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PAPER

A simple strategy for *in situ* fabrication of smart hydrogel microvalve within microchannels for thermostatic control

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Self-regulation of temperature in microchip systems is crucial for their applications in biomedical fields such as cell culture and biomolecule synthesis as well as those cases that require constant temperature conditions. Here we report on a simple and versatile approach for *in situ* fabrication of smart hydrogel microvalve within the microchip for thermostatic control. The thermo-responsive hydrogel microvalve enables “on-off” switch by sensing temperature fluctuations to control the fluid flux as well as the fluid heat exchange for self-regulating the temperature at a constant range. Such temperature self-regulation is demonstrated by integrating the microvalve-incorporated microchip into the flow circulation loop of a micro-heat-exchanging system for thermostatic control. Moreover, the microvalve-incorporated microchip is employed for culturing cells under temperature self-regulation. The smart microvalve shows great potential as temperature controller for applications that require thermostatic conditions. This approach offers a facile and flexible strategy for *in situ* fabricating hydrogel microvalves within microchips as chemostats and microreactors for biomedical applications.

Introduction

Microchips with highly integrated functional units for fluid manipulation are widely used in various fields, such as biological/chemical analysis,¹ disease diagnosis,² high-throughput screening,³ and cell culture,⁴ due to their short analysis time, low cost, and reduced reagent consumption.⁵ Self-regulation of temperature in these microsystems is crucial for their applications in biomedical fields such as cell culture, bio/chemical reactions and those cases that require constant temperature conditions. For example, constant temperature is crucial to ensure efficient gene amplification and cell growth,⁶⁻⁹ and temperature maintenance can also benefit the reaction rate and safety of exothermic reactions. Usually, thermostatic control are achieved by electronic systems; however, these systems require external power supply and complex control algorithms.¹⁰ Alternatively, hydrogel-based microvalves, which enable controlling flow direction and flux via their stimuli-induced shape changes,^{11,12} create new opportunities for developing smart microsystems for

thermostatic control via fluid heat exchange. The hydrogel microvalves integrate both sensing and actuating functions, and require no external power supply; thus, they show great potential in the development of microsystems for thermostatic biomedical applications.

Typically, hydrogel-based microvalves are fabricated by assembly of pre-fabricated hydrogel components into a well-designed microchannel,¹³ or *in situ* synthesis of hydrogel within the microchannel by lithography technique.^{14,15} Micro-sized spherical and cylinder hydrogels have been assembled into the chambers within microchannels to construct in-channel microvalves. The swelling/shrinking changes of such hydrogel microvalves in response to various stimuli such as temperature,¹⁶⁻²³ pH,²⁴⁻²⁷ magnetic field,²⁸ electric field²⁹ and glucose,³⁰ allow controlling the “on/off” switch of microchannels for flow manipulation. Hydrogels with more complex shapes can be *in situ* integrated in microchannels by lithography-based polymerization.³¹⁻⁴¹ Such hydrogels provides more versatility for designing microvalves with flexible shapes for flow control. Although the above-mentioned microvalves allow “on-off” switch in response to a wide range of stimuli for flow manipulation, utilization of such microvalves in microsystems for thermostatic control still remains difficult to be achieved. Moreover, current methods for fabricating microvalves usually require expensive instruments and troublesome multi-step fabrication processes. Therefore, simple techniques for design and fabrication of responsive hydrogel microvalves within microchips for thermostatic control are still essentially required.

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† Electronic Supplementary Information (ESI) available: Figure S1 showing the schematic illustration of the preparation process of the L-shaped chamber, and Figure S2 showing fabrication of the microdevice for cell culture. See DOI: 10.1039/b000000x/

Here we report on a simple and versatile approach for *in situ* fabrication of smart hydrogel microvalve within microchannels for thermostatic control. A microchip with star-shaped microchannels fabricated by simple assembly of coverslips on glass slides is used as the platform for integrating the hydrogel microvalve. For temperature self-regulation, a thermo-responsive hydrogel microvalve, with shape depending on the chamber that is confined by both microchannel and paraffin, is synthesized within the microchip by UV-polymerization. Temperature self-regulation of the hydrogel microvalve is demonstrated by integrating the microvalve-incorporated microchip into the flow circulation loop of a micro-heat-exchanging system for thermostatic control. Moreover, the microvalve-incorporated microchip is employed for culturing cells under thermostatic conditions. The smart microvalve shows great potential as temperature controller for thermostatic biomedical applications. Meanwhile, this approach offers a facile and flexible strategy for controllable creation of hydrogel microvalves within microchips as chemostats and microreactors for cell culture and biochemical reactions.

Materials and methods

Design and fabrication of hydrogel microvalve within microchip

The microchip with star-shaped microchannels was fabricated by assembly of coverslips (thickness: $\sim 150\ \mu\text{m}$) on glass slides according to a previously published method.⁴² The gaps between coverslips create star-shaped microchannels for fluid flow. For *in situ* fabrication of the hydrogel microvalve in the desired site of the microchip, a L-shaped chamber was created in the star-shaped microchannels for hydrogel polymerization (Fig. 1a). Paraffin with melting point of $48\ ^\circ\text{C}$ (Chengdu Kelong Chemical Reagents) was used to selectively block the microchannel, and then redundant paraffin was removed to construct the L-shaped chamber (Please see Fig. S1 in the Supporting Information for detailed schematic illustration). Then, to seal the gaps for flowing fluid, another coverslip was set on the top of the gaps to provide confined microchannels. UV-curable adhesive was used to ensure tight sealing between the coverslips and the glass slide. Needles were set at the inlet and outlet of the microchannel and sealed by epoxy resin for further connecting polyethylene (PE) pipes (Fig. 1a). For synthesis of the hydrogel microvalve, monomer solution containing 10 ml of deionized water, 1.13 g of monomer *N*-isopropylacrylamide (NIPAM, TCI), 0.0308 g of crosslinker *N,N*-methylenebisacrylamide (Chengdu Kelong Chemical Reagents), 0.00452 g of 1-Hydroxy-cyclohexyl-phenylketone (TCI), and 0.00452 g of benzophenone (Tianjin Bodi Chemicals) as photo-initiators were injected into the L-shaped chamber (Fig. 1b). Two risers were set separately at the inlet and outlet of the L-shaped Chamber for injecting the monomer solution and preserving the excess monomer solution from the chamber. Then, the microchip was exposed to UV light in an ice bath for 8 min to polymerize the monomer solution for synthesizing the hydrogel microvalve (Fig. 1c). Next, the risers were sealed by epoxy resin. Finally, the paraffin remained in the microchannel was washed off with hot water, and a hydrogel-microvalve-incorporated microchip was obtained (Fig. 1d). The fabrication process was observed with an optical microscope.

