Thermomodulated cell culture/harvest in polydimethylsiloxane microchannels with poly(N-isopropylacrylamide)-grafted surface

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Cell culture and harvest are the most upstream operation for a completely integrated cell assay chip. In our previous work, thermoresponsive poly(N-isopropylacrylamide) (PNIPAAm) was successfully grafted onto polydimethylsiloxane (PDMS) surface via benzophenone-initiated photopolymerization. In the present work, the PNIPAAm-grafted-PDMS (PNIPAAm-g-PDMS) surface was explored for thermomodulated cell culture and noninvasive harvest in microfluidic channels. Using COS 7 fibroblast from African green monkey kidney as the model cells, the thermomodulated adhering and detaching behaviors of the cells on the PNIPAAm-g-PDMS surfaces were optimized with respect to PNIPAAm-grafting yields and gelatin modification. The viability of the cells cultured on and harvested from the PNIPAAm-g-PDMS surface with the thermomodulated noninvasive protocol was estimated against the traditional cell culture/harvest method involving trypsin digestion. The configuration of the microchannel on the PNIPAAm-g-PDMS chip was evaluated for static cell culture. Using a pipette-shaped PNIPAAm-g-PDMS microchannel, long-term cell culture could be achieved at 37 °C with periodic change of the culture medium every 12 h. After moving the microchip from the incubator set at 37 °C to the room temperature, the proliferated cells could be spontaneously detached from the PNIPAAm-g-PDMS surface of the upstream chamber and transferred by a gentle fluid flow to the downstream chamber, wherein the transferred cells could be subcultured. The thermomodulated cell culture, harvest, and passage operations on the PNIPAAm-g-PDMS microfluidic channels were demonstrated. © 2010 American Institute of Physics. [doi:10.1063/1.3516038]

I. INTRODUCTION

In recent years, cell-based assays on microfluidic chips have attracted increasing attention due to their advantages over conventional macroscale systems, such as low cost, high throughput, and intensive integration. To perform a complete cell-based assay on a microchip, series of cell handling operations are expected to be integrated on one microfluidic chip.¹ Cell culture and harvest are obviously the most upstream operation for a completely integrated cell assay chip. Nevertheless, among the recent published papers on manipulation and assay of cells with microfluidic chips, only a few dealt with cell harvest operation after cells had been proliferated on-chip.²⁻⁶ In these published works, traditional trypsinization was employed for cell detachment from the channel surface, making on-line cell harvest, cell transport, or cell passage operations possible. However,

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the nonspecific protease is harmful to the cells due to its damaging effect toward crucial cell surface proteins.⁷ Therefore, techniques that allow on-chip harvest of cells with little damage effect are in high demand for the development of on-chip-integrated cell assay systems.

Poly(N-isopropylacrylamide) (PNIPAAm) is a type of thermoresponsive smart polymer that exhibits a reversible phase transition at around 32 °C [known as the lower critical solution temperature (LCST)]. At a temperature below its LCST, the polymer chains swell and become hydrophilic, whereas at a temperature above the LCST the chains collapse and become hydrophobic.⁸ When PNIPAAm is grafted onto substrates such as polystyrene (PS),^{9,10} poly(ethylene terephthalate),^{11,12} silicon,¹³ glass,¹⁴ and gold,¹⁵ cells can adhere onto the PNIPAAm-modified surfaces and proliferate therein at a temperature above the LCST. The adhering cells can be detached from the surfaces by lowering the temperature to below the LCST without the help of trypsin digestion. The PNIPAAm-modified surfaces have been successfully applied for noninvasive cell harvest,^{9,11,16} patterned cell coculture,^{17–19} and tissue engineering.^{20,21} Recently, Ernst *et al.*¹⁵ studied thermomodulated and shear-flow assisted detaching behavior of L929 fibroblasts from a poly(N-isopropylacrylamide)-copoly(ethylene glycol) modified gold surface prepared in a microfluidic channel. Reviews on thermomodulated cell detachment from the PNIPAAm surface have been recently published.^{22,23}

