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Review Article

Poly(ethylene glycol) (PEG) Microwells in Microfluidics: Fabrication Methods and Applications

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Abstract Immobilization of bioanalytes (e.g., protein, lipid membrane, and cells) within a microfludic channel is a useful strategy in diverse biological analysis including biosensor, diagnosis, and biochemical reactions. Such microfluidic systems can offer miniaturized platforms with distinct advantages such as reduced use of samples or reagents and increased resolution. In particular, polymeric microfluidic devices (e.g., polydimethylsiloxane (PDMS)) bound to a substrate have been widely used. However the non-specific adsorption of bioanalytes is serious problem for microfluidic biological assays, especially for dilute samples. To overcome such limitations, a simple and widely applicable microfluidic channels combined with polyethylene glycol (PEG) microwells have been developed. In this review, we summarize the methods and application of PEG microwells with a particular emphasis on integrated microfluidic systems. The assembly between PEG microwell and microchannel enables precise delivery and manipulation of the biosamples, leading to development of miniaturized diagnostic assays, microreactors, and multiple screening platforms for tissue engineering and cell biology.

Keywords: Poly(ethylene glycol) (PEG), Microwell, Microchannel, Soft lithography, Bioanalytes arrays

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Introduction

In bioanalytical experiments, immobilized bioanalytes (e.g., cell, lipid membrane, and biomolecules) on a solid substrate of two-dimensional ordered array form is widely used[1-11]. The immobilization enables convenient manipulation of bioanalytes for observation and analysis in a high-throughput manner. Conventionally, solid substrates such as well plates[3], microarray slides[4-8], or blotting membranes[9-11] have been well-developed and widely used in biological laboratories. Such platforms, however, need improvements because they require large sample volume, complex operating devices (e.g., robotic liquid handling system), and complicated processes. To solve the problems, immobilization of bioanalytes on the surface of microfluidic channel has risen as an attractive solution, since it takes the advantages of microfluidic control[12-25]. Microfluidic channels can miniaturize sample volumes and enable rapid, high-resolution, and low cost analysis in diverse bioanalytical fields including biosensor, diagnosis, and biochemical reactions.

To immobilize bioanalytes in the microchannel, several microfabrication methods have been developed including laminar flow patterning[16-22] and photo-induced hydrogel patterning[23-26]. In laminar flow patterning, bioanalytes in the stream are immobilized at the underlying surface of microchannel. Multiple bioanalytes can be separately located on the surface because the streams are not mixed in the laminar flow. This method, however, can only pattern a limited number of shapes, e.g., a line pattern. In photo-induced microfabrication process, prepolymer of hydrogel which embeds bioanalytes in the microchannel can be fabricated by selective UV irradiation by using optical mask and subsequently washing off unexposed prepolymer.

Nevertheless, the direct UV exposure of bioanalytes may lead to unexpected damage, and dense hydrogel network may prevent the precise measurement of bioanalytes.

In order to construct 2D microarrays on a flat surface, several microfabrication methods, such as direct contact printing of bioanalytes[1,2,13,17] and droplet dispensing[1,5,6,27], have been developed. In these methods, damage of the immobilized bioanalytes may occur in the post procedure, including microchannel bonding by oxygen plasma or heating.

To overcome these limitations, several groups have developed a microfluidic system combining microwell arrays that can capture and immobilize the bioanalytes within the predefined sites inside the channel[28-32]. In this system, bioanalytes in the flow is topographically confined inside the microwell structure through passive transport such as simple diffusion, sedimentation or capillary force. At the same time, the wall of microwell acts as a selective barrier inhibiting the binding of biosamples to undesired position. Especially, when the surface of microwell consists of non-biofouling materials such as polyethylene glycol (PEG), the undesired bindings are efficiently suppressed[29]. The sidewall of microstructures further provides a physical barrier protecting the docked biosamples from fluidic shear stress when the height of the microwell is designed sufficiently higher than the size of bioanalytes[30-32]. In this regard, there are two types of microwell: i) wells with the exposed substrate (typically, glass surface) on the bottom, and ii) wells with modified substrate (i.e., the bottom and side of the well are coated with the same material). Exposed microwells have been used for selective immobilization of biosamples at the exposed substrate through surface interactions while surface-modified microwells have been applied to docking of large biosamples such as single cells or aggregates of cells[30-32].

In this review, we introduce methodological approaches to pattern various biosamples using PEG microwells in a microchannel with their fabrication methods and versatile applications to protein, lipid membranes and cell arrays (Figure 1). "PEG microwells in microchannels" generally consist of two layers, a top microchannel layer and a bottom layer constructed with PEG microwells. Indeed, PEG is a suitable material for accompanying the physical integrity of microwell structure along with non-biofouling property. We will first discuss the general material properties of PEG-based hydrogels, and describe the detailed fabrication methods and biological applications of PEG microwells in microchannel.

