that the minimum and maximum hydrogel diameters are achieved. Increasing or decreasing the pH further does not change the diameter. Between a pH of 4 and 9 the hydrogel diameter begins to transition from the contracted state to the expanded state with increasing buffer pH.

The results for the T shaped device are shown in Fig. 4. The images in Fig. 4a and b show that when the hydrogels are expanded the mixing between the two parallel streams increases. This is also illustrated in the graph of the concentration profile shown in Fig. 4c. The slope of the linear portion of the concentration profile decreases when the hydrogels are in the expanded state versus the contracted state. The mixing efficiency for a device with contracted hydrogels is 18.3%. In comparison the mixing efficiency is 34.5% when the hydrogel array is expanded. The mixing efficiency of the same device geometry without a hydrogel array is 11.0%. The mixing profile of the contracted state is very similar to the profile when no hydrogels are present. This indicates that the hydrogels in their contracted state have only a small added effect on the lateral mixing in the device, but the expanded hydrogels increase mixing of the two streams.

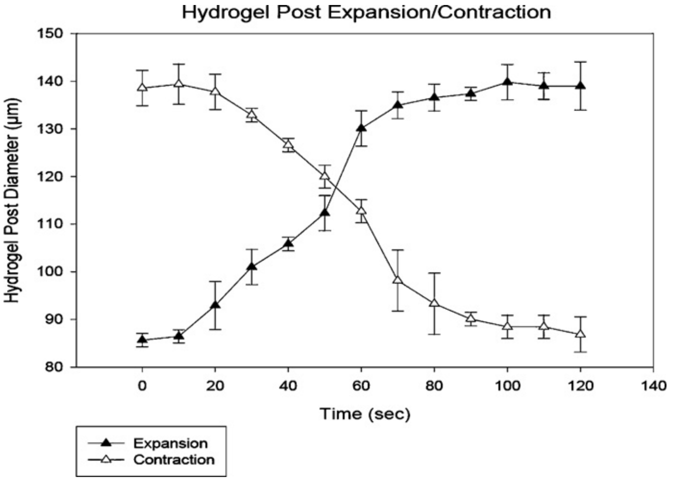

Fig. 2. Rate of hydrogel expansion/contraction. A plot of the measured hydrogel post diameters over time when solution pH is switched from 10.0 to 1.68 for contraction and 1.68 to 10.0 for expansion.

The modified T shaped device also demonstrates the use of the hydrogel array as an obstacle to increase mixing with a modified microfluidic channel geometry. In this device we have added a third fluid stream between the T device inlets. The results for this modified device are shown in Fig. 5. Like the T shaped device, mixing between parallel streams is increased when the hydrogel posts are expanded. This is also demonstrated in the calculation of the mixing efficiencies. In the contracted hydrogel state the device mixing efficiency is 25.0%. When the hydrogel array is expanded the mixing efficiency increases to 72.9%. Also, a device without a hydrogel array has a mixing efficiency of 14.8%. This device shows an increase in overall mixing efficiency over the T-shaped device, some of which is due to the geometry of the flow streams, but also shows a more

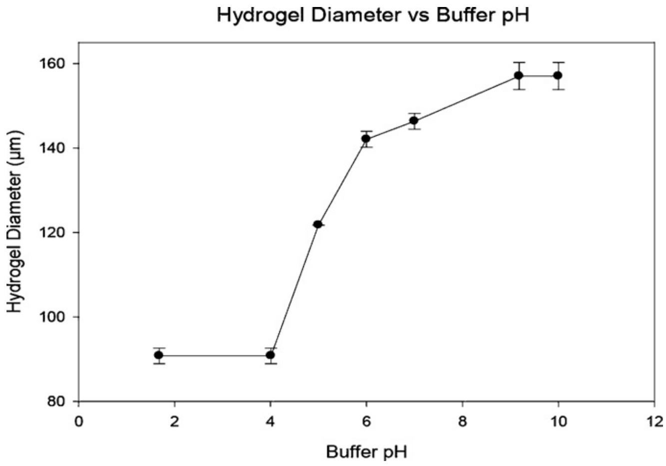

Fig. 3. Hydrogel diameter vs buffer pH. Measured hydrogel post diameters at various buffer solution pH ranging from 1.68 to 10.0.

