Implementation of tetra-poly(ethylene glycol) hydrogel with high mechanical strength into microfluidic device technology

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Hydrogels have several excellent characteristics suitable for biomedical use such as softness, biological inertness and solute permeability. Hence, integrating hydrogels into microfluidic devices is a promising approach for providing additional functions such as biocompatibility and porosity, to microfluidic devices. However, the poor mechanical strength of hydrogels has severely limited device design and fabrication. A tetra-poly(ethylene glycol) (tetra-PEG) hydrogel synthesized recently has high mechanical strength and is expected to overcome such a limitation. In this research, we have comprehensively studied the implementation of tetra-PEG gel into microfluidic device technology. First, the fabrication of tetra-PEG gel/PDMS hybrid microchannels was established by developing a simple and robust bonding technique. Second, some fundamental features of tetra-PEG gel/PDMS hybrid microchannels, particularly fluid flow and mass transfer, were studied. Finally, to demonstrate the unique application of tetra-PEG-gel-integrated microfluidic devices, the generation of patterned chemical modulation with the maximum concentration gradient: 10% per 20 μm in a hydrogel was performed. The techniques developed in this study are expected to provide fundamental and beneficial methods of developing various microfluidic devices for life science and biomedical applications. © 2013 AIP Publishing LLC. [<http://dx.doi.org/10.1063/1.4822033>]

I. INTRODUCTION

Polymeric materials are used to form the structural body of microfluidic devices. Representative examples of polymeric materials include engineering plastics, such as poly(methyl methacrylate) (PMMA),¹ polystyrene (PS)² and polycarbonate (PC),³ and elastomers such as poly(dimethylsiloxane) (PDMS).⁴ The principal differences between plastics and elastomers are their mechanical properties. Namely, elastomers are soft and deformable at room temperature unlike plastics, and their softness is sometimes used to enable certain dynamic functions of actuator valves and pumps,^{5–7} for example. Generally, not only mechanical properties but also various specific properties of materials lead to various device functions. Among other polymer materials, hydrogel polymers have several excellent characteristics for biomedical applications, such as softness, biological inertness and solute permeability and are widely used in biomolecular analysis,⁸ medical device development⁹ and tissue engineering.¹⁰ Hence, integrating hydrogels into microfluidic devices is a promising approach for extending the function of microfluidic devices.^{11,12} Several groups have recently reported the application of microfluidic devices using hydrogels to molecular analysis,¹³ drug screening,¹⁴ and tissue engineering.^{15–19}

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However, there are serious challenges in the integration of hydrogels into microfluidic devices, namely the limitation in device design and fabrication arising from the following reasons. First, hydrogels have insufficient mechanical strength when formed into small size or thin-film shape. This leads to inconvenience in handling hydrogel polymers as part of microfluidic devices. Second, there are no simple and robust bonding methods that can be applied to microstructure assembly. Although bonding is essential for enclosing microfluidic channels to fabricate microfluidic devices, bonding hydrogels onto a solid microstructure is difficult because of their high water content (>90 wt. %) and low surface sliding friction coefficient ($<10^{-3}$).²⁰

Fortunately, recent progress in macromolecular chemistry has solved the problem of the low mechanical strength of hydrogels. Several research groups have reported mechanically superior hydrogels such as slide-ring (SR) gels, nanocomposite (NC) hydrogels, double network (DN) gels, and tetra-poly(ethylene glycol) (tetra-PEG) gels synthesized using unique materials designed to reduce local stress concentration in hydrogels under deformation.^{21–26} The tetra-PEG gel is composed of two symmetrical tetrahedron-like macromers of the same size.²⁷ It has a highly homogeneous network structure, resulting in its high mechanical strength, and has desirable characteristics as a structural material of microfluidic devices. Its maximum compression breaking strength is reported to be over 27 MPa at $M_w = 20 \text{ kg mol}^{-1}$ and a macromonomer concentration of 160 mg ml^{-1} .²⁸ Moreover, it is a chemical gel that generates chemically cross-linked networks, which can overcome bonding-related issues.