Polydimethylsiloxane (PDMS) is one of the most widely used polymer materials in fabrication of microfluidic chips due to its excellent properties such as convenient processability, chemical stability, and optically transparency.²⁴ Friend and Yeo²⁵ presented in detail the techniques for the fabrication of PDMS microfluidic devices and the potential usages of these devices in a recent published paper. The most attractive properties of this material lie in its biological compatibility and gas permeability, which make PDMS preferable to glass or plastic substrates for preparation of microfluidic chips used for cell culture^{26–29} and cell-based assay.^{30,31} Thus, the carbon dioxide and oxygen required by the living cells cultured inside the microchannels can diffuse through the porous PDMS substrate into the microchannels, rendering the cell culture in closed microchannels possible. From this point of view, grafting of PNIPAAm into the inner walls of PDMS microchannels can combine the advantages of PDMS with the merits of PNIPAAm to create a favorable environment for in-channel cell culture/harvest operation. In our previous work, PNIPAAm-grafted-PDMS (PNIPAAm-g-PDMS) surface has been successfully prepared via benzophenone-initiated photopolymerization and its potential application for thermomodulated cell culture was preliminarily demonstrated.³²

The present work is intended to explore the thermoresponsive PNIPAAm-g-PDMS surfaces for in-channel cell culture and noninvasive harvest. The thermomodulated adhering and detaching behaviors of COS7 African green monkey kidney fibroblast cells on PNIPAAm-g-PDMS surface were investigated with respect to PNIPAAm grafted yield and gelatin coating, and the viability of the cells cultured on and harvested from the PNIPAAm-g-PDMS surface was evaluated. A PDMS microfluidic chip with a PNIPAAm-g-PDMS channel was fabricated to demonstrate the in-channel thermomodulated cell culture, harvest, and passage operations.

II. EXPERIMENTAL

A. Fabrication of the PDMS microfluidic chip

The microfluidic device used for cell culture was fabricated by PDMS (Sylgard 184, Dow Corning) using replicate molding and soft lithography. A pipette-shaped microfluidic channel [see Fig. 1(a)] was fabricated by casting the mixture of the PDMS precursor and curing agent (in a 10:1 ratio) against a SU-8 mold and cured at 75 °C for 1.5 h. After curing, the PDMS substrate was peeled from the mold and accessing holes (1.5 mm i.d.) were punched at each terminal of the channel. The channel-structured PDMS substrate was 2 mm in thickness. A flat PDMS cover film with 0.8 mm thickness was prepared by casting the mixture against a clean, flat glass plate. The channel-structured substrate and the cover film were then irreversibly bonded immediately after air plasma treatment. Finally, two reservoirs (2.5 mm i.d.) made of PDMS sheet of 2 mm thick were irreversibly sealed on the two accessing holes of the channel.

FIG. 1. The micro-PDMS device for cell culture. (a) The layout of the microchip. (b) The cross section view of the microchannel. (c) The photopicture of the chip. (d) The schematic diagram of two serially connected microfluidic chips used for cell passage operation (not in scale). U: upstream chip. D: downstream chip. T: fine tubing connecting the two chips.

B. Modification of PDMS channel surfaces with PNIPAAm

A modified procedure for UV-induced grafting PNIPAAm to PDMS channel surfaces was based on our previous work.³² Briefly, the channel was flushed by a stream of 20% benzophenone (Acros Organics) in acetone for 10 min and then washed with copious water. After the microchannel was filled with a mixed monomer solution containing 10% (w/v) N-isopropylacrylamide (NIPAAm, Acros Organics), 0.5 mM NaIO₄, and 0.5% benzyl alcohol, the PDMS chip was exposed to the UV lights (6.75 mW/cm² at 365 nm) emitted from a high-pressure mercury lamp for 25 min. During the photopolymerization grafting, the chip was put on an ice-water heat sink, with the chip temperature kept below 20 °C. Finally, the channel was sequentially flushed with acetone and de-ionized water.