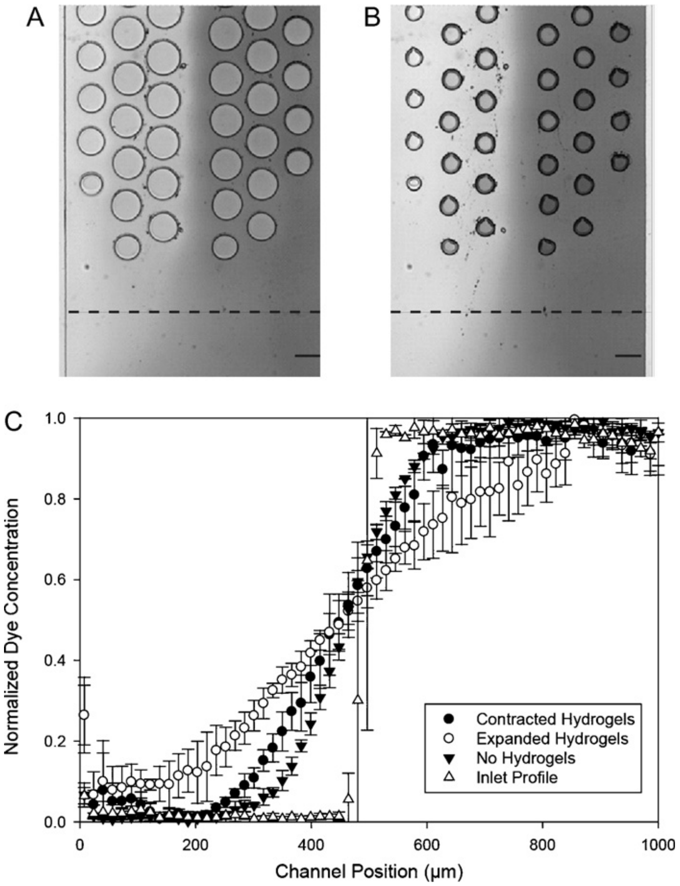

Fig. 4. Diffusion profile comparison in T shaped device. (A) Image of dye profile when hydrogels are in the expanded state. (B) Image of dye profile when hydrogels are in the contracted state. Dashed line indicates position of profile line scan. Scale bars represent 100 μm. (C) Graph comparing dye profile in each hydrogel state as well as a device without hydrogels and inlet profile for comparison.

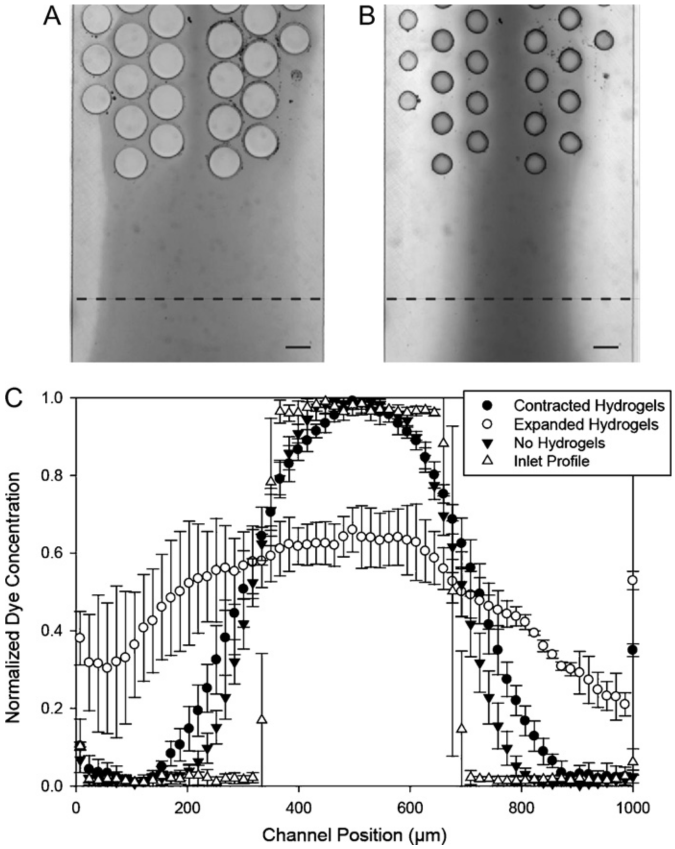

Fig. 5. Diffusion profile comparison in T shaped device with additional center fluid stream. (A) Image of dye profile when hydrogels are in the expanded state. (B) Image of dye profile when hydrogels are in the contracted state. Dashed line indicates position of profile line scan. Scale bars represent 100 μm. (C) Graph comparing dye profile in each hydrogel state as well as a device without hydrogels and inlet profile for comparison.

drastic change in the mixing efficiencies when the hydrogel state is changed. When the hydrogels are contracted the concentration profile is similar to the profile of the device without hydrogels. As before this indicates the contracted hydrogel array has a small added effect on the mixing of the streams. When the hydrogels are expanded the dye concentration profile flattens out across the entire microchannel width. Although perfect mixing is not achieved there is a drastic change in mixing between the two states.