With the above background, here, we studied the implementation of a high-mechanical-strength hydrogel, tetra-PEG gel, into microfluidic device technology. In order to be able to bond the tetra-PEG hydrogel to microstructure assembly, a novel hydrogel reactive microbonding (HRMB) method was developed. Then, fundamental characterization of prototype devices was conducted mainly in terms of fluid flow and mass transfer behaviors. Furthermore, the generation of patterned chemical modulation in the hydrogel was demonstrated as one of the possible applications of tetra-PEG-gel-integrated microfluidic devices.

II. MATERIALS AND METHODS

A. Hydrogel reactive microbonding (HRMB) method

Figures 1(a) and 1(b) show the fabrication sequence of a tetra-PEG gel/PDMS hybrid microchannel using the HRMB method. PDMS sheets with microfluidic channels were fabricated by typical soft lithography. A master mold was fabricated by patterning double layers of a dry film photoresist (TMMF 2045, thickness: $45 \mu\text{m}$, Tokyo Ohka Kogyo Co., Ltd., Tokyo, Japan) laminated on a flat glass plate. A PDMS prepolymer (Sylgard 184, Dow Corning Co., MI, USA) was poured onto the mold and cured at 85°C for 2 h. The cured PDMS was then peeled off the mold. The subsequent surface modification of PDMS involved exposure to vacuum UV at 172 nm (Xe-excimer light sources, 172/120 Z(HV), Heraeus Noblelight GmbH, Germany) in a controlled O_2 atmosphere at 100 Pa for 30 s, followed by immersion in a solution containing 5% (3-aminopropyl)triethoxysilane (APTES) (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), 5% water and 90% ethanol (Kanto Chemical Co., Inc., Tokyo, Japan) for 30 min. Thereafter, PDMS was thoroughly rinsed with ethanol and water, dried under a stream of dry nitrogen, and finally heated at 60°C for at least 2 h.²⁹

Tetra-PEG gel sheets of $30 \times 30 \text{ mm}$ area and $200 \mu\text{m}$ thickness were formed in the gap between two parallel flat glass plates coated with a perfluoropolymer (CytopTM, Asahi Glass Co., Ltd., Tokyo, Japan) to avoid sticking of the hydrogel onto the glass plates. Chemical gelation was initiated by simply mixing two macromonomer solutions of tetraamine-terminated PEG (TAPEG) and tetra-NHS-glutarate-terminated PEG (TNPEG) (NOF Corp., Tokyo, Japan). It is reported that spatially uniform network structures are formed by the formation of amide bonds between the amino groups of TAPEG and the succinimidyl ester groups of TNPEG as shown in Figure 1(a). TAPEG and TNPEG were dissolved in phosphate buffer (PB, pH 7.4) and phosphate-citric acid buffer (CPB, pH 5.8), respectively. A 1:1 mixture of 100 mg ml^{-1} TAPEG ($M_w = 10 \text{ kg mol}^{-1}$) and 100 mg ml^{-1} TNPEG ($M_w = 10 \text{ kg mol}^{-1}$) was moderately

FIG. 1. HRMB method. (a) A tetra-PEG gel is formed by the formation of amide bonds between the amino groups of TAPEG and the succinimidyl ester groups of TNPEG. (b) A tetra-PEG gel sheet was formed through an incomplete cross-linking reaction, and a thin layer of a TAPEG and TNPEG mixture was spin-coated as the bonding adhesive layer. (APTES)-anchored PDMS with microchannels was deposited on the layer. Amide bonds formed between the amino groups of PDMS and the succinimidyl ester groups of TNPEG in the bonding adhesive layer. TAPEG and TNPEG in the bonding adhesive layer also reacted with residual amino groups and the succinimidyl ester groups to form the amide bonds in the tetra-PEG gel sheet.

cross-linked for 10 min at room temperature. After a predetermined gelation time, the resulting tetra-PEG sheet was detached from the supporting glass plates.