C. Cell culture in a 24-well plate

PNIPAAm-g-PDMS slabs, prepared as described in our previous work,³² were cut into disks of 15 mm i.d., and the disks were fit into the bottom of the wells of a 24-well plate, one disk for each well. (Note: To prevent the PNIPAAm-g-PDMS disks from floating up to the surface of culture medium during cell culture, a small amount mixture of PDMS precursor and curing agent was used to glue the disks to the bottom of the well). The well plate with the PNIPAAm-g-PDMS disks was then sterilized by UV lights for 12 h. Before cell seeding, the surfaces of PNIPAAm-g-PDMS disks inside the wells were coated with 0.1% gelatin (Guoyao Chemical Co.) and dried. COS7 cells were suspended in RPMI 1640 medium containing fetal bovine serum (10%), ampicillin (100 units/ml), and streptomycin (100 μg/ml). 1 ml of the cell suspension with a density of approximately 4×10^4 cells/ml was seeded into each well. Cell incubation was performed at 37 °C in a humidified 5% CO₂ atmosphere until the cells reached 90% confluence. Then, the 24-well plate was transferred from the incubator to room temperature. After 15 min, the cultured cells could be detached from the PNIPAAm-g-PDMS surfaces. After the harvested cells were separated into individuals by bubbling with a pipette gun, counting was performed with a haemocytometer. As a control experiment, COS7 cells with the same seeding density as above described were cultured in the 24-well plate with gelatin-coated, native PDMS disks at the bottom of wells. Such cultured cells were harvested after subjected to the treatment of 0.25% trypsin/0.01% EDTA solution for 30 s. The viabilities of the cells harvested with either the thermomodulated, noninvasive method or the trypsin digestion method were examined after staining with 4% (w/v) trypan blue solution and expressed as the mean percentage of nine samples.

D. Cell culture and passage in microfluidic channels

Cells were cultivated in the microchannel under the condition of static medium. The inner surfaces of the PNIPAAm-g-PDMS channels were coated by gelatin before cell culture. Then, COS7 cell suspension with a density of approximately 4×10^6 cells/ml was infused into one microreservoir of the chip. With the synergetic action of capillary and hydrostatic forces, the cell suspension was aspirated into the channel until reaching the other reservoir. After the liquid levels on both reservoirs were adjusted to the same height by supplement of the medium into the reservoirs, the reservoirs were sealed with films to retard evaporation. The chip was put into the incubator that was set at 37 °C and circulated with a humidified 5% CO₂ atmosphere. During cultivation, the culture medium was replaced every 12 h. To prevent the adhering cells from being detached from the channel walls and, subsequently, being flushed out of the channel during the medium replacement, the operation of medium replacement should be finished in less than 5 min, during which the chip was exposed to room temperature. After the cells were cultivated in the PNIPAAm-g-PDMS microchannel for three days, the chip was taken out of the incubator and put on the platform of a microscope at room temperature. After 10–15 min, the cells detached from the bottom of the channel. For cell passage operation, the detached cells were on-line aspirated to a downstream chip, which was connected to the upstream chip via a section of fine tubing [see Fig. 1(d)]. The transferred cells were subcultivated on the downstream chip the same as the above described.