Setup of microvalve-integrated micro-heat-exchanging system

Temperature self-regulation of the hydrogel microvalve was demonstrated by integrating the microvalve-incorporated microchip into the flow circulation loop of a micro-heat-exchanging system for thermostatic control. A micro-heat-exchanger was fabricated by a method similar to that used for fabricating the microchip (Fig. 2a-c). Two reservoirs, each with size of $0.5\ \text{cm} \times 0.5\ \text{cm} \times 150\ \mu\text{m}$, were separated by a coverslip and constructed in the micro-heat-exchanger for respectively flowing hot fluid (lower reservoir) and cold fluid (upper reservoir) (Fig. 2a-c). To create the micro-heat-exchanging system with circulation loop for the cold fluid, the microvalve-incorporated microchip for flux control, and a peristaltic pump (KCP-B, Kamoer Shanghai) for powering flow circulation, were connected with the lower reservoir of the micro-heat-exchanger by PE pipes. A microfluidic flow control system (MFCS) (Fluigent) was used throughout this study to inject cold water with a constant pressure (40 kPa) and measure the flux (Fig. 2d-f).

In the micro-heat-exchanging system, cold water was supplied into the circulation loop via the inlet ($I_{\text{cf},1}$) connected to the peristaltic pump. In the micro-heat-exchanger, hot water with a constant flow rate was injected via the inlet (I_{hf}) by syringe pump (PHD 2000, Harvard Apparatus) and flowed out via the outlet (O_{hf}). In the microchip (Fig. 1c), microchannel-1 was used as the entrance for the circulating cold water, and microchannel-2 and microchannel-4 were used as the exits. A gas-liquid separator was set in microchannel-4 to avoid entrance of air into the loop, and a one-way valve was used downstream the microchannel-2 to avoid backflow. Probes of temperature sensors (TES-1310, TES Electrical Electronics) were respectively incorporated in each of the inlets ($I_{\text{cf},2}$ and I_{hf}) and outlets (O_{cf} and O_{hf}) of the micro-heat-exchanger to monitor the fluid temperature (Fig. 2d-f).

Thermo-responsive switch performance of hydrogel microvalve

The thermo-responsive “on-off” switch behaviour of the hydrogel microvalve was investigated by measuring the flux through the microvalve at different temperatures (Fig. 3). Briefly, microchannel-1 was sealed by blocking the needle with a plug to ensure the fluid flowing through the microvalve. Then, water with temperatures ranging from $28\ ^\circ\text{C}$ to $40\ ^\circ\text{C}$ was respectively injected through microchannel-2 by the MFCS (Fig. 3d). The flux of microchannel-2, which equals to the flux sum of microchannel-3 and microchannel-4, was measured as the flux through the microvalve to indicate the microvalve switch.

The response time of the hydrogel microvalve for thermo-responsive switch was investigated by suddenly changing the cold water ($25\ ^\circ\text{C}$) that flows in microchannel-2 to hot water ($45\ ^\circ\text{C}$) (Fig. 3f). In the microchip, the microchannel-2 was used for injecting water, and all other microchannels were unsealed for draining the water. The flux change of microchannel-2 was continuously monitored during the process to indicate the response time for microvalve switch.

Sealing performance of hydrogel microvalve

The sealing performance of the hydrogel microvalve was studied by measuring the flux through the microvalve when injecting

microchannel-2 with fluid at a constant temperature (25 °C) but different pressures (0–100 kPa) (Fig. 4a). Microchannel-1 was sealed by blocking the needle with a plug to ensure the fluid flowing through the microvalve. Similarly, the flux of microchannel-2 was measured as the flux through the microvalve to indicate the sealing performance. Moreover, to investigate the effect of the protruding section on the sealing performance, microvalves with protruding sections at length of 140 μm, 170 μm, 200 μm, and 230 μm were respectively used for the sealing performance test.

Temperature self-regulation of hydrogel microvalve for thermostatic control

The temperature self-regulation of the hydrogel microvalve was studied by cooling hot fluid with cold fluid in the microvalve-integrated micro-heat-exchanging system (Fig. 2e,f) for thermostatic control. Hot water that flows in the lower reservoir of the micro-heat-exchanger was cooled by cold fluid that flows in the upper reservoir.

Typically, to cool the hot water at different temperatures, air at room temperature (25 °C), water (22 °C) without microvalve-control, and microvalve-controlled circulating water were respectively used as the cold fluid. It is worth noting that, water without microvalve-control was injected via the inlet (I_{cf-2}) in the micro-heat-exchanger; thus the water would flow out of the circulation loop via microchannel-4 due to the absence of microvalve. For both water-cooling methods, cold water with a constant pressure of 100 kPa and a constant temperature of 22 °C was supplied into the circulation loop via I_{cf-1} by the MFCS. Hot water with temperatures ranging from 33 °C to 45 °C was supplied with a constant flow rate of 1000 μL min⁻¹ by the syringe pump.

The effect of the cold-water temperature on the thermostatic control was investigated by using cold water with different temperatures for cooling the hot fluid (38 °C). For both water-cooling methods, cold water with a constant pressure of 100 kPa but different temperatures was used.

Temperature self-regulation with hydrogel microvalve for cell culture

The temperature self-regulation with the smart microvalve for cell culture was investigated by using *Chlorella pyrenoidosa* as the model cell. The cell growth in the reservoir (size: 5mm × 5mm × 3mm) of microdevices with and without the smart microvalve for temperature self-regulation were respectively monitored (please see Fig. S2 in the Supporting Information for schematic illustration showing the fabrication of the microdevice for cell culture). Briefly, two sets of *Chlorella pyrenoidosa* cells were separately cultured in Bold's Basal medium (75 μL) in the reservoirs of two microdevices. For one of the devices, hot fluid (44°C) cooled with microvalve-controlled circulating water (22 °C) was introduced under the reservoir for controlling the culture temperature; while for the other one, hot fluid (44 °C) cooled with air at room temperature was introduced. The fluorescent chloroplasts in *Chlorella pyrenoidosa* allow observation of the cells by confocal laser scanning microscope (CLSM) (SP5-II, Leica) with excitation at approximately 488 nm. The cell growth was monitored by using Leica microscope (Leica DM 4000,

under a Leica I3 filter, set BP 450–490) for observation and cell counting chamber for counting numbers.

