

An organic self-regulating microfluidic system

David T. Eddington,^a Robin H. Liu,^b Jeffrey S. Moore^b and David J. Beebe^{*a}

^a Biomedical Engineering Department, University of Wisconsin—Madison, Madison, WI 53706, USA

^b Beckman Institute, University of Illinois at Urbana—Champaign, Urbana, IL 61801, USA

Received 6th September 2001, Accepted 30th October 2001

First published as an Advance Article on the web 22nd November 2001

In this paper we present an organic feedback scheme that merges microfluidics and responsive materials to address several limitations of current microfluidic systems. By using *in situ* fabrication and by taking advantage of microscale phenomena (e.g., laminar flow, short diffusion times), we have demonstrated feedback control of the output pH in a completely organic system. The system autonomously regulates an output stream at pH 7 under a range of input flow conditions. A single responsive hydrogel component performs the functionality of traditional feedback system components. Vertically stacked laminar flow is used to improve the time response of the hydrogel actuator. A star shaped orifice is utilized to improve the flow characteristics of the membrane/orifice valve. By changing the chemistry of the hydrogel component, the system can be altered to regulate flow based on hydrogels sensitive to temperature, light, biological/molecular, and others.

Introduction

In the human body, control is realized organically at all scales, from the whole body (e.g., temperature) down to the single cell (e.g., membrane potential). Silicon based MEMS have proven well suited to optical and physical sensing applications,^{1–3} but the incorporation of electronic feedback in silicon-based systems increases system complexity, making manufacture costly and prohibitive. In contrast, *in vivo* control is achieved through organic materials and efficient chemical mechanisms. Recently, microfluidic devices have emerged as a useful tool in biological research^{4,5} and clinical medicine,^{6,7} but the ability to economically construct functionally complex systems (systems capable of regulation) stands as one of the obstacles to the realization of practical microsystems for medicine and biology.⁸ Another obstacle is the sensitivity of traditional silicon-based actuators such as electrostatic devices to environmental contaminants (i.e., dust). In order to overcome the limitations of silicon-based microsystems, there has been a growing interest in the use of soft materials for microsystem fabrication.

Methods for constructing soft actuators^{9,10} and soft plumbing^{11–13} have been developed. Feedback systems constructed from disparate materials and components have been demonstrated, but fabrication complexity impedes their application.^{14,15} That is, they rely on conventional system designs (i.e., separate actuator, sensing and signal processing components) that are inherently difficult to assemble at the microscale. In this paper we describe the development of an autonomous organic microfluidic system that is capable of complex function (self-regulation of a fluid stream's pH). A responsive polymeric material replaces the major components (sensors, signal processors and controlling apparatus) that are required for conventional microfluidic pH regulation. The microfluidic system described in this paper can be constructed within one day and operates without electronics or external power. Feedback control is achieved while maintaining system elegance (i.e., complex functionality and ease of construction) through the use of hybrid system designs that combine a three-

dimensional micromolded channel network with *in situ* construction of stimuli responsive components.

The development of microfluidic systems that provide feedback control would open new applications in biology and chemistry. A self-regulating pH system has applications including the sequence determination of proteins, DNA analysis, and others.¹⁶ pH control is necessary when the process itself alters the pH (e.g., base is produced by protein degradation and acid by carbohydrate metabolism¹⁶), causing irreparable damage to biologicals. Many biologicals remain stable only within a small window of pH. For chemical or biochemical processes that consume or produce hydrogen ions, it is therefore desirable to have a system that continuously monitors and regulates the pH of the solution. The rapid fabrication techniques combined with the functionality of stimuli responsive materials allow the feedback characteristics of the system to be readily adapted by changing system parameters (geometries and materials) as needed for different applications. As an example of pH regulation, the system described in this paper demonstrates the regulation of the output pH stream in response to flow perturbations in the input stream.