For both the T shaped device and modified version some asymmetry is seen between the two sides of the channel. These differences are introduced during the fabrication of the device. The hydrogel array mask is aligned by hand under the microscope and the hydrogel array may not be perfectly centered. Also, when the excess hydrogel prepolymer is flushed from the device some of the hydrogel pillars move slightly which can affect the resistance of the right and the left hand sides of the channel differently. These differences are reflected in the data, but were accepted as small enough for the demonstration of the concept of the mixing devices.

4. Discussion

We have shown that the volumetric change of pH sensitive hydrogels can be used to control the mixing profiles in a straight planar microfluidic channel. Expanded hydrogels increase the mixing of dye over contracted hydrogels. The devices are essentially passive mixing devices which use obstacles to increase mixing. The expanded hydrogel obstacle array forces the flow stream to split and recombine multiple times along the length of the channel. When the hydrogels are contracted the array acts less to interrupt the flow within the microchannel and the mixing profile is similar to a device without a hydrogel array. This result is significant because the device acts like an active mixer, where mixing can be turned on and off, but is still a passive mixing device. Use of microscale hydrogel posts allow the buffer solution to more quickly diffuse completely into the hydrogels. The result is a hydrogel array that is able to cycle quickly between the contracted and expanded states. Also, the diameter of the posts varies at intermediate pH values. Although not demonstrated in this paper, these intermediate diameters could allow for a range of mixing efficiencies in one device that would depend on solution pH.

The hydrogel mixing devices are very much like current passive mixing devices which use obstacles within the microchannel to improve mixing efficiencies. Many obstacle geometries have been explored to improve mixing in microfluidic devices. Wang et al. demonstrated that the inclusion of asymmetric obstacles increased mixing by forcing the fluid streams to bend and have a lateral flow component [10]. Bhagat et al. simulated and designed a Y mixer device with diamond shaped obstacles to improve mixing over the standard Y channel [25]. These researchers investigated obstacle geometry, offset from center, height, and repition pattern. The results show that all four parameters have an effect on mixing in the device and can be optimized. Rectangular obstacles have also been used to increase mixing of larger particles [9]. Other designs move the obstacles to the channel walls to force lateral movement of the fluid [26,27]. Dense pillar arrays have also been demonstrated for mixing of various solutions [28].

Since obstacle geometry is important, hydrogel swelling geometry would also be of consideration in any design. Beebe et al. compared the swelling of circular and rectangular hydrogels [29]. Optimized hydrogel geometry could produce more complete mixing in a shorter device length as well as a larger difference between the expanded and contracted states. Obstacle mixer designs reproduced in hydrogel form may produce devices with improved, predictable mixing profiles, which are stimuli responsive based on hydrogel polymer type chosen.

Absorption of molecules into the hydrogel posts may need to be a consideration of future designs. The diffusion of molecules into and out of the hydrogel array posts may affect the mixing efficiency and concentration profile in our device. Reduction of undesired diffusion and absorption of molecules into the hydrogel array would need to be balanced against the necessary diffusion of molecules for swelling and contraction of the hydrogel array. The intended use of the hydrogel array would determine which strategies are used to control absorption into the hydrogel array. Some of the factors that should be considered when designing the hydrogel array to prevent undesired diffusion include: monomer chemistries of the hydrogel matrix, pore size, density of crosslinks, and the size and charge of the diffusing molecule [30]. Changing one of these properties will determine how a specific molecule and the hydrogel interact, but may also change the swelling properties of the hydrogel.

As mixing is such a ubiquitous and important aspect of many microfluidic lab on a chip or diagnostic applications, this work demonstrates a new possibility that could improve previously demonstrated techniques. Being able to control the on/off capability of the solution to be mixed could also allow new device possibilities that were previously not possible with standard planar mixers.

5. Conclusion

In this paper we demonstrated the use of pH sensitive hydrogels in a microfluidic device to control fluid interface diffusion and mixing in a microfluidic device. The device design incorporated an array of hydrogels as stimuli-responsive obstacles to split and recombine the fluid flow in the device which in turn increased diffusion and mixing when hydrogels were swollen. This results in a passive mixing device which can be turned on and off like an active mixing device. The design could find use in a diagnostic device with the changing diffusion interface as an output readout. The simple hydrogel array could be incorporated into current devices which take advantage of the interface between two or more fluids or be used with current obstacle mixing designs. Although this work was demonstrated with pH sensitive hydrogels, alternate device designs are only limited by the currently available stimuli responsive hydrogel chemistries.

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