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Stimuli-responsive hydrogel patterns for smart microfluidics and microarrays

Do Hyun Kang,†[a] Sang Moon Kim,†[a] Byungjun Lee,[a] Hyunsik Yoon*[b] and Kahp-Yang Suh*[a]

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In this review, we highlight the properties, functions and applications of stimuli-responsive hydrogel patterns in bioanalytical applications. Stimuli-responsive hydrogel patterns can be realized by well-established micro- and nanofabrication technologies such as photolithography and micromolding, and are currently adopted as active components for manipulation of flow and biosamples in microchannel and microarray systems. We overview the properties of stimuli-responsive hydrogel materials and their fabrication methods along with some representative examples in microfluidics and microarrays.

1 Introduction

Recent advances in materials science and miniaturization technology have brought innovative outcomes in bioanalytical applications for biological research, healthcare, and food industry.[1–7] In particular, micro- and nanofabrication methods have enabled the miniaturization of conventional bioanalytical systems for novel diagnostic or therapeutic systems utilizing various bioanalytes such as DNA, proteins, cells, and tissues.[1–7] In these systems, polymers have been frequently employed due to their inherent biocompatibility as well as easy and low-cost processability.[6–8] Two representative bioanalytical platforms in association with these developments are: (i) microchannels and (ii) microarrays. Microchannels, which are used to guide a flow containing biosamples, are one of the essential components in miniaturized bioanalytical devices.[2–4] In addition, microarrays, which are spatially ordered arrays in discrete spots on a solid matrix, have enabled rapid and high-throughput screening of bioanalytes in a quantitative manner.[9–12]

Despite increasing demands for such miniaturized bioanalytical systems, they still have obstacles for practical applications partly due to their relatively complicated and expensive operation schemes. For example, current microfluidic devices frequently require external tubing and pumps along with an additional integration to the device for the overall miniaturization and portable uses. Also, the precise manipulation of flows and biosamples with biocompatibility is a remaining issue in the current system.[4,13] In this regard, we pay attention to hydrogel materials which are responsive to external signals. A stimuli-responsive hydrogel is a kind of signal transducer which

[a]*School of Mechanical and Aerospace Engineering WCU Program for Multiscale Mechanical Design, Seoul National University, Seoul, 151-744, Korea. Fax: +82-2-883-0179*

[b]*Department of Chemical and Biomolecular Engineering, Seoul National University of Science & Technology, Seoul 139-743, Korea. E-mail: hsyoon@seoultech.ac.kr*

† These authors equally contributed to this work.

Do Hyun Kang received his BS degree from the school of mechanical and aerospace engineering at Seoul National University (SNU) in 2009, and is currently a PhD candidate in the same department at SNU under the supervision of Prof. Kahp-Yang Suh. He is a fellow of the "Global PhD Fellowship", a prize fellowship program supported by National Research Foundation of Korea. His research interest is the fabrication of stimuli-responsive polymeric patterns for biosensors and biomedical applications.

Sang Moon Kim received his BS degree from the school of mechanical and aerospace engineering at Seoul National University (SNU) in 2010, and is currently a PhD candidate in the same department at SNU under the supervision of Prof. Kahp-Yang Suh. His research interest is focused on the directional physicochemical properties of anisotropic micro/nano structures and stimuli-responsive polymeric patterns for energy/actuation system applications.

can convert chemical, thermo, optical, mechanical, electrical or magnetic stresses into a different type of signal.[14–17] The hydrogel materials have been developed for a broad range of applications including drug delivery systems,[18,19] special surfaces interacting with their environment,[20–22] sensing systems of bioanalytes,[14,23–25] and actuators[26,27] inspired from natural systems. This review focuses on the patterned structures of the stimuli-responsive hydrogels for microchannel and microarray applications. They can be simply incorporated into a miniaturized bioanalytical system through well-established micro- and nanofabrication methods such as photolithography and micromolding.

The applications of stimuli-responsive hydrogel patterns can be classified into two categories (Fig. 1): (i) surface and (ii) volumetric responses. In the surface response, a stimulus-responsive hydrogel pattern can exhibit the transition of various surface properties such as wettability and chemical functionality, thereby controlling the direction of liquid flow and the binding of biological molecules, respectively.[20–22,25,28] In this case, one can take advantage of the high surface-to-volume ratio of the hydrogel pattern at such a small scale. In the volumetric response, in contrast, a hydrogel pattern is deformed collectively in response to the change of temperature, pH, light, electric field, *etc.*, so that a liquid flow or a biosample can be manipulated in an appropriate way.[26,27,29] In Table 1, representative examples of stimuli-responsive hydrogels are summarized with their signal-receiving components, inputs/outputs, and signal transduction mechanisms.

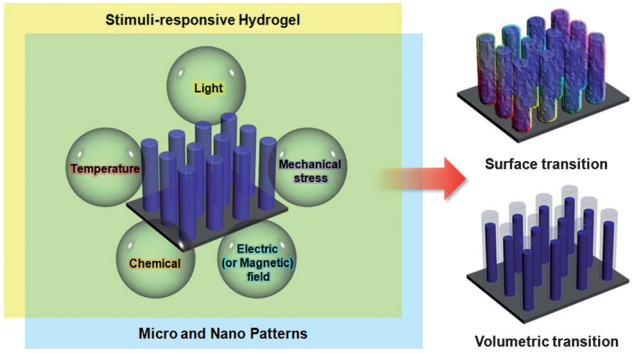

Fig. 1 Concept of surface and volumetric transitions of stimuli-responsive hydrogel patterns for bioanalytical manipulation. It is noted that the hydrogel patterns are not limited to pillar arrays; other types of patterns such as rectangular lines and microwells are also included.