Results and discussion

Fabrication of hydrogel microvalve within microchip

For *in situ* fabrication of thermo-responsive hydrogel microvalve, a microchip with star-shaped microchannels is constructed. To create the microvalve in the desired site of the microchip, a L-shaped chamber that confined by the microchannel and paraffin is prepared in the microchannel for hydrogel synthesis (Fig. 1e). Injection of the monomer solution in the chamber followed with *in situ* UV-polymerization produces a thermo-responsive poly(*N*-isopropylacrylamide) (PNIPAM) hydrogel microvalve with L-shape in the microchip. The hydrogel microvalve is dyed with rhodamine B to clearly show the L-shape (Fig. 1f). Such a strategy provides a versatile approach for *in situ* fabrication of hydrogel microvalve with different compositions in microchips. Moreover, since the chamber shape can be simply changed by adjusting the microchannel shape and the position of paraffin, this approach allows creation of hydrogel microvalves with flexible shapes.

Thermo-responsive switch of the hydrogel microvalve for flow control

The PNIPAM networks that used for constructing the hydrogel microvalve allow reversible volume changes when temperature changes across the volume phase transition temperature (VPTT) (~32 °C).⁴³ This thermo-responsive volume transition enables changing the microvalve shape by sensing temperature fluctuations to control the “on-off” of the microchannel for flux manipulation (Fig. 3a-c). Such a thermo-responsive switch behaviour is systematically studied by flowing the microchannel with deionized water at different temperatures for flow rate measurement (Fig. 3d). As shown in Fig. 3e, when water (28 °C) is injected, the hydrogel microvalve swells and blocks the microchannel; thus the flow rate through the microvalve is ~0 μL min⁻¹, indicating the complete “off” state of the microvalve. When the water temperature increases, the hydrogel microvalve gradually shrinks and opens the microchannel for water flowing, leading to an increased flow rate. A significant increase of the flow rate is observed near 32 °C, due to the dramatic volume change of PNIPAM near its VPTT. Especially, the critical temperature for opening the microvalve is observed at about 29–30 °C. This temperature mainly depends on the VPTT of the hydrogel because the VPTT affects the shrinking/swelling behaviours. When the water temperature further increases to higher than 35 °C, a maximum flow rate is obtained, indicating that the shrunken hydrogel microvalve reaches its equilibrium state for complete open of the microchannel.

The response time for switching the microvalve from “off” state to “on” state is studied by suddenly changing the flowing cold water (25 °C) to hot water (45 °C) (Fig. 3f). After suddenly switching cold water to hot water, the water temperature increases and results in shrinking of the hydrogel microvalve for an increased water flux (Fig. 3g). Especially, a rapid increase of the flow rate is observed after 6 s, due to the significant increase of the water temperature. Then, it takes ~6 s for the flow rate to

achieve ~80 % of the maximum flow rate, indicating a fast response. Such a dramatic increase of the flow rate shows the excellent “on-off” switch behaviour of the hydrogel microvalve. Further improvement of the response rate can be achieved by simply tuning the architecture of the polymeric networks in the hydrogel microvalve, e.g. using nanogels as crosslinkers.⁴³ Moreover, this “on-off” switch is reversible and repeatable due to the reversible and repeatable swelling/shrinking volume changes of PNIPAM hydrogel.²⁴

Sealing performance of hydrogel microvalve

The sealing performance against pressure is one of the key factors for evaluating a microvalve. To achieve excellent sealing performance, a protruding section is designed in our hydrogel microvalve to withstand the pressure impact (Fig. 4a). We investigate this sealing performance by measuring the flux through the microvalve when injecting microchannel-2 with cold water (25 °C) at different pressures (Fig. 4a). When the swollen microvalve in “off” state closes the microchannel, this protruding section is first deformed under the pressure impact of the flowing water, thus protects the microvalve from detaching from the microchannel wall. The protruding section with longer length can provide better protection for the microvalve to achieve excellent sealing performance. As shown in Fig. 4b, for microvalves respectively with protruding section of 140 μm and 170 μm, a significant increase of water flux that leaks through the microvalve occurs when increasing the water pressure from 0–100 kPa. By contrast, for microvalves with protruding section of 200 μm and 230 μm, their leaked water flux remains very low (~1 μL min⁻¹) under the pressure of 0–100 kPa, showing excellent sealing performance.

Temperature self-regulation of the hydrogel microvalve for thermostatic control

The hydrogel microvalve that enables thermo-responsive switch for flow control provides novel platforms based on fluid heat exchange for thermostatic control. We demonstrate this by incorporating the hydrogel-microvalve-integrated microchip into a micro-heat-exchanging system to self-regulate the water temperature in the micro-heat-exchanger for thermostatic control (Fig. 2d-f). To highlight the advantage of our hydrogel microvalve for temperature self-regulation, three cooling methods are compared, as shown in Fig. 5. For the air-cooling, the water temperature at the outlet (T_{O-hf}) remains almost the same as that at the inlet (T_{I-hf}), showing low cooling efficiency. Moreover, the variation of T_{O-hf} from 33.5 °C to 42.7 °C indicates the poor thermostatic control of the air-cooling style (Fig. 5a). For the water-cooling without microvalve-control, the T_{O-hf} largely decreases due to the efficient heat exchange between hot and cold water (Fig. 5a). However, the results still display limited thermostatic control, showing temperature variation of ~4 °C when cooling hot water with temperature change of ~10 °C. As compare to these two cooling styles, the microvalve-controlled water-cooling shows excellent temperature self-regulation for thermostatic control. As shown in Fig. 5b, with increasing T_{I-hf} from ~34 °C to ~44 °C, the T_{O-hf} remains nearly constant at ~30 °C after cooling with microvalve-controlled water. This excellent thermostatic control is due to the thermo-responsive switch of the

hydrogel microvalve for flow control. In the micro-heat-exchanger, the T_{O-cf} increases with increasing T_{I-hf} due to heat exchange. Such an increase of T_{O-cf} makes the microvalve shrink and leads to part of the water draining out of the microchip through microchannel-4. To compensate the water loss for maintaining the pressure, another flow of cold water (22 °C) enters into the circulation loop via I_{cf-1} and mixes with the circulating water for cooling the water temperature. Since the flux of I_{cf-1} is in direct proportion to the water loss through microchannel-4, the thermo-responsive switch of the microvalve allows adjusting the temperature of circulating cold water, which then controls the heat exchange in the micro-heat-exchanger for thermostatic control.