Bonding between the tetra-PEG gel sheet and the PDMS sheet was performed as follows. A TAPEG and TNPEG mixture was spin-coated at 4000 rpm for 20 s onto the tetra-PEG gel sheet as a sort of adhesive layer within 30 s after mixing of TAPEG and TNPEG. The adhesive layer is speculated to be a few micro meters in thickness. Then, the spin-coated tetra-PEG gel surface was placed over the patterned PDMS surface. The reactive groups of freshly coated TAPEG and TNPEG adhesive layer could readily react with the amino groups modified on the APTES-modified PDMS surface and with the residual active groups in the tetra-PEG gel sheet. Here, the amount of residual active groups could be controlled by adjusting the gelation time of the tetra-PEG gel sheet and was fixed at 10 min in this study.

To examine bonding quality, the tetra-PEG gel/PDMS bonding interface at the microchannel structure was observed using an environmental scanning electron microscopy (ESEM) system equipped with a thermoelectronic cooling stage (Philips/FEI XL30 ESEM-FEG, OR., USA). ESEM observation was conducted while the tetra-PEG gel was being hydrated at a saturation pressure of water vapor (653 Pa, 1 °C).

To evaluate the mechanical properties of tetra-PEG gel membranes as well as the strength of bonding between the tetra-PEG gel and PDMS sheets, pressure bulge test was conducted using the experimental setup schematically shown in Figure 2. The test structure comprised three layers with a 30 × 30 mm area: a 100-μm-thick tetra-PEG gel membrane, a 5-mm-thick PDMS sheet with a 2.7-mm-diameter orifice, and a 0.5-mm-thick slide glass. The tetra-PEG gel layer was bonded to the PDMS sheet by HRMB, while the PDMS sheet was tightly attached to the slide glass by standard oxygen plasma treatment.³⁰ A uniform pressure was applied to one

FIG. 2. Schematic of experimental setup for pressure bulge test. Pressure is applied to a $100\text{-}\mu\text{m}$ -thick tetra-PEG gel membrane. The sample geometry is shown, where P is the applied pressure, d is the deflection of the tetra-PEG gel membrane, t is the thickness of the membrane, and a is the radius of the membrane.

side of the circular tetra-PEG gel membrane over the orifice using a syringe pump (BAS Bee pump, Bioanalytical Systems, Inc., IN, USA), and the deflection of the membrane was measured as a function of applied pressure, which was measured by a pressure sensor (DT-8890 A, CEM Corp., Matthews, NC, USA).

B. Characterization of fluid flow of tetra-PEG gel/PDMS hybrid microchannel

A prototype device, shown in Figure 3, was used for characterizing the fluid flow in the tetra-PEG gel/PDMS hybrid microfluidic device. It comprises two PDMS sheets, each fabricated with a straight microchannel ($h = 90\text{ }\mu\text{m}$, $w = 100\text{ }\mu\text{m}$, $L = 15\text{ mm}$) and a flatbed microreactor ($5 \times 5\text{ mm}$ area and 0.15 mm depth), and a tetra-PEG separator membrane of $100\text{ }\mu\text{m}$ in thickness sandwiched between them. Both the microchannel and the microchamber were filled with phosphate-buffered saline (PBS) (Gibco, Invitrogen Corporation, NY, USA) with the addition of a trace amount of 10 nM fluorescein (Kanto Chemical Co., Inc., Tokyo, Japan). One end of the microchannel was connected to a syringe pump (BAS Bee pump, Bioanalytical Systems, Inc., IN, USA), and the PBS was supplied at a flow rate of $10\text{ }\mu\text{l min}^{-1}$. The flow velocity distributions in both the microchannel and the microchamber were measured by fluorescence

FIG. 3. Schematic illustration of device for characterizing fluid flow in tetra-PEG gel/PDMS hybrid microfluidic device. The microchannel and microchamber are separated by a tetra-PEG gel membrane.

correlation spectroscopy (FCS). The reason for using FCS is that FCS provides a high resolution of flow velocity in the z -axis and enables measurement in hydrogels where tracking beads cannot penetrate, compared with particle image velocimetry (PIV) or particle tracking velocimetry (PTV). A confocal microscope with an FCS measurement unit (Zeiss LSM-510 META Confocal Microscope, ConfoCor, Carl Zeiss Inc., Jena, Germany) and a water immersion lens (C-Apochromat NA1.2 40 $\times$, Carl Zeiss, Inc., Jena, Germany) were used for FCS measurement. Fluorescein was excited at 488 nm, and emission was detected at 530–610 nm.