In this tutorial review, we overview the properties, functions, and applications of stimuli-responsive hydrogel patterns towards smart microchannels and microarrays. Specifically, stimuli-responsive hydrogel patterns can control the flow in microchannels as pumps, valves, or mixers through their volumetric actuation (closed channel) or the change of surface properties (open channel). Furthermore, they can tune the transport, reaction, and encapsulation of bioanalytes in a microarray format *via* various actuation mechanisms. Although stimuli-responsive hydrogels can be used in sensory systems due to their inherent responsive behaviors,[14,23–25] those applications are ruled out in this review since they do not necessarily

Byungjun Lee received his BS degree in mechanical and aerospace engineering at Seoul National University (SNU) in 2012, and he is currently a PhD candidate in the school of mechanical and aerospace engineering at the same university (SNU) under the supervision of Prof. Kahp-Yang Suh. His research interest is the fabrication and analysis of various types of polymeric micro/nano-patterned structure for microfluidic applications and bio-sensing devices.

Hyunsik Yoon studied chemical engineering in SNU and he obtained his PhD degree in 2004. As an industrial experience, he worked for Samsung Electronics until 2007 in the area of display devices including liquid crystal display (LCD) business. After returning to SNU as a postdoctoral researcher and a research professor, he wrote about 30 papers in peer-reviewed journals. He began his independent career in 2012 as an assistant professor in the department of chemical and biomolecular engineering at Seoul National University of Science & Technology. His current interests include the bioinspired structures and their applications.

Kahp-Yang Suh obtained his PhD degree from the school of chemical and biological engineering at Seoul National University (SNU) in 2002. After the postdoctoral research in Massachusetts Institute of Technology (MIT), he began his independent career in 2004 as an assistant professor in the mechanical and aerospace engineering at SNU. He wrote 192 papers in peer-reviewed journals and 25 US or domestic patents. He won a number of awards, including the TR100 young innovator award (2004) from MIT technology review. While attending a conference in the USA, he passed away in June, 2013.

Table 1 Examples of stimuli-responsive hydrogels with their signal-receiving components, inputs, possible outputs, and signal transduction mechanisms

Components	Examples	Inputs	Possible outputs	Mechanism
Responsive chemical groups	Acid and base groups	pH[38–41]		
	Ion-binding groups	Metal ions[42]		Change of hydrophilicity or charge by molecular interaction Swelling and deswelling by change of hydrophilicity, charge or crosslinking
	Antigen-binding groups	Antigen[43]		
	Sugar-binding groups	Sugar[44]	Change in surface wettability Mechanical actuation	
	Enzyme-binding groups	Enzyme[45]		
	Polymers with LCST behavior (*e.g.* PNIPAAM)	Temperature[14,15,46]		Change of hydrophilicity by LCST behavior Swelling and deswelling by change of hydrophilicity
	Photosensitive molecules	Light[47–52]		Photochemical reactions
Additives	Magnetic particles	Magnetic field[31] AC magnetic field[37]	Mechanical actuation	Attractive force to magnet Heating *via* the magnetization reversal process in thermo-responsive hydrogels
	Photothermal particle	Light[32–34]	Change in surface wettability Mechanical actuation	Heating *via* the photothermal effect in thermo-responsive hydrogels
	Electrically heated material	Voltage[35,36]		Electric heating in thermo-responsive hydrogels

require an array of hydrogel patterns. Also, other types of stimuli-responsive materials such as self-assembled monolayers, grafted brushes, and thin films are also excluded, for which detailed information is available elsewhere.[16,17,30] We first summarize stimuli-responsive components of hydrogels and developed fabrication methods for ordered hydrogel patterns. Next, current research and applications of smart stimuli-responsive hydrogel patterns are highlighted with the emphasis on microchannels and microarrays.

2 Stimuli-responsive hydrogels

Hydrogels are three-dimensional polymer networks which have hydrophilic parts and can be swollen by absorbing a certain amount of water. The chemical or physical linking in the networks prevents hydrogels from dissolving in water, therefore maintaining a three-dimensional shape with mechanical stability.[17] These hydrogel structures are attractive for various applications such as bioanalysis, tissue engineering, drug delivery, sensors, and actuators due to the following reasons: (i) the aqueous structure and biocompatibility of the hydrogel provide a favorable environment to various biosamples such as DNA, proteins, cells, and tissues. Such biosamples can retain their biological activities without damage or denaturing of the hydrogel. (ii) The hydrogel can be easily processed by various well-developed top-down fabrication methods without complex alternations. For example, hydrogel formation through photo- or thermo-initiated radical polymerization of acrylate or methacrylate monomers is highly compatible with photolithography or micromolding.

Based on the above advantages, stimuli-responsive hydrogels with chemical, thermal, optical, electrical or magnetic stresses have been reported with great attention.[14–17,23,24,31–37] Stimuli-responsive properties of hydrogels are generally achieved by incorporating special components in the polymer chain of hydrogels, which can receive and respond to input signals. The components can either be a specific chemical structure of a polymer chain,[14–17,23,24] or an additive in polymer,[31–37] being inserted into the polymer network during or after polymerization. Then, converting to output signals can be attained by a direct change of the component or several accompanying steps including interactions with neighboring environments or chemicals. The internal changes of hydrogel structures mostly originate from the interaction between the hydrogel and absorbed water. As shown in Fig. 2 and Table 1, two schemes are generally adopted, which are change of wettability on the hydrogel surface and actuation of the hydrogel due to swelling and deswelling of water.

Specific chemical groups or molecules incorporated in the chains of polymer can play an important role in receiving stimuli such as chemical, thermal, and optical signals.[14–17,23,24] For an example of chemical inputs, pH-responsive hydrogels have acid or base groups that can be protonated or deprotonated according to the pH of environmental solution, and the degree of protonation changes the conformation and the hydrophilicity of the polymer accordingly. Furthermore, within the specific hydrogel structure, these changes also induce bulky mechanical motion *via* swelling (expansion) and deswelling (contraction) in water. In the pH-responsive hydrogels, the pK_a value of the chemical groups is an important parameter which