We also investigate the effect of the cooling water temperature on the thermostatic control by cooling hot water with a constant temperature of 38 °C (Fig. 5c,d). For water-cooling without microvalve control, both T_{O-hf} and T_{O-cf} decreases with decreasing T_{I-cf} , showing poor thermostatic control with temperature variation of ~6 °C (Fig. 5c). By contrast, the microvalve-controlled water-cooling exhibits excellent thermostatic control with temperature variation within ~0.7 °C (Fig. 5d), due to the thermo-responsive switch of microvalve for flow manipulation.

Temperature self-regulation with hydrogel microvalve for cell culture

The potential of the microvalve-integrated microchip for thermostatic biomedical applications is demonstrated by culturing *Chlorella pyrenoidosa* cells (Fig. 6a) under temperature self-regulation with the microvalve. With conventional air cooling for temperature control, the number of the cells remains nearly unchanged after 48 h (Fig. 6b,d). By contrast, with microvalve-controlled water cooling for temperature control, the cells grow a lot within 48 h (Fig. 6c,d). Compared with the temperature condition provided by the air cooling, the temperature condition provided with the microvalve-controlled water cooling is much more suitable for the growth of *Chlorella pyrenoidosa* cells.^{44,45} The results exhibit the excellent performance of temperature self-regulation with the microvalve-integrated microchip for cell culture.

Conclusions

In summary, we present a facile and versatile approach for *in situ* fabrication of smart hydrogel microvalve within microchannels for thermostatic control. The microvalve that consists of PNIPAM hydrogel enables thermo-responsive “on-off” switch for flow control, thus manipulates the heat exchange for self-regulating fluid temperature in micro-systems at a constant temperature. Such smart microvalves integrated in microchips are promising as temperature regulators for applications that require thermostatic conditions such as cell culture and biomolecule synthesis. Moreover, since copolymerization of PNIPAM with hydrophilic or hydrophobic monomers allows VPTT adjustment,⁴⁶ the self-regulated constant temperature that depends on the VPTT can be easily tuned. Besides, the hydrogel microvalves can also be flexibly developed for “on-off” switch in response to other stimuli such as pH,⁴⁷ glucose⁴⁸ and Pb²⁺ ion⁴⁹

by incorporating corresponding functional monomers in the hydrogel. Therefore, our approach offers a promising strategy for *in situ* fabrication of flexibly structured hydrogel microvalves within microchips as chemostats and microreactors for biomedical applications.