The test device for characterizing solute diffusion in the tetra-PEG gel/PDMS hybrid microfluidic device is shown in Figure 4. It comprises a PDMS sheet fabricated with a straight microchannel ($h = 90\ \mu\text{m}$, $w = 100\ \mu\text{m}$, $L = 15\ \text{mm}$) and a tetra-PEG gel sheet of 1 mm in thickness. A transient observation of diffusion phenomena in the device, as well as numerical simulation, was experimentally performed. In the transient diffusion experiment, $5\ \mu\text{M}$ fluorescein in PBS was introduced into the microchannel using a syringe pump at a flow rate of $20\ \mu\text{l min}^{-1}$. Fluorescence images were acquired every 2 min for 30 min by two-photon laser scanning microscopy (TPLSM) (Zeiss LSM 510 META, Carl Zeiss, Inc., Jena, Germany, and Mai Tai Ti:sapphire lasers, Spectra Physics Inc., CA, USA) with an EC Plan-Neo fluar 10 $\times$ lens (Carl Zeiss, Inc., Jena, Germany). Fluorescein was excited at 800 nm, and emission was detected at 500–610 nm. Image analyses including fluorescence intensity quantification were carried out using ImageJ (National Institutes of Health, MD, USA). It was experimentally confirmed that the attenuation of fluorescence intensity with increasing depth was negligible.

A numerical simulation of fluid flow and solute diffusion in the device was carried out using a commercial software package (CFD-ACE+, ESI Group, Paris, France). A porous medium model in which any solute can move freely within and between pores of its porous material was used to analyze diffusion in hydrogels. The diffusion coefficient of fluorescein in PBS ($D_{\text{fluor, w}} = 2.7 \times 10^{-10}\ \text{m}^2\ \text{s}^{-1}$)³¹ and the experimentally obtained effective diffusion coefficient of fluorescein in $100\ \text{mgml}^{-1}$ tetra-PEG gel ($D_{\text{fluor, g}} = 0.8 \times 10^{-10}\ \text{m}^2\ \text{s}^{-1}$) were used in the simulation.

C. Generation of patterned chemical modulation in hydrogels

To demonstrate the possible application of tetra-PEG-gel-integrated microfluidic devices, we fabricated a tetra-PEG gel/PDMS hybrid microfluidic device with interdigitated microchannel patterns, as shown Figure 5. Two parallel and alternately folded microchannels had a width and a height of $50\ \mu\text{m}$ and $45\ \mu\text{m}$, respectively, and a space between microchannels of $50\ \mu\text{m}$. Two fluorescent solutions of $5\ \mu\text{M}$ fluorescein and $5\ \mu\text{M}$ sulforhodamine B (Kanto Chemical

FIG. 4. Schematic illustration of device for characterizing solute diffusion in tetra-PEG gel/PDMS hybrid microfluidic device. Solute in the microchannel transfers into the tetra-PEG gel by diffusion.

FIG. 5. Illustration of design of interdigitated microchannels of tetra-PEG-gel-integrated microfluidic device for generation of patterned chemical modulation in hydrogels. Interdigitated microchannels have a $50\text{ }\mu\text{m}$ line-and-space pattern.

Co., Inc., Tokyo, Japan) were introduced into different microchannels at a flow rate of $10\text{ }\mu\text{l min}^{-1}$ using syringe pumps. The fluorescent molecules diffused into the hydrogel were observed by TPLSM using an EC Plan-Neo fluar $10\times$ lens. Fluorescein and sulforhodamine B were excited at 800 nm , and emission was detected at $500\text{--}550\text{ nm}$ and $565\text{--}615\text{ nm}$, respectively.