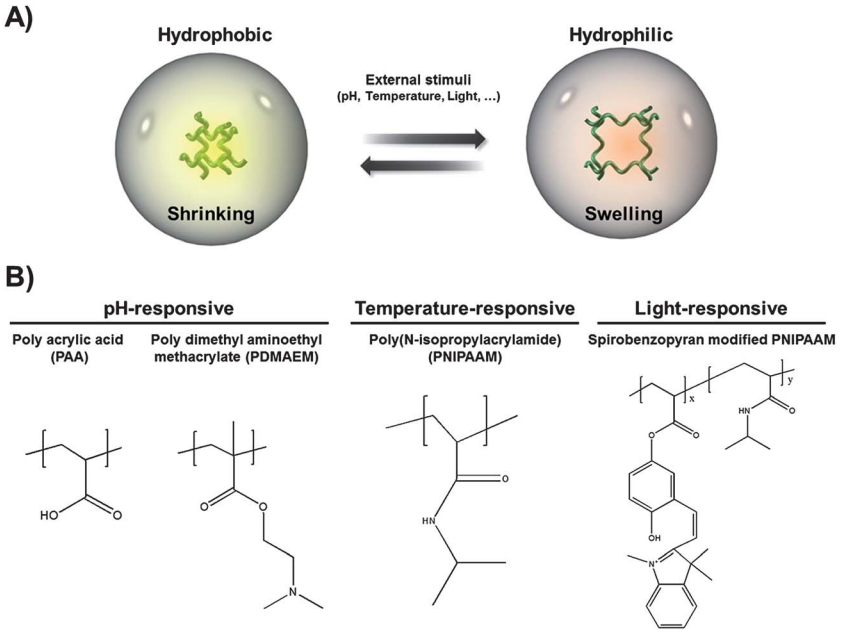

Fig. 2 (A) Schematic illustration of the responsive behavior of stimuli-responsive hydrogels in terms of wettability switching. (B) Representative examples of stimuli-responsive hydrogels (pH-, temperature-, and light-responsive hydrogels).

is related to the degree of protonation, and thus defines the aspect of response, at a certain environmental pH. Additionally, polyacid hydrogels absorb water at pH > pK_a and expel water at pH < pK_a, while polybase hydrogels show the opposite behavior.[14,38] Therefore, according to the desired operating pH and behavior, various pH-responsive hydrogels such as acidic poly(acrylic acid),[39,40] acidic poly(vinyl pyridine),[41] and basic poly(dimethyl aminoethyl methacrylate)[39,41] have been developed and used for switching of hydrophilicity or volumetric change. Similarly, ion-responsive hydrogels[42] and analyte-responsive hydrogels[42–45] which can bind to ions or analytes due to the presence of pendant groups or molecules have been broadly used for chemical or mechanical output signals.

Alternatively, certain polymers show responsive behavior through changes of inter and intra molecular interactions of chemical groups within the polymer depending on temperature. A well-known thermoresponsive polymer, poly(*N*-isopropylacrylamide) (PNIPAAM) generates a response through the change of inter and intra molecular interactions between its pendant groups and water molecules.[14,15,46] Below the critical temperature called lower critical solution temperature (LCST), PNIPAAM exhibits hydrophilic properties due to the hydrogen bonding between the pendant group and water molecules. When the temperature is higher than the LCST, the bonding is broken, thus the polymer becomes hydrophobic. Therefore, the PNIPAAM hydrogel can generate chemical or mechanical signals at different temperatures. The LCST of PNIPAAM is near 32 °C and the temperature is very compatible with bioanalytical and biomedical applications.

In the case of optical signals, photosensitive chemical groups such as spirobenzopyran for photoswelling (*e.g.* spirobenzopyran-functionalized PNIPAAM),[47] azobenzene for photoisomerization (*e.g.* azobenzene- and α-CD-functionalized polyacrylamide),[48,49] nitrobenzene for photolysis (*e.g.* nitrodopamine-functionalized polyethylene glycol),[10,50] and cinnamoyl groups for reversible photocrosslinking[51,52] have been used. Electro-[53] and mechano-sensitive[54,55] chemical groups have also been reported. In the above cases, stimuli-responsive properties are incorporated into the hydrogel by the polymerization of monomers including responsive chemical groups, or modification of premade polymeric structures with chemical groups. By controlling the composition, the degree of responsiveness can be tuned to a certain degree. In addition, by incorporating several kinds of responsive chemical groups sequentially, dual or multiple responses could be obtained.[20]

A different strategy on creating stimuli-responsive hydrogels is to insert an additive to the gel. For example, embedding magnetic particles is generally used for making magneto-responsive hydrogels.[31] Besides, heat-generating additives such as photothermal nanomaterials (*e.g.* iron oxide nanoparticles, graphene, CNTs),[32–34] electrically heated nanomaterials,[35,36] and magnetic nanoparticles in an alternating magnetic field[37] can give rise to additional responsive properties in a thermorepsonsive hydrogel. In such an application, the dispersity of additives in the polymer network is important for their high-quality responses.

3 Patterning methods for stimuli-responsive hydrogels

Stimuli-responsive hydrogel patterns can be achieved with the use of well-established micro- and nanofabrication methods over large areas.[56–59] Here, the fabrication methods are divided into two categories: (i) photolithography and (ii) micromolding techniques. These methods have been used exclusively or cooperatively to fabricate stimuli-responsive hydrogel patterns,

Table 2 Classification of fabrication methods of stimuli-responsive hydrogel patterns. For each method, the formation mechanism, resulting structure, and processable polymer are summarized[a]

Classification	Examples	Patterning methods	Resulting structures	Processable polymers
Photolithography and its related techniques	Photolithography[39,56–58]	Selective crosslinking or cleavage of polymer by UV exposure through a photomask	Micro/nanostructures having height depending on the photomask design	Photo-curable
	Multiphoton lithography[60–62]	Selective crosslinking or cleavage of polymer on the focal spot of light	Complex 3D structures	Photo-curable
Micromolding techniques	Replica molding[68]	Thermal crosslinking of prepolymer filling the cavity of the master mold	Reverse structures of the master mold	Thermo-curable
	UV molding[22,67]	UV crosslinking of prepolymer filling the cavity of the master mold		Photo-curable
	Nano-imprinting[64,66]	Pressure-induced deformation of polymer above T_g		Thermo-deformable
	Capillary force lithography[11,65]	Capillary rise of polymer in the cavity of the master mold		Photo-curable/ solvent-laden polymer

[a] Photo-curable: poly(acrylic acid)-based, poly(dimethyl aminoethyl methacrylate)-based, and poly(*N*-isopropylacrylamide)-based polymers and their copolymers, polyethylene glycol (PEG)-based copolymers, and others. Thermo-curable: poly(*N*-isopropylacrylamide)-based polymers and their copolymers, polyethylene glycol (PEG)-based copolymers, and others. Thermo-deformable: poly(vinyl alcohol)/poly(acrylic acid) blends. Solvent-laden polymer: poly[(3-trimethoxysilyl) propyl methacrylate]-*r*-poly[(ethylene glycol)-methyl ether methacrylate] based polymers.

as summarized in Table 2. From a geometric point of view, the structure can either be formed as a pure responsive structure in an ordered array format or a composite structure integrated on the as-formed non-responsive pattern.