Hydrogels consist of a broad range of polymers that have a high water content. Within this class of materials exist polymers that undergo volumetric changes in response to changes in their local environment.¹⁰ Since the temporal response of organic systems is often diffusion limited (e.g., muscle contraction, hydrogel response), moving to the micro scale facilitates functionality. In the case of hydrogels, this allows for their use as active components in feedback systems, similar to fluid transport in xylem vessels of plants.^{17,18} Other similarities between *in vivo* systems and systems incorporating hydrogels exist such as their inherent non-linearity and hysteresis. Through appropriate system design and by leveraging unique fluid phenomena at the micro scale (e.g., laminar flow), we have been able to achieve stable feedback control in a completely organic micro fluidic system. By using a stimuli responsive hydrogel post as the single active element in the system, we eliminate the need for external power and electronics. The hydrogel acts both as the pH sensor and actuator by changing volume at different pH values. At low pH values the hydrogel constricts to its shrunken volume while at high pH it fully expands.

In the self-regulating system described here, this volume change is coupled to deform a thin impermeable poly-(dimethylsiloxane) (PDMS) membrane. The PDMS membrane deformation partially occludes an orifice (based on the size of the hydrogel) to regulate the feedback stream of compensating buffer solution. Fig. 1 illustrates the flow paths and operation of the system. Hydrogels have been previously used to regulate fluid flow, but in those larger scale devices¹⁹ the compensating flow was either on or off. However, our device throttles the compensating flow to yield variable feedback as shown in Fig. 1b.

Fabrication

Construction of the system shown in Fig. 1 combines compression micromolding, layered manufacturing¹¹ and *in situ* liquid

phase photopolymerization.⁹ Through compression micro-molding of PDMS elastomer, three two-dimensional (2-D) layers were fabricated. A mixture of PDMS prepolymer and curing agent (Sylgard 184 silicone elastomer kit, Dow Corning, Midland, MI) was prepared in a 10:1 ratio and poured onto an epoxy based photoresist (EPON) mold master with a transparency, glass, and aluminium discs placed on top. Under 100 lb compression, this stacked structure was held at 75 °C for 2 h to realize thru holes in the PDMS layers. The top 2-D PDMS layer was processed under reduced compression (1 lb) to allow a 30 μm PDMS membrane to form over the EPON mold master. The 30 μm membrane was then manually removed from the top layer, except over the actuation chamber, to allow compensating buffer to enter the top channel.

The completed device consists of a 3-D PDMS channel network and a pH sensitive hydrogel. The channel network is fabricated by first patterning a thick (1 cm) bottom with access holes cored out with a 12 gauge syringe needle, then three 2-D channel layers and a coverslip are sequentially stacked on the thick layer and bonded with a No. 2 coverslip as the top. To achieve bonding, the layers are activated in an oxygen plasma and aligned under a stereoscope using methanol as a surfactant between the layers. Alignment was realized by manual manipulation aided by surface tension effects. Fig. 1 shows the completed 3-D PDMS microfluidic network.

Once the microfluidic network is fabricated (channel dimension of $200 \times 200 \mu\text{m}$), the hydrogel sensor/actuator is photopolymerized *in situ*.²⁰ The hydrogel monomer components were 11% AA (acrylic acid), 84% HEMA (2-hydroxyethyl methacrylate), 1% EGDMA (ethylene glycol dimethacrylate), and 3% DMPA [2,2-bis(hydroxymethyl)propionic acid]. The exposure was 120 s at 365 nm. Methanol was used to flush out any unpolymerized prepolymer from the device.