III. RESULTS AND DISCUSSION

A. Fabricated structures of tetra-PEG gel/PDMS hybrid microchannel

In bonding processes for enclosing microfluidic channels, there are potential problems of structural failures such as microchannel clogging and dead volume.³² To check the quality of our bonding method, the bonding interface between the structured PDMS surface and the tetra-PEG gel was observed by optical microscopy and by ESEM. As seen in Figures 6(a) and 6(b), no overflows of spin-coated tetra-PEG into microchannel structures were observed, and hence it was confirmed that tetra-PEG gel/PDMS hybrid microchannels of a $50\text{ }\mu\text{m}$ line-and-space pattern can be formed successfully using the HRMB method with high reproducibility. It is noteworthy that with this method, microchannels with sizes down to $10\text{ }\mu\text{m}$ in height can be fabricated without any clogging of the microchannel structures with the adhesive layer.

Next, the bulge test was carried out to examine the properties of thin tetra-PEG sheets. The deflection of the tetra-PEG sheet under the action of a uniform pressure applied to one side was

FIG. 6. Structures of tetra-PEG gel/hybrid microchannels. (a) Photograph of tetra-PEG gel/PDMS hybrid microchannels. The channel width (w) and height (h) were $50\text{ }\mu\text{m}$ and $45\text{ }\mu\text{m}$, respectively, and the width of the adhesive area of the hydrogel and PDMS was $50\text{ }\mu\text{m}$. (b) ESEM image of tetra-PEG gel hybrid microchannel. The tetra-PEG gel was hydrated at the saturation pressure of water vapor (653 Pa , $1\text{ }^{\circ}\text{C}$).

FIG. 7. High deformability of tetra-PEG gel membrane. (a) Side-view photographs of deformed tetra-PEG gel membrane ($t = 100 \mu\text{m}$) at 0, 4, and 7 kPa. (b) Pressure-deflection curve. Vertical error bars resulted from the resolution limit of the CCD camera used for imaging deflected membrane sample. Measurement error in pressure was ± 0.02 kPa.

measured as a function of applied pressure, as shown in Figure 7(a) from which the stress–strain relationship can be evaluated. As shown in Figure 7(b), in the pressure range of 0–5.0 kPa, the deflection of tetra-PEG gel gradually increased, showing an elastic deformation behavior (strain: $\varepsilon = 0$ –0.2, stress: $\sigma = 0$ –43 kPa). Here, the Young’ modulus of tetra-PEG was evaluated to be 113 kPa, which is 10 times higher than that of a polyacrylamide gel at the same concentration.³³ When pressure was increased to approximately 5.0 kPa, the deflection showed a sudden rise due to the plastic deformation and the bulge finally ruptured at $P = 7.0$ kPa. At this rupture point, a relatively large values of breaking strain $\varepsilon = 0.6$ and large breaking stress $\sigma = 93$ kPa were recorded. It is estimated that a peel force per unit length of 5.1 N/m was applied to the interface of tetra-PEG gel and PDMS when the rupture in the tetra-PEG gel sheet occurred; however, the bonded interface was not broken. Although another peel-off test for the evaluation of the force of adhesion between tetra-PEG and PDMS was attempted, difficult was encountered because of the breaking in the bulk gel during the test. At least, it was found to be larger than the 22 N/m peel force per unit (data not shown).