Photolithography has been widely used for making volumetric hydrogel structures that can be polymerized or crosslinked upon exposure to light.[56–58] The method is fast and highly reproducible with monomers having photopolymerizable groups (*e.g.* acrylate and methacrylate). Traditionally, a selective UV light exposure through a photomask with transparent and opaque regions has been used for the patterning as shown in Fig. 3A. Recently, a more sophisticated photolithographic method termed "multi-photon lithography" has been introduced for fabricating complex 3D structures.[60–62] For high-resolution patterning (<100 nm), e-beam lithography may be used, but the method is potentially limited due to high surface tension of hydrogels.[63]

Micromolding techniques such as replica molding, nano-imprinting, and capillary force lithography (CFL) are versatile tools for generating micro- and nanopatterns of hydrogels.[56–59,64–68] These techniques have been developed with various types of polymeric materials such as thermoplastic and thermoset polymers, solvent-laden polymers, and UV-curable polymers. In the process (Fig. 3B), a master mold is placed on a spin-coated or a drop-dispensed polymer layer (fluidic phase) in the form of a melted polymer above glass transition temperature (T_g), solvent-laden polymer, or UV-curable prepolymer, and then an external pressure or capillary force makes the polymer molded into the void space of the master mold. After solidifying the polymer *via* cooling (temperature-directed molding), solvent evaporation (solvent-assisted molding), or UV curing (UV-assisted molding), complementary polymeric patterns are generated after removal of the mold. These methods offer a cheap, low-expertise route to fabrication of micro- and nanopatterns in a typical laboratory setting over relatively large areas.

In the solvent-assisted molding, a good or a poor solvent can be used. First, a good solvent is used to soften hydrogel layers at the time of contact, thereby forming a replica of the original mold by solvent absorption or capillarity. If the mold is polydimethyl siloxane (PDMS) and the prepolymer of the hydrogel is dissolved in ethanol (contact angle <90°), a simple micromolding takes place upon placement of the PDMS mold.[69] If a poor solvent is used (contact angle >90°), on the other hand, the process can be mediated by dewetting in such a way that the hydrogel in the void region recedes downwards until exposure of the substrate.[11,70] Using this concept, very small nanowells (~50 nm) of polyethylene glycol (PEG) hydrogels were achieved on a glass substrate and used as reservoirs for nanoarrays of single lipid vesicles.[11]

4 Bioanalytical manipulation using stimuli-responsive hydrogel patterns

Bioanalytical devices require controlled transport, adsorption, or release of biosamples for achieving timely analytic reactions at desired locations.[2–5] To this end, stimuli-responsive hydrogel patterns can be useful for manipulating various bioanalytes in microfluidics and microarrays in a spatio-temporal fashion. For example, microfluidic devices require controlled transport of samples dissolved in water. Furthermore, processing within microarrays involves multiple steps such as transport, reaction, and encapsulation of analytes for applications in various

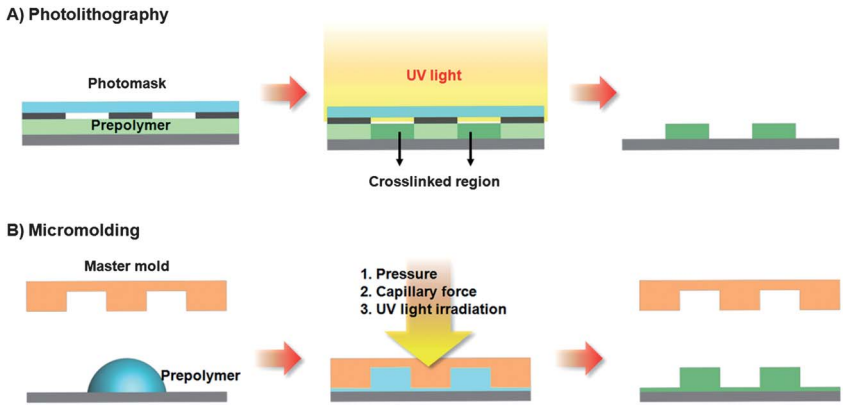

Fig. 3 Schematic illustration of fabrication methods for stimuli-responsive hydrogel patterns: (A) photolithography and (B) micromolding techniques.

diagnostic and therapeutic fields. Stimuli-responsive patterns have contributed to those bioanalytical systems in various ways as described below.

4.1 Manipulation of flow with microchannels

In order to exploit the advantages of miniaturization in bioanalytical applications, a precise control of tiny volume of water (micro- to femtoliter) containing biosamples is essential. In the past decades, micro- and nanofabrication technologies have successfully been used to construct microfluidic components such as channels, pumps, valves, mixers, and so on.[71–75] Such conventional microfluidic components, however, would pose a potential challenge to practical applications because they require a complex external setup for power supply and flow control, as well as an additional integration to the device. During the operation, several issues such as biocompatibility of components and autonomous response to environmental fluctuation are also limiting the expected bioanalytical results. To overcome these obstacles, many researchers have recently reported stimuli-responsive hydrogel patterns included within microfluidic devices with simple integration methods. As a result, the hydrogel patterns which can alter their volumetric structure by suitable stimuli have been applied to microfluidic components such as valves, pumps, or mixers.[29,39,53,76–81] Furthermore, the direction of flow or wettability was made switchable inside a microfluidic channel or on the surface of the hydrogel with a programmed operation.[47,82–84]