III. RESULTS AND DISCUSSION

A. Thermomodulated culture and harvest of cells on PNIPAAm-g-PDMS surfaces

1. Gelatin coating

In preliminary tests, it was observed that COS7 cells could not properly spread on the PNIPAAm-g-PDMS surfaces after two-day cultivation, either still on round status or aggregated together. Liu and co-workers³³ reported that gelatin coating would promote adhesion of fibroblast cells on their prepared thermoresponsive surfaces. Therefore, in the present work, the effect of gelatin coating on adhering behavior of COS7 cells on the newly developed PNIPAAm-g-PDMS surfaces was investigated. Test showed that the adhering and spreading behavior of the COS7 cells on the gelatin-coated PNIPAAm-g-PDMS surfaces was significantly improved. One night after seeding, the seeded cells spread fully on the surface, and four days after seeding the proliferated cells reached above 90% confluence. Of particular interest was the observation that the gelatin coating did not hamper thermomodulated cell detachment, as the adhering cells could be detached from the gelatin-coated PNIPAAm-g-PDMS surfaces by leaving the surface at room temperature for about 10 min. In the following experiments, PNIPAAm-g-PDMS surfaces of either bulk films or channel inner walls were coated with gelatin before cell seeding.

2. PNIPAAm-grafting yield

It has been reported that the yields of PNIPAAm grafted on such substrates as PS and silicon have a crucial effect on cell adhering and detaching behaviors.^{10,13,34,35} However, no literature has reported this issue for the PNIPAAm-g-PDMS surface.

In our previous work, it has been observed that the thickness of PDMS substrate was a critical factor influencing the yields of PNIPAAm-grafting, the thinner the substrate the higher the grafting yields.³² To obtain PNIPAAm-g-PDMS surfaces with different PNIPAAm yields, PDMS substrates of three different thicknesses (0.5, 0.8, and 1.0 mm) were subjected to the UV-induced grafting,³² and the grafting yields were determined with attenuated total reflection Fourier transform infrared (ATR-FTIR) spectrometer (a model Nexus-670 FTIR spectrometer, Nicolet, USA).^{10,36} With this analytical method, the relative yield of PNIPAAm-grafting was estimated with the peak height ratio of the amide carbonyl peak at 1660 cm⁻¹ derived from the grafted PNIPAAm chains to the internal reference peak at 2965 cm⁻¹ that corresponds to the methyl

FIG. 2. The fluorescence images of the cells cultivated for three days on the PNIPAAm-g-PDMS surfaces with the relative grafting yields of 7.28 ± 0.61 (a), 3.55 ± 0.99 (b), and 0.88 ± 0.11 (c). The PNIPAAm-g-PDMS surfaces were precoated with gelatin. Before being observed under a fluorescence microscope, the cells were fixed with 95% ethanol and stained afterward by acridine orange (AO).

group of the PDMS substrate. Thus, the relative yields of PNIPAAm chains grafted to the PDMS substrates with the thickness of 0.5, 0.8, and 1.0 mm were measured at 7.28 ± 0.61 ($n=3$, the same below), 3.55 ± 0.99 , and 0.88 ± 0.11 , respectively.

After COS7 cells were cultivated on the PNIPAAm-g-PDMS surfaces with various PNIPAAm yields for three days, the morphology of the cells was observed. On the PNIPAAm-g-PDMS surface with the highest yield (7.28 ± 0.61), a few cells spread on the surface, with most cells being clustered and aggregated with each other as shown in Fig. 2(a). On the surface with the moderate yield (3.55 ± 0.99), however, the cells spread well and reached 80%–90% confluence [see Fig. 2(b)]. On the surface with the lowest PNIPAAm-grafting yield (0.88 ± 0.11), the cells reached almost complete confluence [see Fig. 2(c)]. It is suggested that the lower the PNIPAAm-grafting yield, the better the cells proliferate on the PNIPAAm-g-PDMS surface. The influence of PNIPAAm-grafting yield on cell detachment caused by lowering temperature was also examined. In the experiment, when the PNIPAAm-g-PDMS slabs where cells had been cultured for three days were removed from the incubator to the platform of an inverted microscopy at room temperature, timing began. When almost all the cells floated from the PNIPAAm-g-PDMS surface, timing ceased. For each PNIPAAm-grafting yield, the average detaching time duration was the mean of three independent samples. As shown in Fig. 3, the detaching time duration was increased with the increase of the grafting yield. The observation that the cells tended to adhere to the PNIPAAm-g-PDMS surface with low PNIPAAm yield might be ascribed to that higher PNIPAAm-grafting yield may lead to more hydration of the grafted polymer even at 37 °C, preventing cell from adhesion. This observation is similar to those reported by Refs. 10 and 13 for endothelial cells¹⁰ and hepatoma cells,¹³ respectively. Compromised between cell adhesion and detachment behaviors, the thermoresponsive surface with the PNIPAAm-grafting yield of 3.55 ± 0.99 was used in the following experiments.