Results

The device was operated under an optical microscope (Olympus, BX-60). Phosphate buffer with ionic strength adjusted to 0.2 M through the addition of NaCl was used in the experiment at pH 2 (input) and pH 12 (compensating). A syringe pump (Harvard apparatus, PHD 2000) provided control of the input stream solution flow and a 40 cm water column provided constant pressure for the compensating buffer solution. The pH of the output stream was measured by a LAZAR PHR-146 micro combination pH electrode and assumed to be completely mixed due to the dimensions of the output collection tube. Initially the hydrogel was made to swell completely and seal off the pH 12 buffer flow. Next, the input flow rate was set to 25 $\mu\text{L min}^{-1}$ and the output pH was measured every 60 s. A microtiter plate was used to gather the output with each column representing each minute and each row representing a specific input flow rate. The input flow rate was ramped up in steps of 25 $\mu\text{L min}^{-1}$ to 100 $\mu\text{L min}^{-1}$, then back down to 25 $\mu\text{L min}^{-1}$ in 25 $\mu\text{L min}^{-1}$ decrements, while measuring the output every 60 s. These results are shown in Fig. 2. The small oscillations in the regulated output flow in Fig. 2b may be due to unsteady syringe pump flow rates.

Discussion

Successful operation of the pH regulation system described here depends on the geometry of the valve orifice. The initial valve design consisted of a circular orifice that displayed an oscillatory response under constant input stream flow. These

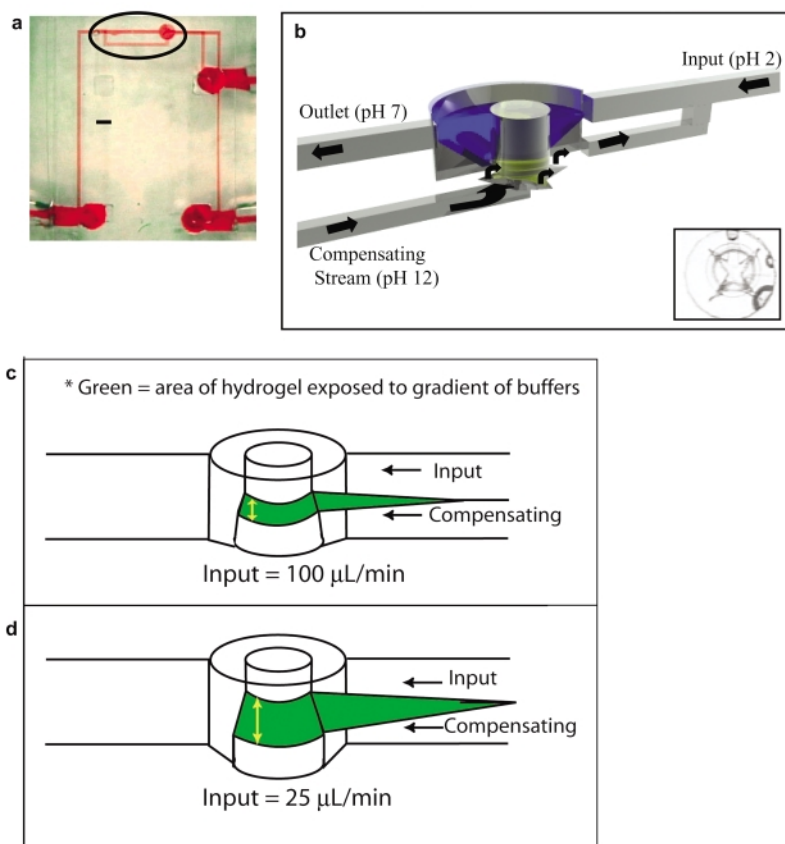


Fig. 1 a, Magnified view of one device, the black line is 1 mm. The device utilizes five layers to realize the required channel network. The circled region corresponds to the section shown in b. b, Schematic of the microfluidic network. The hydrogel post is orange, the 30 μm PDMS membrane is blue, and the star orifice is shown in red. A top view of the actual device is shown in the lower right corner. c, Schematic representation of the hydrogel exposed to input at 25 $\mu\text{L min}^{-1}$. d, Input at 100 $\mu\text{L min}^{-1}$. The input was a pH 2 buffer and the compensating flow was a pH 12 buffer. The green areas represent the diffusion region.

observations suggested a functioning feedback loop lacking stability. Several key parameters dictate system stability other than orifice morphology, including chamber height, membrane thickness, fluid path lengths, swelling kinetics and laminar flow orientation. Chamber height and membrane thickness modifications improved the time response of the system, but the circular orifice still caused on/off control of the feedback stream. When the hydrogel expands, it completely seals the small orifice, stopping the compensating flow. Lack of feedback shrinks the hydrogel, continuing the oscillatory cycle.