In general, swelling and shrinking of a stimuli-responsive hydrogel through absorption and expulsion of water has been used for volumetric control in microfluidics. As described earlier, the responsive hydrogel can be easily integrated into a microfluidic device, and guide the flow of a liquid into the desired direction. As shown in Fig. 4A, Beebe and co-workers

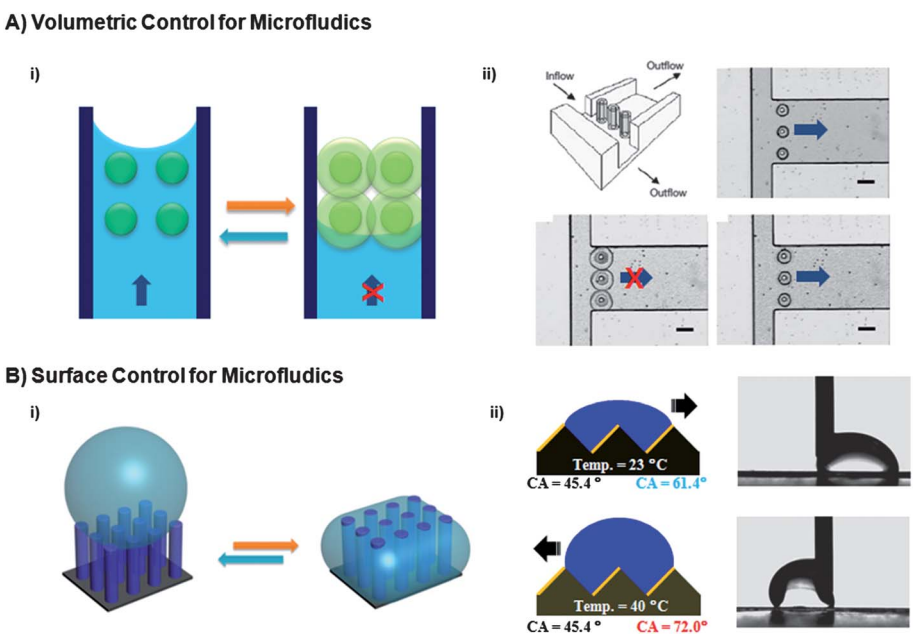

Fig. 4 Schemes representing microfluidic control by using stimuli-responsive hydrogel patterns. (A) (i) Schematic illustration of volumetric control for microfluidics and (ii) its representative example of microvalve in a microchannel. (B) (i) Schematic illustration of surface control for microfluidics and (ii) its representative example of control of the flow direction on a patterned surface. (A) Reprinted with permission from ref. 39. (B) Reprinted with permission from ref. 22.

have reported autonomous flow control in a microfluidic channel using pH-responsive hydrogel structures.[29,39,76] The hydrogel structure directly formed in a microchannel *via* photolithography reversibly changes its volume in a certain pH range. The hydrogel structure in the swelling state can block the path of fluid ("off state"), and reversibly the structure opens the path in the shrunken state ("on state"), resulting in the controlled passage of a specific pH solution in the designated region. For broader applications, two kinds of hydrogels showing opposite swelling and deswelling behaviors were used. A polyacid hydrogel based on acrylic acid is swollen at high pH (>6.8) and deswollen at low pH (<5.7), while a polybase hydrogel synthesized from 2-(dimethylamino) ethyl methacrylate is actuated oppositely. In parallel, being inspired from blood vessels, an asymmetric valve was demonstrated for on–off states actuated by backward and forward pressures with a thin hydrogel strip.[76] In addition to microvalves, the swelling and deswelling of the hydrogel has been applied to micropumps and micromixers with various stimuli such as chemicals, heat, and light.[76–80] Furthermore, the electro-responsive rotation of the hydrogel structure in a microfluidic channel was also reported as the operating mechanism for microvalve[53] and micropumps.[81]

Apart from the volumetric actuation, the change of wettability has been used as a versatile mechanism for flow manipulation in microfluidics. The degree of interactions between water and the surface governs the fluidic behaviors due to its high surface-to-volume ratio in the microfluidic channel. In particular, hydrophilic surfaces can attract the flow in a microfluidic channel through capillary forces, while hydrophobic surfaces resist against the flow through hydrophobic interactions with water. Therefore, the stimuli-responsive hydrogels that can alter their wettability in response to an external stimulus provide a facile way of flow control in the microchannel. Recently, by utilizing the water permeable property of hydrogels, microfluidic channels embedded with responsive hydrogels have been reported.[47,82,83] In these approaches, the hydrogels changed their wettability with controllable penetration of flow *via* various stimuli such as heat and light. In addition, a hydrogel network has also been used as a nanofluidic channel bridging microchannels in order to control and amplify the ion concentration.[84]

In a separate approach from closed microchannels, the change of wettability allows for manipulation of the mechanism of flow control in a surface-based microfluidic system (*i.e.* open channel microfluidics).[85–87] Such an open-channel system usually consists of a selective hydrophilic region on a hydrophobic substrate, thereby restricting the liquid flow to a narrow stream. Also, a natural force such as surface tension is usually employed as a driving potential to actuate a liquid sample. In a surface-based microfluidic system, surface roughness or topography generated by micro- and nanofabrication is a useful tool for controlling the wettability since the increase of roughness amplifies the inherent wetting property of materials. Namely, the hydrophilic (or hydrophobic) materials become more hydrophilic (or hydrophobic) when a surface roughness exists according to the Wenzel model.[88,89] Furthermore, in the case of a surface having anisotropic topography (*i.e.* a surface with a different degree of roughness according to the direction), the wetting behavior also becomes anisotropic and the resulting anisotropic wetting would be useful for control of the flow direction.[90] This phenomenon can have a synergic effect with responsive materials which can reversibly alter their surface wettability in response to an external stimulus for flow manipulation. Therefore, there has been much research regarding engineered surface topography coated with a responsive material. Of these, the polymeric materials with LCST behavior have been applied to thermoresponsive transition of the contact angle in the presence of anisotropic micro- or nanostructures.[89,91–93] Various stimuli-responsive materials including pH-, chemical-, and photo-responsive polymeric patterns have been reported for wettability control.[91,93,94] The above-mentioned surfaces, however, may have difficulties in robust integration to the microfluidic channel due to their bottom-up based fabrication and coating processes.