FIG. 3. Time durations required by the thermomodulated detachment of the cells from the PNIPAAm-g-PDMS surfaces with the relative grafting yields of 7.28 ± 0.61 (a), 3.55 ± 0.99 (b), and 0.88 ± 0.11 (c). The PNIPAAm-g-PDMS surfaces were precoated with gelatin. The cells were cultivated at 37 °C in a humidified 5% CO₂ atmosphere for three days. The thermomodulated detachment of the cultivated cells was conducted at room temperature. The error bar indicates the standard deviation for the average of three samples.

3. Cell viability

Despite the fact that thermomodulated culture and harvest of cells on the PNIPAAm grafted surface are commonly recognized more friendly to the cells than the traditional culture and harvest method involving trypsin digestion, few works have been reported on the quantitative comparison of the viability of the cells cultured and harvested by the two different methods. In the present work, the viability of cells cultured and harvested with the two types of techniques were estimated and compared. It was found that the mean survival rate of the COS7 cultured on the PNIPAAm-g-PDMS surface at 37 °C and harvested from it by lowering temperature to about 20 °C was $89.1 \pm 6.0\%$ ($n=9$), and that of COS7 cultured on PDMS surface and harvested from the surface by trypsin digestion was $80.6 \pm 7.3\%$ ($n=9$), the former being significantly higher than the latter ($P=0.016$). Separate 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) test indicated that there was no significant difference in the viability, estimated before harvest, between the cells cultured on native PDMS and that cultured on PNIPAAm-g-PDMS. This superiority would be more outstanding ($97.2 \pm 2.6\%$ versus $75.7 \pm 7.3\%$) when the developed method was used to culture and harvest highly sensitive human mesenchymal stem cells.³⁷

B. Cell culture and harvest in PNIPAAm-g-PDMS microchannels

1. Culture mode and microchannel architecture

Cell culture in microfluidic chips can be conducted either in continuous perfusion mode^{38–40} or in static mode.^{41,42} With the continuous mode, a culture medium with certain ingredients is continuously infused into the culture chamber, supplying the nutrition and removing of toxic metabolites.⁴³ However, this mode requires complicated experimental setup. Moreover, the continuous perfusion flow exposes cells to shear stress that may have negatively effects on cell proliferation.^{43–45} With the consideration of simplicity in operation and to achieve high viability for cells, the static culture mode with periodic (every 12 h) changing of the culture medium was adopted in the present work.

In the static culture mode, the architecture of the microchannel is crucial. Initially, a simple straight microchannel (400 μm wide, 70 μm deep, and 2 cm long) was employed to cultivate the cell therein. It was observed that the cells seeded on the central section of the channel were atrophied within two days, whereas those on the sections closed to both reservoirs were well-proliferated. Obviously, as the cells gradually proliferated, the cells on the central section of channel suffered a shortage of nutrition and surfeit of metabolite waste in the culture medium due to the limitation of diffusion-based mass transport under the static condition. Therefore, a pipette-shaped architecture with an enlarged culture chamber in the center and accessing channels at both sides, as shown in Figs. 1(a) and 1(b), was designed. Compared with the initial straight channel, the pipette-shaped channel network was shorter, wider, and deeper. With such channel architecture, cells in the culture chamber can well spread and vigorously proliferate until reaching confluence [see Figs. 4(a) and 4a'].