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References

- P. A. Auroux, Y. Koc, A. deMello, A. Manz, and P. J. R. Day, *Lab Chip*, 2004, **4**, 534-546.
- S. Nagrath, L. V. Sequist, S. Maheswaran, D. W. Bell, D. Irimia, L. Ulkus, M. R. Smith, E. L. Kwak, S. Digumarthy, A. Muzikansky, P. Ryan, U. J. Balis, R. G. Tompkins, D. A. Haber, and M. Toner, *Nature*, 2007, **450**, 1235-1241.
- P. S. Dittrich, and A. Manz, *Nat. Rev. Drug Discovery*, 2006, **5**, 210-218.
- S. C. Baker, G. Rohman, J. Southgate, and N. R. Cameron, *Biomaterials*, 2009, **30**, 1321-1328.
- G. M. Whitesides, *Nature*, 2006, **442**, 368-373.
- C. Spits, C. L. Caignec, M. D. Rycke, L. V. Haute, A. V. Steirteghem, I. Liebaers, and K. Sermon, *Nat. Proto.*, 2006, **1**, 1965-1970.
- A. R. Connolly, and M. Trau, *Angew. Chem. Int. Ed.*, 2010, **122**, 2780-2783.
- N. Sahiner, H. Ozay, O. Ozay, and N. Aktas, *Appl. Catal. B*, 2010, **101**, 137-143.
- M. Godin, F. F. Delgado, S. Son, W. H. Grover, A. K. Bryan, A. Tzur, P. Jorgensen, K. Payer, A. D. Grossman, M. W. Kirschner, and S. R. Manalis, *Nat. Methods*, 2010, **7**, 387-390.
- L. Dong, and H. Jiang, *Soft Matter*, 2007, **3**, 1223-1230.
- D. J. Beebe, J. S. Moore, J. M. Bauer, Q. Yu, R. H. Liu, C. Devadoss, and B. H. Jo, *Nature*, 2000, **404**, 588-590.
- L. Ionov, *Adv. Funct. Mater.*, 2013, **23**, 4555-4570.
- Y. S. Song, *Carbon*, 2012, **50**, 1417-1421.
- D. T. Eddington, and D. J. Beebe, *Adv. Drug Delivery Rev.*, 2004, **56**, 199-210.
- D. Kim, and D. J. Beebe, *Lab Chip*, 2007, **7**, 193-198.
- C. Yu, S. Mutlu, P. Selvaganapathy, C. H. Mastrangelo, F. Svec, J. M. and J. Fréchet, *Anal. Chem.* 2003, **75**, 1958-1961.
- A. Richter, S. Howitz, D. Kuckling, and K. F. Arndt, *Sens. Actuators B*, 2004, **99**, 451-458.
- A. Richter, A. Türke, and A. Pich, *Adv. Mater.*, 2007, **19**, 1109-1112.
- K. F. Arndt, D. Kuckling, and A. Richter, *Polym. Adv. Technol.*, 2000, **11**, 496-505.
- A. Richter, D. Kuckling, S. Howitz, T. Gehring, and K. F. Arndt, *J. Microelectromech. Syst.*, 2003, **12**, 748-753.
- A. Richter, J. Wenzel, and K. Kretschmer, *Sens. Actuators B*, 2007, **125**, 569-573.
- J. Wang, Z. Y. Chen, M. Mauk, K. S. Hong, M. Y. Li, S. Yang, and H. H. Bau, *Biomed. Microdevices*. 2005, **7**, 313-322.
- Y. P. Zhang, S. Kato, and T. Anazawa, *Sens. Actuators B*, 2008, **129**, 481-486.
- D. Kuckling, A. Richter, and K. F. Arndt, *Macromol. Mater. Eng.*, 2003, **288**, 144-151.
- C. W. Liu, J. Y. Park, Y. G. Xu, and S. H. Lee, *J. Micromech. Microeng.*, 2007, **17**, 1985-1991.
- D. J. Kim, S. R. Kim, J. Y. Park, J. Y. Baek, S. J. Kim, K. Sun, T. S. Lee, and S. H. Lee, *Sens. Actuators A*, 2007, **134**, 321-328.
- J. Y. Park, H. Ji. Oh, D. J. Kim, J. Y. Baek, and S. H. Lee, *J. Micromech. Microeng.*, 2006, **16**, 656-663.
- N. S. Satarkar, W. Zhang, R. E. Eitel, and J. Z. Hilt, *Lab Chip*, 2009, **9**, 1773-1779.
- G. H. Kwon, Y. Y. Choi, J. Y. Park, D. H. Woo, K. B. Lee, J. H. Kim, and S. H. Lee, *Lab Chip*, 2010, **10**, 1604-1610.
- A. Baldi, Y. Gu, P. E. Loftness, R. A. Siegel, and B. Ziaie, *J. Microelectromech. Syst.*, 2003, **12**, 613-621.
- S. Sugiura, K. Sumaru, K. Ohi, K. Hiroki, T. Takagi, and T. Kanamori, *Sens. Actuators A*, 2007, **140**, 176-184.
- G. F. Chen, F. Svec, and D. R. Knapp, *Lab Chip*, 2008, **8**, 1198-1204.
- E. J. Geiger, A. P. Pisano, and F. Svec, *J. Microelectromech. Syst.*, 2010, **19**, 944-950.
- D. C. Duffy, J. C. McDonald, O. J. A. Schueller, and G. M. Whitesides, *Anal. Chem.*, 1998, **70**, 4974-4984.
- Q. Yu, J. M. Bauer, J. S. Moore, and D. J. Beebe, *Appl. Phys. Lett.*, 2001, **78**, 2589-2591.
- D. J. Beebe, J. S. Moore, Q. Yu, R. H. Liu, M. L. Kraft, B. H. Jo, and C. Devadoss, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 13488-13493.
- R. H. Liu, Q. Yu, and D. J. Beebe, *J. Microelectromech. Syst.*, 2002, **11**, 45-53.
- Q. Z. Luo, S. Mutlu, Y. B. Gianchandani, F. Svec, and J. M. J. Fréchet, *Electrophoresis*, 2003, **24**, 3694-3702.
- D. T. Eddington, and D. J. Beebe, *J. Microelectromech. Syst.*, 2004, **13**, 586-593.
- S. R. Sershen, G. A. Mensing, M. Ng, N. J. Halas, D. J. Beebe, and J. L. West, *Adv. Mater.*, 2005, **17**, 1366-1368.
- D. S. Kim, and D. J. Beebe, *Sens. Actuators A*, 2007, **136**, 426-433.
- N. N. Deng, Z. J. Meng, R. Xie, X. J. Ju, C. L. Mou, W. Wang, and L. Y. Chu, *Lab Chip*, 2011, **11**, 3963-3969.
- L. W. Xia, R. Xie, X. J. Ju, W. Wang, Q. M. Chen, and L. Y. Chu, *Nat. Commun.*, 2013, **4**, 2226.
- C. Yang, Z. Ding, and K. Zhang, *World J. Microbiol. Biotechnol.*, 2008, **24**, 2919-2925.
- R. W. Eppley, *Fish. Bull.*, 1972, **70**, 1063-1085.
- R. Xie, Y. Li, L. Y. Chu, *J. Membrane Sci.*, 2007, **289**, 76-85.
- Y. J. Lee, and P. V. Braun, *Adv. Mater.*, 2003, **15**, 563-566.
- M. J. Zhang, W. Wang, R. Xie, X. J. Ju, L. Liu, Y. Y. Gu, L. Y. Chu, *Soft Matter*, 2013, **9**, 4150-4159.
- B. Zhang, X. J. Ju, R. Xie, Z. Liu, S. W. Pi, L. Y. Chu, *J. Phys. Chem. B*, 2012, **116**, 5527-5536.

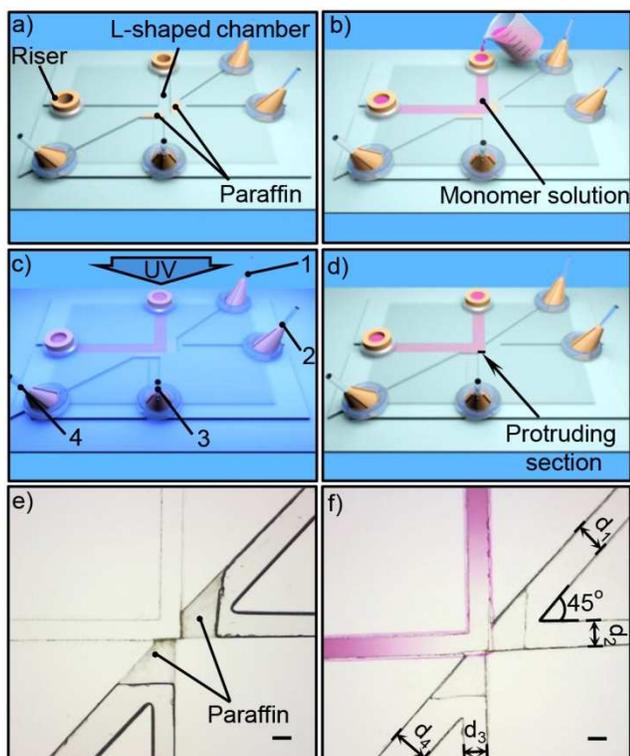


Fig. 1 *In situ* fabrication of hydrogel microvalve within a microchip. (a-d) Schematic illustration showing the fabrication process: First, construct a L-shaped chamber in a star-shaped microchannel for hydrogel synthesis (a); then, inject monomer solution in the L-shaped chamber (b) followed with UV-polymerization (c) and paraffin removal for producing the hydrogel microvalve (d). (e,f) Optical micrographs showing the L-shaped chamber (depth: 150 μm , width: 350 μm) confined by paraffin (e) and the L-shaped hydrogel microvalve labelled with rhodamine B (f). The widths of the microchannels are: $d_1 = \sim 460 \mu\text{m}$, $d_2 = \sim 380 \mu\text{m}$, $d_3 = \sim 370 \mu\text{m}$, $d_4 = \sim 550 \mu\text{m}$, and all the depths are $\sim 150 \mu\text{m}$. The scale bars are 300 μm .