While recognizing the above problems, responsive hydrogel patterns would yield a facile solution for manipulation of switchable flow on a micropatterned hydrogel surface.[20–22] Recently, reversible switching of the liquid flow direction has been demonstrated by combining the surface wettability control and patterning as shown in Fig. 4B.[22] Here, a switchable and unidirectional liquid flow was achieved with physical symmetry and chemical asymmetry of micro-prism arrays consisting of thermo-responsive PNIPAAm hydrogels, one face of which was covered with metal films *via* oblique metal deposition (*i.e.*, two-face prism arrays). The alternating change of surface wettability turned out to be efficient in inducing the reversal of the flow direction with temperature variation. These results suggest that the switching of a flow direction is even possible in an open-channel system without resorting to an integrated electrode array as found in electrowetting.[95,96]

4.2 Manipulation of biosamples with microarrays

Microarrays, two-dimensional ordered patterns of biosamples on a solid matrix (typically a microscope slide), are important platforms for assaying various biological behaviors in a high-throughput manner. The development of microarray technologies has led to convenient and rapid experimental settings in diverse bioanalytical fields including biosensors, diagnosis, and biochemical reactions.[10,11,97] Conventionally, hydrogels have been attractively used in microarrays due to several reasons: (i) three-dimensional structures of hydrogels contain large quantities of biosamples, (ii) the aqueous environment of hydrogels prevents the damage or denaturing of biosamples, and (iii) the hydrophilicity of hydrogels is suitable to suppress the non-specific binding during sample processing.[11,98–100] It is noted in this regard that polyethylene glycol (PEG) hydrogels have been frequently used to minimize non-specific binding of biomolecules such as proteins and cells.[11,100]

With these advantages, novel microarray systems combining ordered patterns of a responsive hydrogel have recently been reported for controlled and automated assays without involving the need for skillful and sophisticated procedures.[31,40,100–106] In particular, such a smart microarray has great potential for

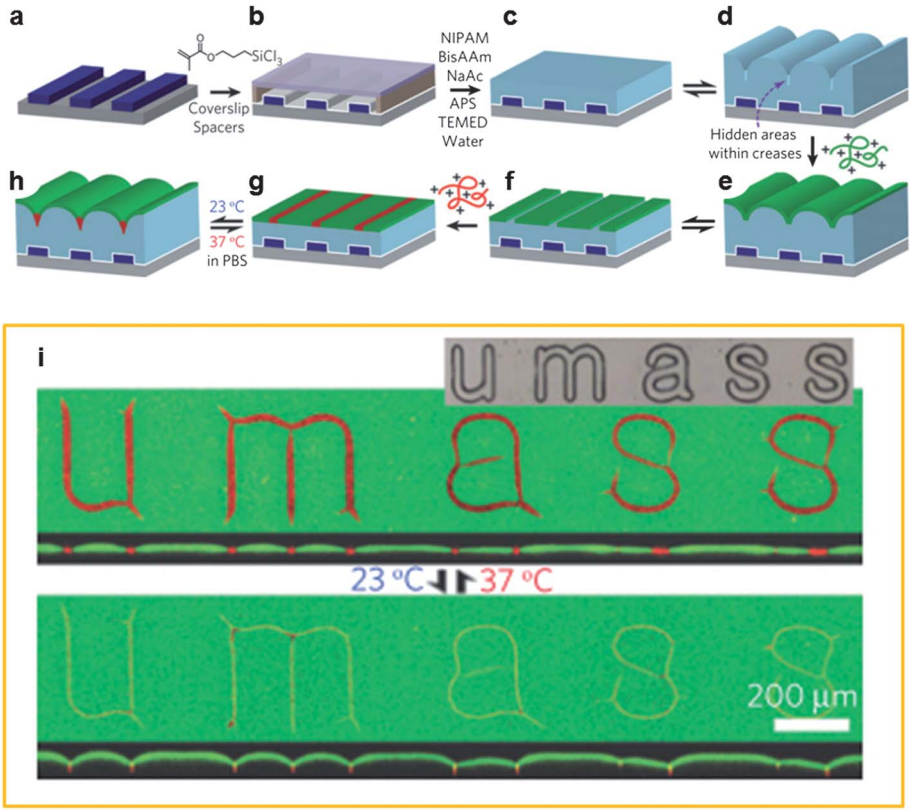

Fig. 5 Example of smart microarrays for dynamic patterning of biosamples utilizing the actuation of thermo-responsive hydrogels. (a–h) Fabrication scheme of microarrays and creasing actuation with temperature change, in which an example of dynamic patterning of the red fluorescent electrolyte is shown in (i). Reprinted with permission from ref. 101.

manipulation of biosamples at desired time, which includes transport, reaction, and other modifications of biosamples. To this end, the actuation of stimuli-responsive hydrogels by shrinkage or bending has provided a versatile route to complex spatial control of biosamples, which would be difficult in other types of microarray systems.

As shown in Fig. 5, Hayward and co-workers have demonstrated a thermoresponsive polymeric array utilizing dynamic creases which can block or unblock access to functional moieties immobilized on the actuating crease.[101] For the fabrication, a thin thermoresponsive PNIPAAM hydrogel was introduced by capillary action on the topographically pre-patterned rigid substrate, and then the swelling of the hydrogel resulted in the formation of creases by elastic creasing instability. The creases can be reversibly folded or unfolded by thermoresponsive swelling or deswelling of the hydrogel. A polyelectrolyte, poly(L-lysine)-*g*-poly(ethylene glycol) (PLL-*g*-PEG) terminated with functional moieties (red fluorescence in Fig. 5), was selectively back-filled in the region of creases at the unfolding state, after the exposed areas had been coated by untreated PLL-*g*-PEG (green fluorescence in Fig. 5) by masking at the folding state. As a result, the functional moieties coated on the creases were repeatedly exposed or hidden according to unfolding or folding of the creases induced by a temperature change. By utilizing this technique, controlled binding and release of streptavidin-coated beads to biotin-coated creases, and cell encapsulation in RGD peptide-coated creases were demonstrated. The idea was further expanded to light-sensitive dynamic creases, by combining the thermoresponsive hydrogel and photothermal nanoparticles.[32] This light-sensitive pattern enabled individual switching of a crease by spatially localized light, offering better control capabilities in comparison to the thermoresponsive pattern.