2. Cell passage operation on microfluidic chips

The thermomodulated on-line cell culture, harvest, and passage operations were demonstrated with two serially connected microfluidic PNIPAAm-g-PDMS chambers [see Fig. 1(d)]. For comparison, the same operation was also conducted on two serially connected microfluidic PDMS chambers of the same geometry but without surface grafted PNIPAAm chains. The native PDMS chambers were also coated with gelatin as the PNIPAAm-g-PDMS chambers before cell seeding. Figure 4 shows the morphology of the cells at different stages of the cell passage operation conducted on both the PNIPAAm grafted and native PDMS microchambers. After being cultivated on the upstream chambers for three days, the cells on both the PNIPAAm-g-PDMS and native PDMS chambers were almost confluent [see Figs. 4(a) and 4a']. Both the chips were moved out of the incubator and left at room temperature. After 15 min, the cells in the PNIPAAm grafted chamber spontaneously shrunk, split into several small clusters, and were detached from the surface [Fig. 4(b)]. The detached cells could be transferred to the downstream chamber via a

FIG. 4. Morphology of the cells cultivated in both PNIPAAm grafted (a)–(e) and native (a')–(e') PDMS microchannels during a cell passage operation. (a) and (a'): three days after the cells had been cultivated on the upstream chamber. (b) and (b'): 15 min after (a) and (a') were left at the room temperature. (c) and (c'): after the culture chamber was flushed with a gentle medium flow of about 1.5 mm/s. (d) and (d'): the cells transferred to the downstream chamber. (e) and (e'): two days after the cells had been subcultivated on the downstream chamber. Both the PNIPAAm grafted and native PDMS channels were precoated with gelatin.

gentle medium flow of about 1.5 mm/s [Figs. 4(c) and 4(d)]. The cell clusters transferred to the downstream chamber well spread and vigorously proliferated [Fig. 4(e)]. In contrast, the cells cultivated in the native PDMS chamber still adhered in the upstream chamber after leaving at room temperature for 15 min [Fig. 4b'] and could not be transferred and subcultivated in the downstream chamber [see Figs. 4c'–4e']. This experiment result indicates that the cells cultivated in the PNIPAAm-g-PDMS microfluidic channels at 37 °C can be noninvasively harvested by lowering temperature and the harvested cells are able to adhere and proliferate again. Without use of the trypsin/EDTA solution, the harvested cells suffer no damage, and no immediate separation of the harvested cells from the spent trypsin digestion solution is required.

IV. CONCLUSIONS

PNIPAAm-g-PDMS surfaces can be used as the substrate for thermomodulated cell culture and harvest without trypsin digestion. PNIPAAm-grafting yield has significant impact on the performances of PNIPAAm-g-PDMS surfaces for the thermomodulated cell culture and harvest, and a moderate grafting yield is desirable. Gelatin coating enhances cell adhering to the grafted surface. The viability of cells cultured and harvested with the thermomodulated technique is significantly higher than that with the conventional cell culture and trypsin-assisted harvest method. Long-term cell culture in static, periodically replaced medium can be achieved in PNIPAAm-g-PDMS microchips with appropriate culturing chamber configuration. The proliferated cells can be spontaneously detached from the PNIPAAm-g-PDMS surfaces simply by moving the microchip from the incubator to room temperature, and thereafter transported with a gentle flow to the downstream chambers where the harvested cells can be subcultured again. The thermomodulated cell cultivation, noninvasive harvest, and passage operations conducted on the microfluidic chip demonstrate the potential for the PNIPAAm-g-PDMS channel to be used as an upstream operational unit in integrated microchips for cell-based bioscience research such as cell-based assays, cell-based drug screen, and stem-cell culture and differentiation.

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¹A. K. Price and C. T. Culbertson, *Anal. Chem.* **79**, 2614 (2007).