Although some research groups have studied hydrogel bonding on a solid surface, to the best of the authors’ knowledge, there are as yet no reports on bonding methods applicable to microstructured surfaces. Gong *et al.* reported hydrogel bonding on a porous glass surface using the physical anchoring effect. They used DN gels with a high mechanical strength, which were formed from a tightly cross-linked rigid polyelectrolyte as the first network and a sparsely cross-linked flexible neutral polymer as the second network. A strong hydrogel-porous solid interface was achieved by the effect of anchoring DN gels in micropores.³⁴ However, from the viewpoint of microfabrication, this physical bonding method is not feasible owing to the risk of the undesirable occupancy of microstructures by hydrogel. The other approach using chemical bonds was also reported in the literature and involves the use of an active wound dressing (AWD) device comprising a bilayer of porous poly(hydroxyethyl methacrylate) (pHEMA) and PDMS, as reported by Cabodi *et al.* They spray-coated a solution of a photoinitiator and a cross-linker as a photocurable glue over the PDMS surface to form a covalent seal between two sheets.³⁵ However, the use of such liquid glues is often beset by the problem of filling microstructures. In comparison to the above methods, the HRMB method is a combination of two approaches. Namely, the interface with a tetra-PEG sheet is linked by both chemical bonds and molecular anchoring of spin-coated macromonomers, while the interface between the APTES-modified PDMS surface and the adhesive layer is linked only by chemical bonds. Therefore, no severe problems of clogging were observed on the PDMS surface with microstructures.

B. Characterization of fluid flow of tetra-PEG gel/PDMS hybrid microchannel

One of the greatest advantages in the implementation of polymer gels into microfluidic device lies in their semipermeable feature, namely, fluid flow can be isolated among different microchannels or microchambers while allowing mass transfer through the diffusion of solute

FIG. 8. Fluid flow distribution in tetra-PEG gel/PDMS hybrid microfluidic device. Distributions of flow velocities along z -axis in tetra-PEG gel/PDMS hybrid microchannel and microchamber. The flow velocities in the microchannel and microchamber were measured by FCS.

molecules. To check the capability of fluid flow isolation using a tetra-PEG membrane separator in microfluidic devices, a qualitative measurement of flow velocity inside the prototype device shown in Figure 3 was carried out. The distribution of the flow velocity along the z -axis in the microchannel with a hydrostatic flow and in the neighboring microchamber separated by the tetra-PEG membrane is shown in Figure 8. As expected, a parabolic velocity profile was measured in the microchannel, while no fluid flow was detected in the adjacent microchamber. Thus, no leakage of fluid flow was observed. As a perfusion membrane, a porous membrane is often used in microfluidic devices, but flow sometimes leaks from a channel through its pores. It is considered that a tetra-PEG gel with very small pores of about 1–3 nm³⁶ will prevent such fluid flow leakage.

Subsequently, to evaluate mass transfer in the tetra-PEG gel/PDMS hybrid microfluidic device, the transient diffusion of fluorescein molecules in the device was investigated experimentally and numerically using the device. Figure 9(a) shows typical fluorescence images of a cross section of the microchannel after the introduction of 5 μ M fluorescein for 10 min. Figure 9(b) shows the concentration distribution of fluorescein calculated by numerical simulation under the corresponding conditions. Fluorescein concentrations estimated from the fluorescence intensity measured at various depths of the hydrogel below the channel (50, 100, and 200 μ m) are plotted

FIG. 9. Solute diffusion in tetra-PEG gel/PDMS hybrid microfluidic device. (a) Fluorescence images of cross-section of microchannel after delivery of 5 μ M fluorescein for 10 min. (b) Concentration distribution of fluorescein after delivery of 5 μ M fluorescein for 10 min calculated by simulation (SIM). (c) Experimentally measured fluorescence intensities ($C_{\text{Exp.}}$; symbols) and computationally calculated fluorescein concentrations (C_{SIM} ; solid lines) at various depths (50 μ m, 100 μ m, 200 μ m). Error bars show the standard deviations.

against time in Figure 9(c) using closed circles, where the concentration is normalized by $5.0\ \mu\text{M}$, the initial concentration of fluorescein in the microchannel. In addition, the fluorescein concentration numerically predicted is also plotted as a full line in the same figure. The fluorescein concentration in the gel showed a rapid increase immediately after the introduction of the fluorescein solution into the microchannel, and then showed a saturation tendency after 5 min. The normalized concentrations at 30 min after fluorescein introduction were approximately 0.8, 0.6, and 0.4 at the depth of 50, 100, and $200\ \mu\text{m}$, respectively. Very good agreement between the experimental and simulated results may well reflect the perfection of fabrication processes for tetra-PEG gel/PDMS microfluidic devices, including the HRMB method.