In addition to on/off switching of the biosample reaction *via* crease patterns, a striking demonstration of the regulating enzymatic reaction or sample transport has recently been reported from Aizenberg and co-workers by exploiting actuation of pillar arrays.[40,103–105] In this approach, stimuli-responsive micropillar arrays called "hydrogel-actuated integrated responsive systems (HAIRS)" were used. Non-responsive micropillar arrays were embedded in a responsive hydrogel, demonstrating bending actuations from swelling and contraction of the hydrogel in response to the change of humidity,[102,103] temperature,[104] or pH.[103–105] By designing micropillars with an anisotropic geometry, the actuation was generated in collective motion to one direction.[105] More recently, the system was applied to a smart microarray called "self-regulated mechanochemical adaptively reconfigurable tunable systems (SMARTS)" as shown in Fig. 6. In this work, the micropillar array was integrated with a microfluidic channel and offered fast mechanical actuation of biosamples immobilized on top of pillars according to pH or thermal signals.[40,104] In particular, it was demonstrated that the enzymatic reaction was regulated *via*

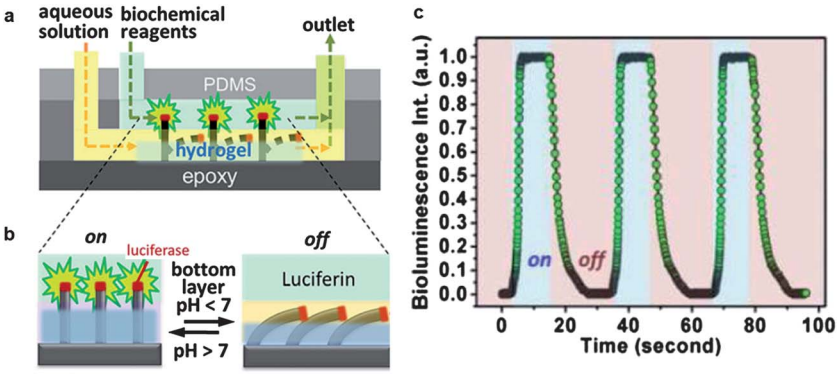

Fig. 6 Example of smart microarrays for regulating the enzymatic reaction, which uses bending of micropillars located in the hydrogel. (a and b) Fabrication methods and the operating mechanism of the actuating pillar arrays integrated inside a microchannel. (c) An example of regulation of the enzymatic reaction in which the bioluminescence reaction was used for the arrays as a model enzymatic reaction and oscillating emission of bioluminescence was observed. Reprinted with permission from ref. 40.