²H. Hufnagel, A. Huebner, C. Gulch, K. Guse, C. Abell, and F. Hollfelder, *Lab Chip* **9**, 1576 (2009).

³B. Y. Zhang, M. C. Kim, T. Thorsen, and Z. H. Wang, *Biomed. Microdevices* **11**, 1233 (2009).

⁴I. Barbulovic-Nad, S. H. Au, and A. R. Wheeler, *Lab Chip* **10**, 1536 (2010).

- ⁵ K. Ziolkowska, E. Jedrych, R. Kwapiszewski, J. Lopacinska, M. Skolimowski, and M. Chudy, *Sens. Actuators B* **145**, 533 (2010).
- ⁶ P. J. Hung, P. J. Lee, P. Sabounchi, R. Lin, and L. P. Lee, *Biotechnol. Bioeng.* **89**, 1 (2005).
- ⁷ R. M. P. Da Silva, J. F. Mano, and R. L. Reis, *Trends Biotechnol.* **25**, 577 (2007).
- ⁸ E. S. Gil and S. A. Hudson, *Prog. Polym. Sci.* **29**, 1173 (2004).
- ⁹ T. Okano, N. Yamada, H. Sakai, and Y. Sakurai, *J. Biomed. Mater. Res.* **27**, 1243 (1993).
- ¹⁰ Y. Akiyama, A. Kikuchi, M. Yamato, and T. Okano, *Langmuir* **20**, 5506 (2004).
- ¹¹ X. H. Cheng, Y. B. Wang, Y. Hanein, K. F. Bohringer, and B. D. Ratner, *J. Biomed. Mater. Res. Part A* **70A**, 159 (2004).
- ¹² O. H. Kwon, A. Kikuchi, M. Yamato, Y. Sakurai, and T. Okano, *J. Biomed. Mater. Res.* **50**, 82 (2000).
- ¹³ L. H. Li, Y. Zhu, B. Li, and C. Y. Gao, *Langmuir* **24**, 13632 (2008).
- ¹⁴ K. Fukumori, Y. Akiyama, M. Yamato, J. Kobayashi, K. Sakai, and T. Okano, *Acta Biomater.* **5**, 470 (2009).
- ¹⁵ O. Ernst, A. Lieske, M. Jager, A. Lankenau, and C. Duschl, *Lab Chip* **7**, 1322 (2007).
- ¹⁶ N. Yamada, T. Okano, H. Sakai, F. Karikusa, Y. Sawasaki, and Y. Sakurai, *Makromol. Chem., Rapid Commun.* **11**, 571 (1990).
- ¹⁷ M. Yamato, C. Konno, M. Utsumi, A. Kikuchi, and T. Okano, *Biomaterials* **23**, 561 (2002).
- ¹⁸ M. Yamato, O. H. Kwon, M. Hirose, A. Kikuchi, and T. Okano, *J. Biomed. Mater. Res.* **55**, 137 (2001).
- ¹⁹ Y. Tsuda, A. Kikuchi, M. Yamato, A. Nakao, Y. Sakurai, M. Umezu, and T. Okano, *Biomaterials* **26**, 1885 (2005).
- ²⁰ M. Harimoto, M. Yamato, M. Hirose, C. Takahashi, Y. Isoi, A. Kikuchi, and T. Okano, *J. Biomed. Mater. Res.* **62**, 464 (2002).
- ²¹ T. Shimizu, M. Yamato, A. Kikuchi, and T. Okano, *Biomaterials* **24**, 2309 (2003).
- ²² M. A. Cooperstein and H. E. Canavan, *Langmuir* **26**, 7695 (2010).
- ²³ Y. Kumashiro, M. Yamato, and T. Okano, *Ann. Biomed. Eng.* **38**, 1977 (2010).
- ²⁴ J. C. McDonald and G. M. Whitesides, *Acc. Chem. Res.* **35**, 491 (2002).