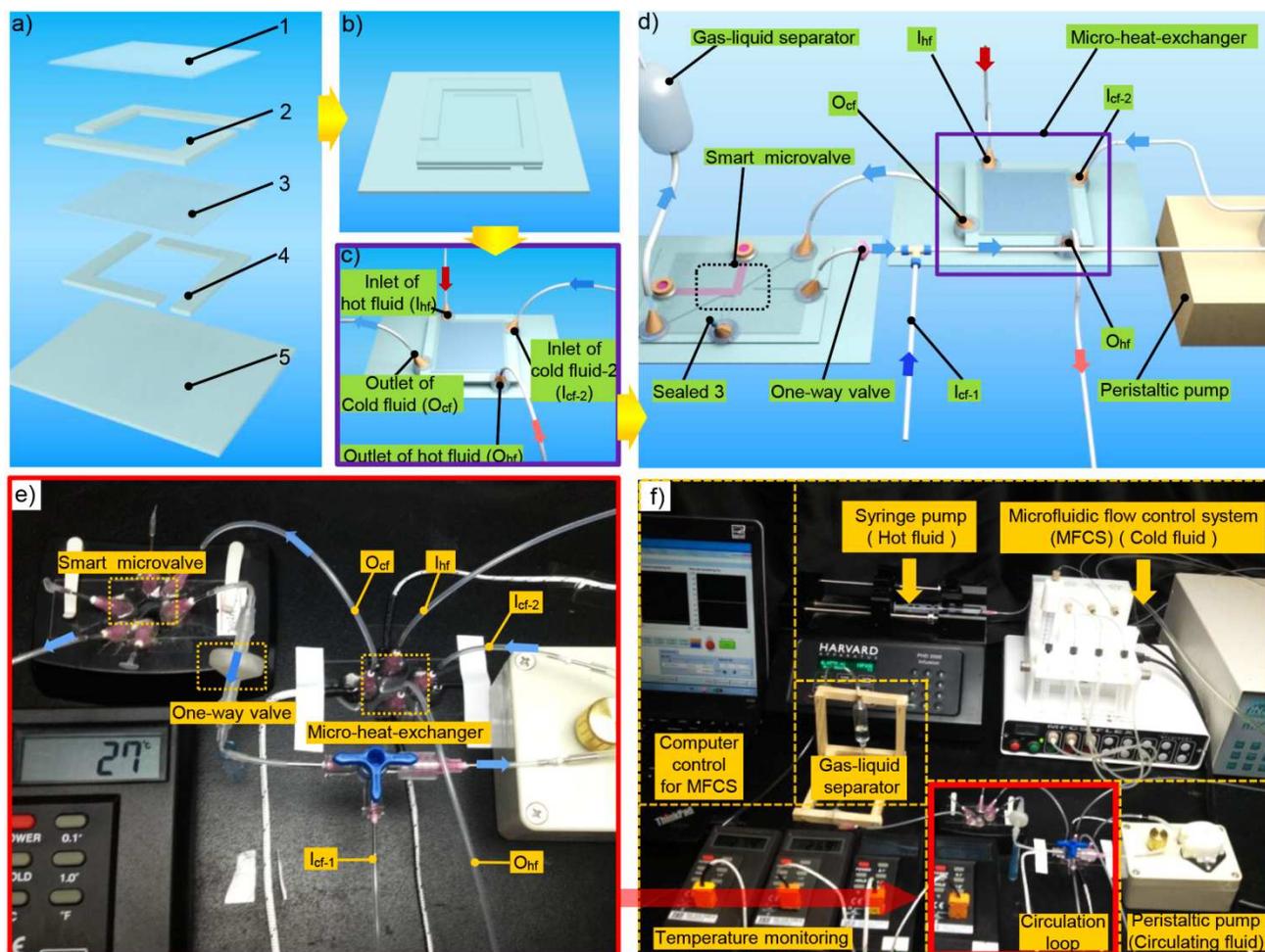


Fig. 2 Hydrogel-microvalve-integrated micro-heat-exchanging system. (a-c) Schematic illustration showing the structure of the micro-heat-exchanger (b,c) made of four coverslips (1-4) and a glass slides (5) (a). (d) Schematic illustration showing the flow circulation loop that contains the smart microvalve, the micro-heat-exchanger, the microvalve-integrated microchip and a peristaltic pump. (e) Magnified photograph of the flow circulation loop in the micro-heat-exchanging system. (f) Photograph of the micro-heat-exchanging system.