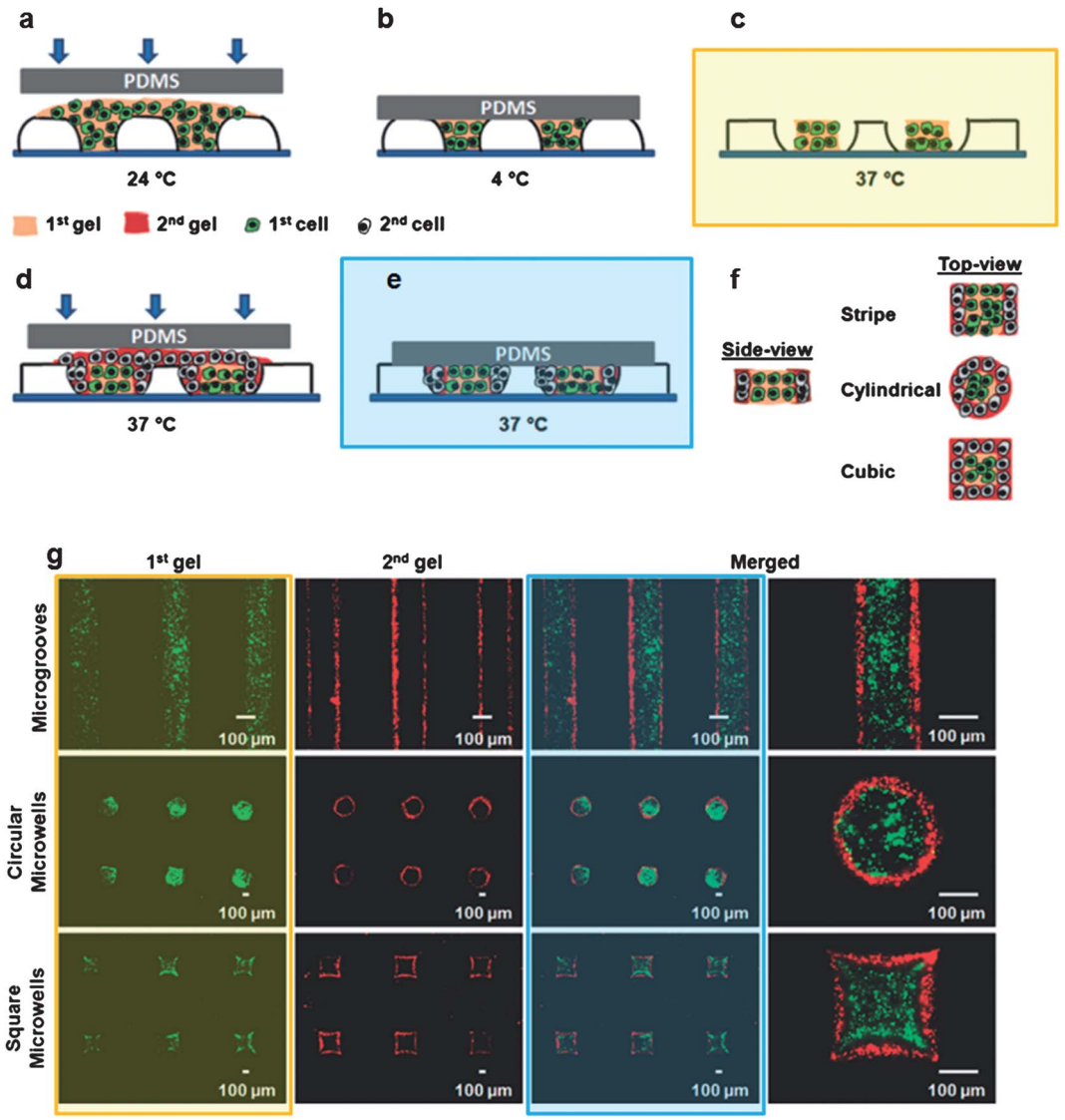

Fig. 7 Example of smart cellular microarrays for serial patterning of hydrogel microstructures embedded with different cell types, utilizing shrinking of the thermoresponsive hydrogel. (a–f) Microarray protocol for the sequential patterning and spatial organization of two cell types. (g) Fluorescent images of sequentially molded microgels within responsive microgrooves, circular microwells, and square microwells. The cells with green and red fluorescence were sequentially encapsulated within the first and second gels, respectively. Reprinted with permission from ref. 114.

reversible switching of micropillar arrays in two streams of laminar flow.[40] These actuations were integrated to more sophisticated responsive systems such as feedback control of chemical reactions, without any complex electronic system.[104] In addition to on/off actuation of micropillars, self-oscillation of micropillars was applied to the spatial control of biosamples.[68,106] In addition, Kokufuta and co-workers have demonstrated pillar arrays composed of the poly(NIPAAM-*co*-$Ru(bpy)_3^{2+}$) hydrogel for generating dynamic rhythmic motion through the Belousov–Zhabotinsky reaction.[68] In the system, oscillating states of oxidized Ru(III) and reduced Ru(II) within the substrate solution (malonic acid, acid, and oxidant) induced swelling and deswelling of the gel repeatedly, which may be useful for the self-beating microactuator for biosample transport with the propagation of wave motion.

Stimuli-responsive hydrogel microarrays are also useful for encapsulating various bioanalytes such as tissues, cells, and lipid bilayers without damage or denaturing over a period of time. Especially, the bioanalytes can reside in a more *in vivo* like environment on or inside hydrogels, which is important for the analysis of biological events in cell-based assays and tissue engineering.[107–112] To address these needs, various microarrays have been reported to preserve and manipulate various biosamples.[113–116] Khademhosseini and co-workers have reported three-dimensional cell culture methods using thermoresponsive PNIPAAM hydrogel microwells or molds to construct tissue-like structures.[113–115] By utilizing the volumetric shrinkage of thermoresponsive structures, they achieved forming and retrieving cell aggregates in the microwells,[113] as well as patterning cell-laden hydrogels by direct micromolding.[114,115] As shown in Fig. 7, their methods were expanded to generate spatial distribution of multiple cells in hydrogels with various shapes towards the analysis on cell–cell interactions in the tissue-mimetic cellular assemblies.[114,115]

5 Summary and outlook

Stimuli-responsive hydrogel patterns have proven to be useful as an efficient platform in smart microchannels and microarrays and they are used as automatically active components for the manipulation of flow control or bioanalytes. When suitably incorporated, stimuli-responsive polymeric patterns can control the direction of flow in a microfluidic system in two ways: volumetric control in a closed channel *via* shrinkage or expansion and surface control in an open channel *via* a wettability change. Also, the hydrogel pattern can be utilized to manipulate various sample characteristics such as transport, reaction, and encapsulation.

Despite recent advances in the preparation and implementation of hydrogel patterns, several issues need to be addressed. First, the thermoresponsive hydrogels such as PNIPAAM are most widely used but their response time is relatively slow on the order of a few seconds for structures of ~10 μm scale as compared to the rapid transition of the electrically triggered mechanism. In this sense, the incorporation of CNTs having high thermal conductivity into the hydrogel reduced the response time (~5 times enhancement) as demonstrated by Javey and co-workers,[34] which could be one of the potential solutions.

Second, it is important to understand the properties, limitations, and applications of various hydrogels in generating micro- and nanostructures.[59,117–119] For example, it is easier to form microstructures (>10 μm) made of PNIPAAM and PEG as their intermediate mechanical properties (Young's modulus < 10 MPa) allow for structural stability at the microscale. For fine patterning below ~1 μm, however, the structures are not stable upon swelling and start to collapse *via* various routes such as mating or clumping. As one of the solutions to strengthen the mechanical stability of soft polymeric materials, Yoon *et al.* demonstrated a simple concept of depositing a thin metal layer in the lower stem region of the polymeric nanopillars, so that the patterns were stable for a long period of time without structural collapses.[120] It is noted in this regard that very soft hydrogels such as gelatin and polyacrylamide, which have been extensively used for tissue scaffolds, are not appropriate to generate micro- or nanopatterns due to their low mechanical rigidity (Young's modulus < 100 kPa).

Third, it is potentially of great benefit to design new hydrogel materials with multiple functionalities. As demonstrated by Hayward *et al.*, the synthesis of multiple materials is useful to respond to multiple stimuli, allowing for a more smart flow control or sample manipulation.[32,101] Combining a modular assembly with inherent responsive properties would also be useful to exploit hydrogel patterns, as the shape itself yields additional control capability in a lego-like assembly of hydrogel building blocks. A similar idea is well demonstrated in a recent work from Khademhosseini and co-workers in a tissue-mimetic assembly of cell-laden hydrogels.[121]

Finally, future studies are needed to develop a robust operation scheme of hydrogel patterns in a more stable, reproducible, and noise-tolerant manner. A simple method of amplifying the input or output signal *via* a novel transduction mechanism would be always welcome as it is directly related to the performance of bioanalytical systems. Integration to bioanalytical systems without losing responsive properties should also be actively pursued for reliable practical applications. It is envisioned that smart stimuli-responsive hydrogel patterns would bring about many innovations in future diagnostic and therapeutic fields such as bioanalytical systems, tissue engineering, and drug delivery.

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