By incorporating a star shaped orifice, a continuous throttling of the compensating stream was achieved (in contrast to the discrete on/off functionality of a circular orifice). This is due to the gradual opening of the star orifice compared to the instantaneous opening of the smaller circular orifice. Other geometries may also work in this system, such as a curved star, but the star orifice seems to be the most logical choice. The compensating buffer flow rate was now directly coupled to the size of the hydrogel post providing analog control of the compensating stream leading to system stability. As the diffusion region broadens at lower flow rates, the total height of the hydrogel post increases. This increase in height causes a downward deformation of the PDMS membrane. The flow rate of compensating buffer is created by the changing deformation of the membrane, which is directly correlated to the size of the hydrogel. For a high input stream flow the hydrogel shrinks, causing the membrane to seal off a smaller area of the star opening, allowing more compensating buffer solution to pass through. Vertical stacking of the input and compensating

streams is also employed such that only a fraction (slice) of the hydrogel is exposed to a changing environment, effectively reducing the size of the hydrogel and improving the time response of the system. The incorporation of the star orifice, the optimization of other geometrical parameters, and the use of vertical fluid stacking, result in the stable regulation of the output stream over a range of flow rates.

The compensating buffer is injected into the input stream from the bottom. Because the system operates at a low Reynolds number ($Re \sim 100$), laminar flow causes the two streams to stack vertically with the input stream on top and the compensating buffer on the bottom. The intersection of the two streams creates a diffusion region. The height of the diffusion region varies inversely with the input flow rate (increasing flow rate decreases the height of the diffusion region). Two input flow conditions and their effect on the hydrogel post are shown schematically in Fig. 1 d, e. Note that if the compensating and input streams mix rapidly, the entire hydrogel would expand and contract uniformly to deform the membrane. However, this device uses laminar flow to minimize the active region of the hydrogel actuator. Previous experiments correlate a smaller hydrogel volume (diffusion region) with improved time response.⁹ A theoretical diffusion profile was calculated for the interface between the vertically stacked laminar flow streams in a microchannel²¹ and found to be approximately 60 and 120 μm for the fastest and slowest flow conditions, respectively. Thus vertical stacking reduces the effective size of the hydrogel post by a factor of ~ 10 and also improves the time response by a factor of ~ 10 . Without vertical stacking, the system requires 50