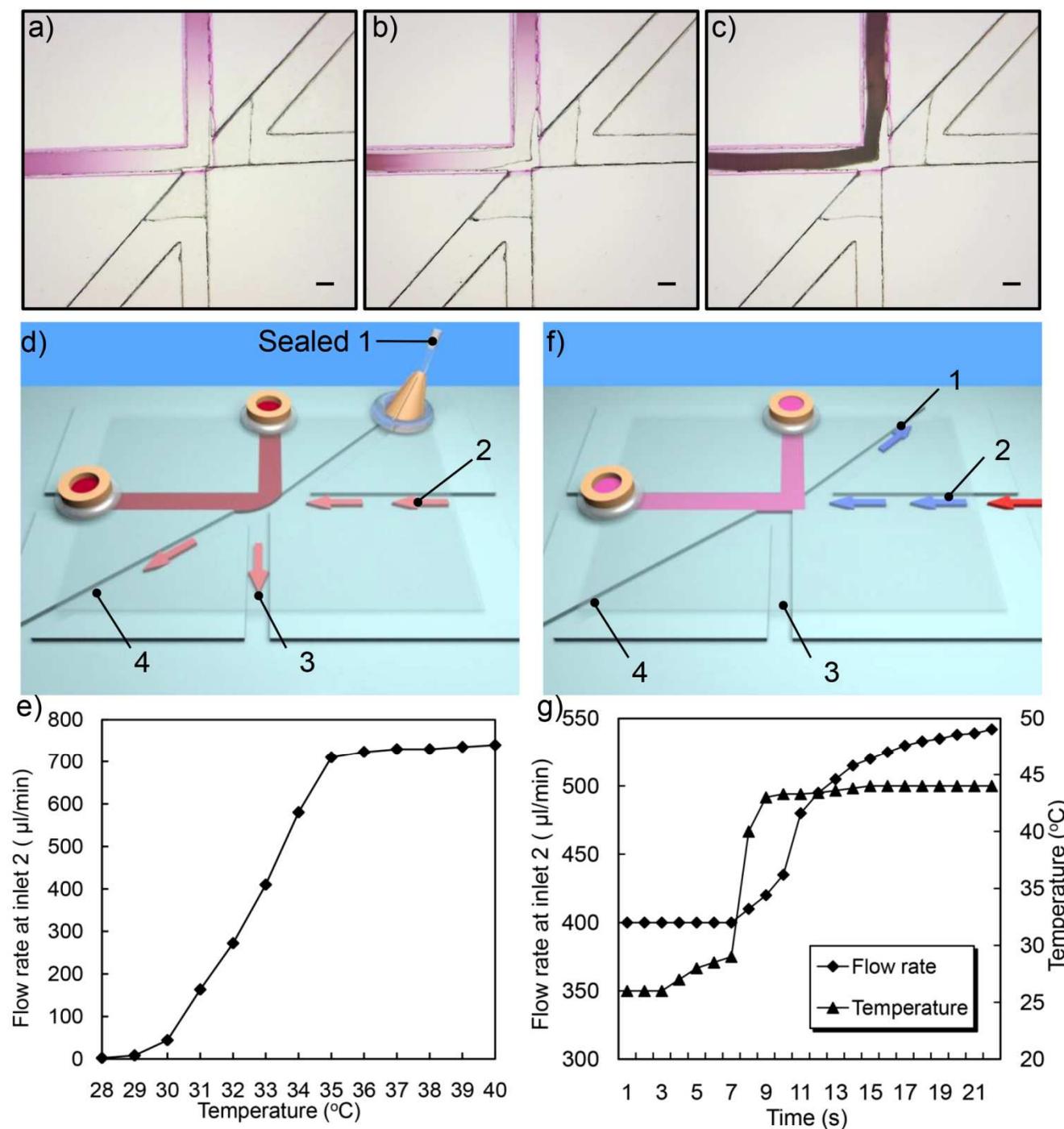


Fig. 3 Thermo-responsive switch of the hydrogel microvalve. (a-c) Optical micrographs showing the volume change of the hydrogel microvalve at 29 °C (a), 32 °C (b) and 35 °C (c). The scale bars are 300 μm. (d,e) Schematic illustration showing the microvalve switch when flowing water with different temperatures (d) for controlling the water flux through the microvalve (e). (f,g) Schematic illustration showing the microvalve switch when suddenly changing the cold water (25 °C) into hot water (45 °C) (f) for controlling the water flux through the microvalve (g). The lengths of the protruding sections in (d, f) are 230 μm.

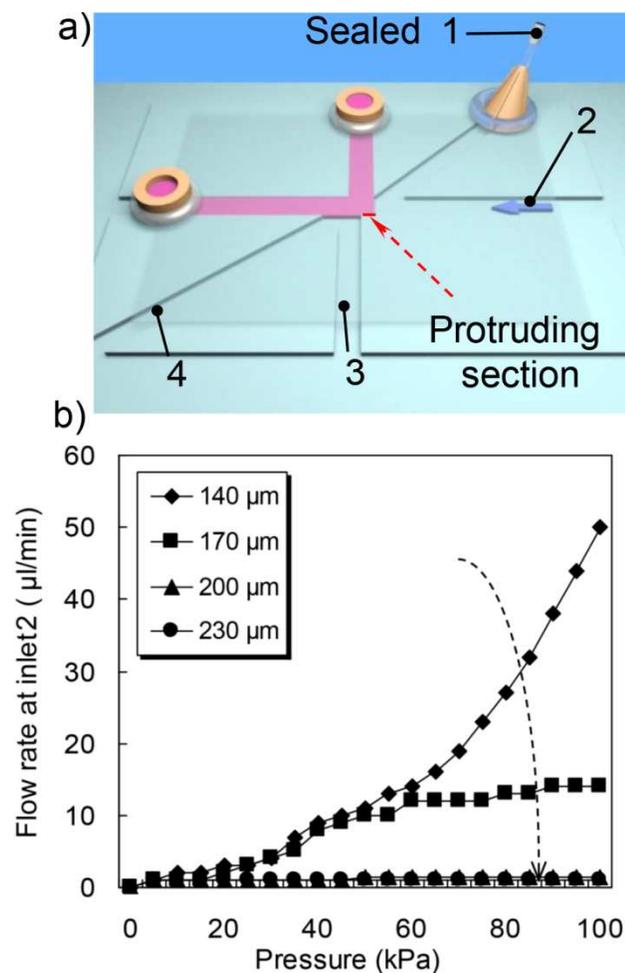


Fig. 4 Sealing performance of the hydrogel microvalve. (a) Schematic illustration showing the experiment setup for testing the sealing performance, in which the flux through the microvalve under different flow pressures is measured. (b) Effect of flow pressure on the water flux through the microvalve, which contains protruding section with length of 140 μm , 170 μm , 200 μm and 230 μm respectively.