C. Generation of patterned chemical modulation in hydrogels

To demonstrate the possible application of tetra-PEG gel/PDMS hybrid microfluidic devices, a prototype device for generating chemical concentration patterns in hydrogels was fabricated as shown in Figure 5. Over the last decade, the use of microfluidic devices has enabled the generation of chemical concentration modulation on the scales of tens of micrometers and has been applied to chemical biology research.³⁷ Although laminar flow in microchannels can readily provide the condition with markedly different chemical concentration within the range of tens of micrometers, continuous fluid flow inevitably induces the mechanical stimulation of the cells. Therefore, the generation of chemical concentration modulation via the hydrogel is an attractive technique for overcoming such a problem.

By flowing $5\ \mu\text{M}$ fluorescein and $5\ \mu\text{M}$ sulforhodamine B, fine concentration modulation patterns, which correspond to microchannels, were observed in the hydrogel, as shown in Figure 10(a). Figure 10(b) shows the horizontal profile of fluorescence intensity at 500–550 nm, corresponding to the emission peak wavelength of fluorescein, and at 565–615 nm, corresponding to that of sulforhodamine B, which was measured at a depth of $25\ \mu\text{m}$ from the tetra-PEG gel/PDMS interface. Note that the signal at 565–615 nm includes a slight contribution of the long-wavelength tail of fluorescein fluorescence. From this figure, the fluorescein concentrations just beneath the source channel (the channel filled with fluorescein solution) and just beneath the sink channel (the channel without fluorescein solution) were estimated to be $4.9 \pm 0.4\ \mu\text{M}$ and $2.2 \pm 0.3\ \mu\text{M}$, respectively. Hence, the relative concentration ratio was 50% or more, and the concentration gradient observed between the hill and the valley was 10% per $20\ \mu\text{m}$. In principle, chemical concentration modulation can be controlled by designing microchannel patterns on the basis of the predicted mass transfer of solutes. This will be investigated in future studies. In addition, tetra-PEG gel shows no cytotoxicity.^{27,38} Thus, the development of the technique presented here will open new opportunities to study the response of cells to spatial

FIG. 10. Patterned chemical modulation in hydrogels. (a) Cross-sectional image of interdigitated tetra-PEG gel/PDMS hybrid microchannels. Green and red correspond to fluorescein and sulforhodamine B, respectively. Lateral image of PDMS layer including microchannels and lateral image of tetra-PEG gel immediately under microchannel. (b) Fluorescence intensity gradient pattern in tetra-PEG gel layer in lateral image shown in (a).

and temporal variations of chemical concentrations just by growing cells on top of the tetra-PEG gel integrated microfluidic device shown in Fig. 5 and allowing chemicals to pass through from the cells to the detector and vice versa.

IV. CONCLUSIONS

To overcome the technical difficulties in the implementation of hydrogels into microfluidic devices, we have proposed the introduction of a tetra-PEG gel synthesized recently, a polymeric hydrogel with a high mechanical strength, and have developed a simple and robust bonding method called hydrogel reactive microbonding (HRMB). By using the HRMB method, the fabrication of tetra-PEG gel/PDMS hybrid microchannels of a 50 μm line-and-space pattern was successfully demonstrated. Moreover, a comprehensive characterization of a tetra-PEG gel/PDMS hybrid microfluidic device was conducted with some prototype devices. The deformability of a thin tetra-PEG gel membrane of 100 μm thickness was confirmed by a bulge test. Flow velocity distribution and diffusive mass transfer were also numerically and experimentally analyzed.

Moreover, to demonstrate the unique application of tetra-PEG gel/PDMS hybrid microfluidic device, we generated a chemical modulation pattern with the maximum concentration gradient: 10% per 20 μm in a hydrogel. The use of high-mechanical-strength hydrogels explored in this study is expected to provide new designs and functions not limited by the poor strength of conventional hydrogels in microfluidic device technology, especially in tissue engineering and drug screening.

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