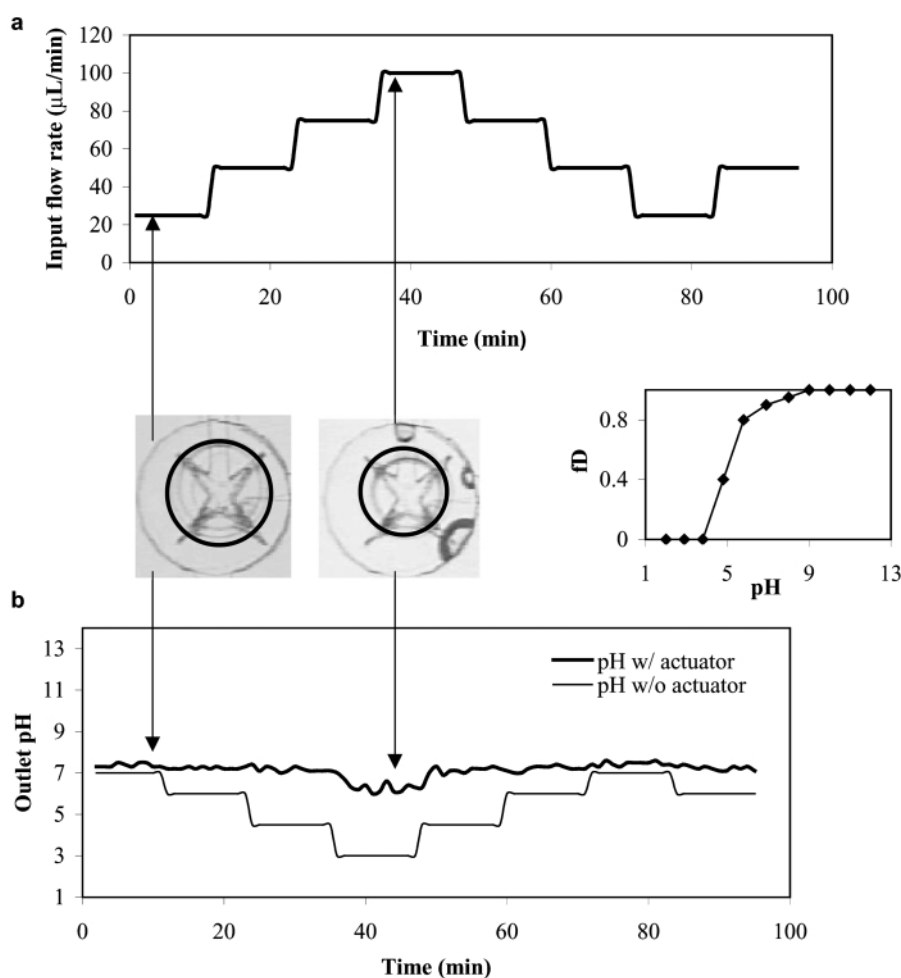


Fig. 2 Graphs showing a, flow rate of input stream *versus* time (which is the same for the two devices described below). This flow rate was varied stepwise using an external syringe pump. b, pH of the output stream *versus* time for two separate devices, one with a hydrogel sensor/actuator and one without (control). The two pictures between the graphs illustrate top views of the hydrogel sensor/actuator at two separate flow rates. The edge of the hydrogel has been highlighted for clarity. The arrows point to when the picture was taken during the experiment. The graph next to the pictures represents the fractional diameter (fD) of the hydrogel with respect to pH.

min to equilibrate and with vertical stacking it only takes a few minutes.

As shown in Fig. 2, the pH is regulated by the hydrogel sensor/actuator at pH 7 for input flow rates between 25–100 $\mu\text{L min}^{-1}$. The device regulates to a level of pH 7 due to the response curve of the hydrogel, which is intrinsic to the type of hydrogel used, the orifice geometry, and the pH of the input and compensating streams. The transition point for the hydrogel used in this system is at a pH of 5.3; however, if a hydrogel with a different chemistry and transition point were used, it would regulate to a different pH. As can be seen in Fig. 2, the system does not effectively regulate pH once the input stream rises above 100 $\mu\text{L min}^{-1}$. At this elevated input flow, insufficient diffusion between the two vertically stacked buffers shrinks the active region of the hydrogel too much to accurately regulate the pH.

Conclusion

The design rules of systems at the micro scale must consider the scale and the characteristics of the responsive materials to fully utilize their functionality. The appropriate use of micro scale phenomena (vertical stacking) and geometries (orifice shape) give the designer parametric control. Other hydrogels have been developed that change with exposure to temperature, light, and biologicals.^{22,23} Thus, we believe the principles demonstrated with pH can be applied to regulate many other parameters, facilitating use across many applications. For example, a hydrogel sensitive to biologicals, such as glucose concentration of a patient's blood, could provide the input stream to regulate an output stream carrying the desired amount of insulin into the blood. The simplicity of system construction (less than one day), the autonomous operation (no power supply) and the flexibility of the system design (adaptable to other applications) allow the concepts described here to be widely and readily incorporated into microsystems. The organic approach to achieving control of complex function at the micro scale combined with the development of biologically responsive materials²² will allow for the creation of biomimetic systems that capture nature's ability to be sensitive and specific while maintaining the robustness and design flexibility of synthetic systems.