- ²⁵ J. Friend and L. Yeo, *Biomicrofluidics* **4**, 026502 (2010).
- ²⁶ M. S. Kim, J. H. Yeon, and J. K. Park, *Biomed. Microdevices* **9**, 25 (2007).
- ²⁷ E. Leclerc, Y. Sakai, and T. Fujii, *Biomed. Microdevices* **5**, 109 (2003).
- ²⁸ W. Dai, Y. Zheng, K. Q. Luo, and H. Wu, *Biomicrofluidics* **4**, 024101 (2010).
- ²⁹ M. Nagai, H. Asai, and H. Fujita, *Biomicrofluidics* **4**, 034109 (2010).
- ³⁰ W. H. Huang, W. Cheng, Z. Zhang, D. W. Pang, Z. L. Wang, J. K. Cheng, and D. F. Cui, *Anal. Chem.* **76**, 483 (2004).
- ³¹ N. N. Ye, J. H. Qin, W. W. Shi, X. Liu, and B. C. Lin, *Lab Chip* **7**, 1696 (2007).
- ³² D. Ma, H. W. Chen, D. Y. Shi, Z. M. Li, and J. F. Wang, *J. Colloid Interface Sci.* **332**, 85 (2009).
- ³³ H. C. Liu and Y. Ito, *J. Biomed. Mater. Res. Part A* **67A**, 1424 (2003).
- ³⁴ Y. Akiyama, A. Kushida, M. Yamato, A. Kikuchi, and T. Okano, *J. Nanosci. Nanotechnol.* **7**, 796 (2007).
- ³⁵ A. Mizutani, A. Kikuchi, M. Yamato, H. Kanazawa, and T. Okano, *Biomaterials* **29**, 2073 (2008).
- ³⁶ C. Q. Qiu, F. Xu, Q. T. Nguyen, and Z. H. Ping, *J. Membr. Sci.* **255**, 107 (2005).
- ³⁷ D. Y. Shi, D. Ma, F. Q. Dong, C. Zong, L. Y. Liu, D. Shen, W. J. Yuan, X. M. Tong, H. W. Chen, and J. F. Wang, *Biosci Rep.* **30**, 149 (2010).
- ³⁸ B. G. Chung, L. A. Flanagan, S. W. Rhee, P. H. Schwartz, A. P. Lee, E. S. Monuki, and N. L. Jeon, *Lab Chip* **5**, 401 (2005).
- ³⁹ A. Tourovskaia, X. Figueroa-Masot, and A. Folch, *Lab Chip* **5**, 14 (2005).
- ⁴⁰ S. K. Mahto, T. H. Yoon, and S. W. Rhee, *Biomicrofluidics* **4**, 034111 (2010).
- ⁴¹ K. Sugiura, N. Kaji, Y. Okamoto, M. Tokeshi, and Y. Baba, Thirteenth International Conference on Miniaturized Systems for Chemistry and Life Sciences, 2009, p. 1243.
- ⁴² K. I. Kamei, S. L. Guo, Z. T. F. Yu, H. Takahashi, E. Gschweng, C. Suh, X. P. Wang, J. G. Tang, J. McLaughlin, O. N. Witte, K. B. Lee, and H. R. Tseng, *Lab Chip* **9**, 555 (2009).
- ⁴³ L. Kim, Y. C. Toh, J. Voldman, and H. Yu, *Lab Chip* **7**, 681 (2007).
- ⁴⁴ Y. S. J. Li, J. H. Haga, and S. Chien, *J. Biomech.* **38**, 1949 (2005).
- ⁴⁵ Z. R. Healy, N. H. Lee, X. Q. Gao, M. B. Goldring, P. Talalay, T. W. Kensler, and K. Konstantopoulos, *Proc. Natl. Acad. Sci. U.S.A.* **102**, 14010 (2005).