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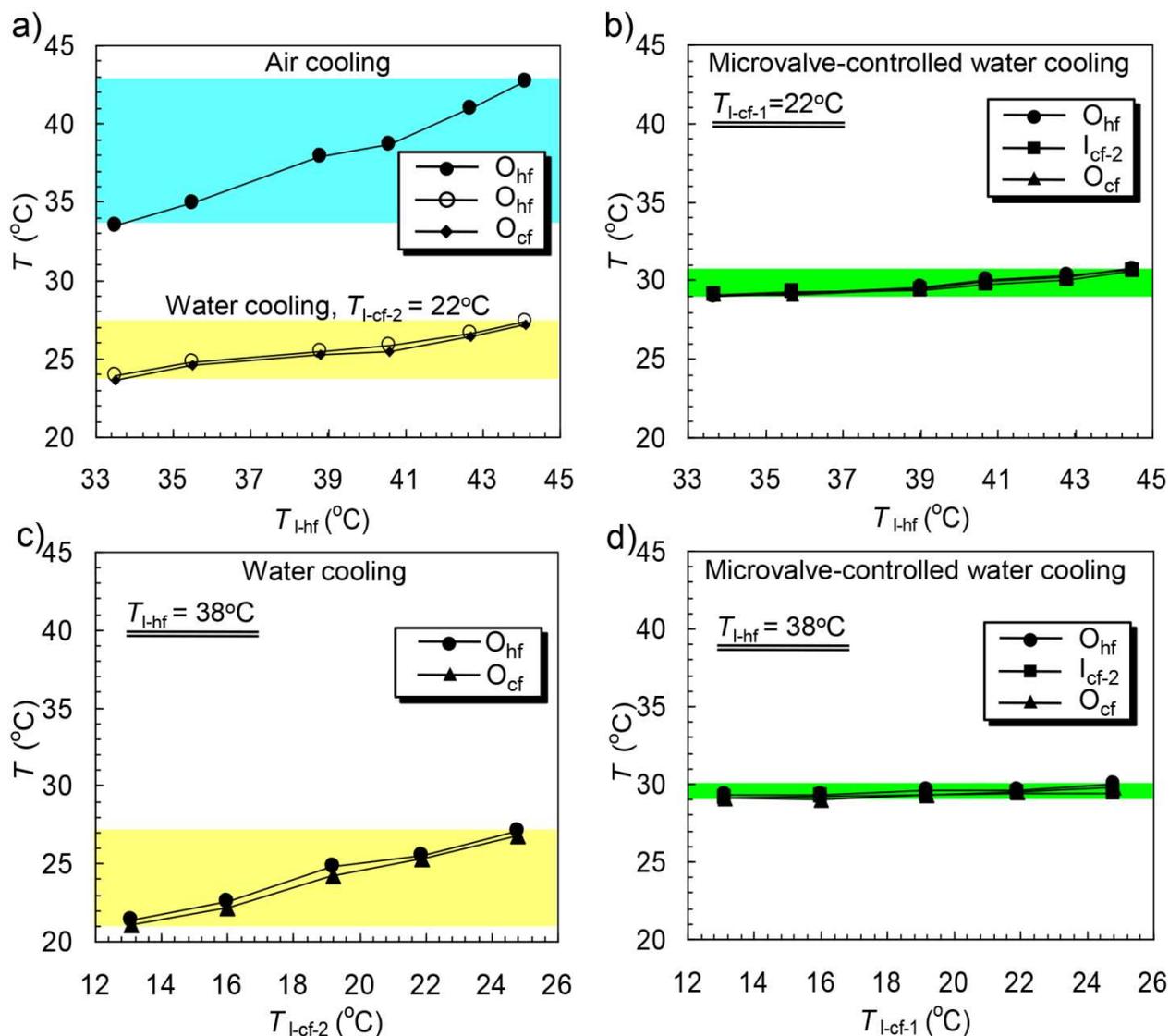


Fig. 5 Hydrogel microvalve for self-regulating the temperature of hot water for thermostatic control. (a,b) Hot water at different temperatures are respectively cooled with air (25 °C) (a, upper), water without microvalve-control ($T_{I-cf-2}=22^\circ\text{C}$) (a, lower) and microvalve-controlled circulating water ($T_{I-cf-1}=22^\circ\text{C}$) (b). (c,d) Hot water with constant temperature of 38 °C is respectively cooled with water without microvalve-control (c) and microvalve-controlled circulating water, each with different temperatures (d). The lengths of the protruding sections are 230 μm .

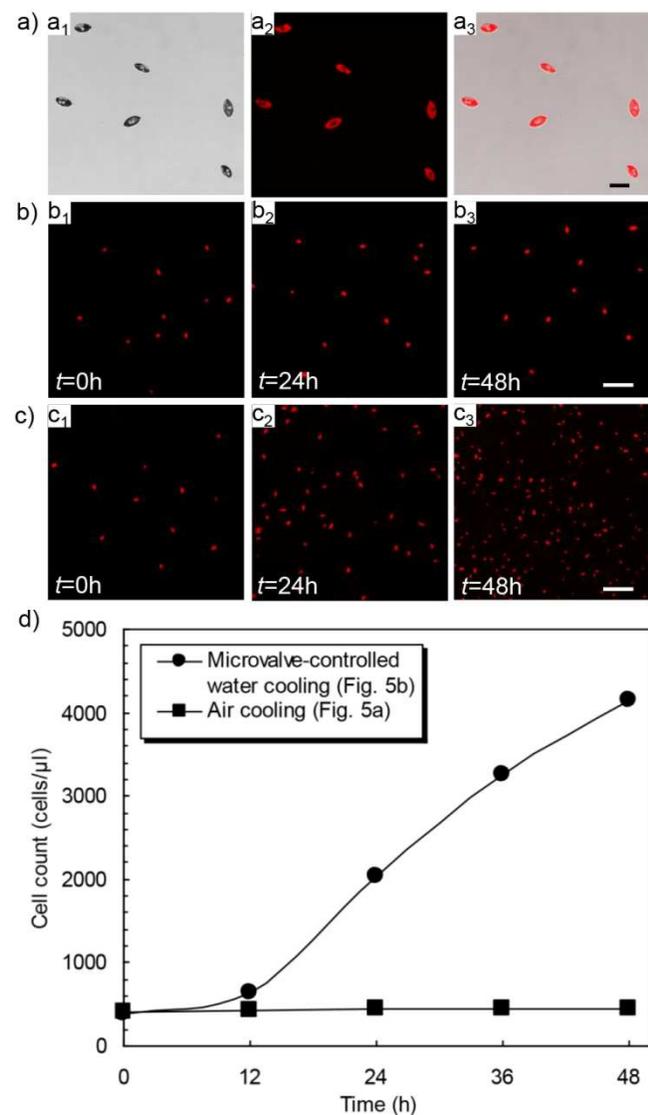


Fig. 6 Temperature self-regulation with hydrogel microvalve for cell culture. (a) Optical (a_1), fluorescent (a_2) and overlapped (a_3) CLSM images of the *Chlorella pyrenoidosa* cells. (b,c) Fluorescent images showing the growth of *Chlorella pyrenoidosa* cells at 0 h, 24 h, and 48 h, under air cooling (b) and microvalve-controlled water cooling (c). (d) The growth curves of *Chlorella pyrenoidosa* cells under air cooling and microvalve-controlled water cooling. The scale bars are 10 μm in (a) and 100 μm in (b,c).

Entry for the graphical Contents

5 Title: **A simple strategy for *in situ* fabrication of smart hydrogel microvalve within microchannels for thermostatic control**

By *Shuo Lin, Wei Wang,* Xiao-Jie Ju, Rui Xie, and Liang-Yin Chu**

10 A smart hydrogel microvalve is *in situ* fabricated within microchip and integrated in the flow circulation loop of a micro-heat-exchanging system for thermostatic control.