Acknowledgements

Support from DARPA-MTO is gratefully acknowledged.

References

- 1 G. H. Siewell, W. R. Boucher and P. H. McClelland, *Hewlett-Packard J.*, 1985, 33.
- 2 R. E. Suloff, *Transducers '91: the 1991 International Conference on Solid-state Sensors and Actuators*, San Francisco, CA, 1991, p. 170.
- 3 W. R. Wu, R. O. Gale, L. J. Hornbeck and J. B. Sampsell, *Proc. SPIE—Int. Soc. Opt. Eng.*, 1988, 24.
- 4 S. Takayama, J. C. McDonald, E. Ostuni, M. N. Liang, P. J. A. Kenis, R. F. Ismagilov and G. M. Whitesides, *Proc. Natl Acad. Sci. USA*, 1999, **96**, 5545.
- 5 A. Hatch, A. E. Kamholz, K. R. Hawkins, M. S. Munson, E. A. Schilling, B. H. Weigl and P. Yager, *Nature Biotechnol.*, 2001, **19**, 461.
- 6 M. Hagmann, *Science (Washington, D. C.)*, 2000, **290**, 82.
- 7 J. Ramsey, *Nature Biotechnol.*, 1999, **17**, 1061.
- 8 P. Mitchell, *Nature Biotechnol.*, 2001, **19**, 717.
- 9 D. J. Beebe, J. S. Moore, J. M. Bauer, Q. Yu, R. H. Liu, C. Devadoss and B.-H. Jo, *Nature (London)*, 2000, **404**, 588.
- 10 T. Tanaka, D. Fillmore, S. Shao-Tang, I. Nishio, G. Swislow and A. Shah, *Phys. Rev. Lett.*, 1980, **45**, 1636.
- 11 B.-H. Jo, L. M. Vanderberghe, K. M. Motsegood and D. J. Beebe, *J. Microelectromech. Syst.*, 2000, **9**, 76.
- 12 M. Unger, H.-P. Chou, T. Thorsen, A. Scherer and S. Quake, *Science (Washington, D. C.)*, 2000, **288**, 113.
- 13 D. C. Duffy, J. C. McDonald, O. J. A. Schueller and G. M. Whitesides, *Anal. Chem.*, 1998, **70**, 4974.
- 14 S. Bohm, W. Olthuis and P. Bergveld, *J. Biomed. Microdevices 1:2*, 1999, 121.
- 15 C. Laritz and L. Pagel, *Sensors Actuators*, 2000, **84**, 230.
- 16 R. Switzer and L. Garrity, *Experimental Biochemistry*, W. H. Freeman and Company, New York, 1999.
- 17 M. A. Zwieniecki, P. J. Melcher and N. M. Holbrook, *J. Exp. Bot.*, 2001, **52**, 257.
- 18 M. A. Zwieniecki, P. J. Melcher and N. M. Holbrook, *Science (Washington, D. C.)*, 2001, **291**, 1059.
- 19 N. B. Graham, *Second European Conference on Smart Structures and Materials*, Glasgow, 1994, p. 31.
- 20 R. H. Liu, Q. Yu and D. Beebe, *J. Microelectromech. Syst.*, 2001, in the press.
- 21 S. Williams, S. Levin, T. Lenczycki and C. Giddings, *Ind. Eng. Chem. Res.*, 1992, **32**, 2172.
- 22 T. Miyata, N. Asami and T. Urugami, *Nature (London)*, 1999, **399**, 766.
- 23 K. Kataoka, H. Miyazaki, M. Bunya, T. Okano and Y. Sakurai, *J. Am. Chem. Soc.*, 1998, **120**, 12694.