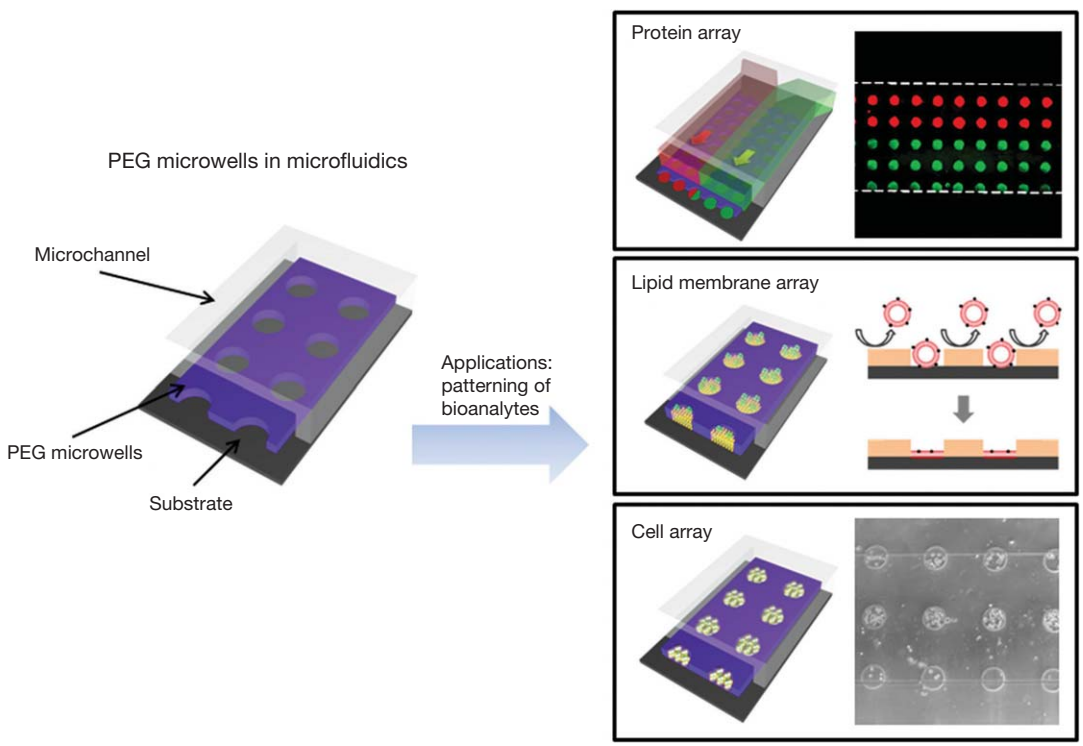

Figure 1. A schematic illustration of PEG microwells in a PDMS microchannel and their uses for protein, lipid membrane, and cell patterning. Reproduced from 29, copyright 2004, with permission from American Chemical Society, and 32, copyright 2004, with permission from Royal Society of Chemistry.

Material properties of PEG-based hydrogels

The microwell structures in the microchannel have been fabricated with various polymeric materials such as PDMS (polydimethylsiloxane)[33-36], PUA (polyurethane acrylate)[30,37], PMMA (polymethyl methacrylate)[31,34,35,38], PVA (polyvinyl alcohol)[39], HA (hyaluronic acid)[29,40], and PEG (polyethylene glycol)[29,41,42]. Among them, PEG is one of the most attractive materials used in bioanalytical devices due to its strong non-biofouling property and versatile processibility[41,43]. Biofouling is a big problem deteriorating the performance of analysis in surface-based assays which uses immobilized bioanalytes on the substrate. When a rare biological sample is to be analyzed, non-specifically bound substance causes critical false signal in the experiment. PEG can resolve such problem through its inherent non-biofouling property. The inhibition of bio-fouling on PEG surface is related to its high hydrophilicity (contact angle: ~0°). Since water molecules strongly bind to PEG surface it blocks the adsorption of biosamples[43].

Surface modifications with PEG have been considered as a traditional way to minimize non-specific adsorption of biomolecules. The modification of silicon-based surface (e.g., silicon, glass, quartz, and PDMS) with PEG has been achieved by physical adsorption[44], chemical immobilization such as grafting[45-47], or gas phase techniques[48]. Although these approaches have shown reliable non-specific binding property resulting from polymer chain mobility and steric stabilization force of PEG at surface, inherent defects on the coating and long-term resistance to biofouling (i.e., modified surfaces slowly recover their original hydrophobicity) are still remaining problems[49].

To overcome the limitations, fabrication techniques for monolithic microwells entirely comprised of cross-linked PEG have been developed[28,29,32]. These approaches have following advantages: i) robust non-biofouling property due to the monolithic PEG structures, ii) simplified fabrication process through eliminating surface modification process, and iii) high resolution of structures even in nanometer scale. Indeed, sub-hundred nanometers of PEG nanowells were achieved on glass substrate for nanoarrays of single lipid vesicles (~50 nm)[50].

Fabrication of PEG microwells in microchannels

Construction of PEG microwells in microchannels includes three-process: i) fabrication of the top microchannel layer, ii) fabrication of the PEG microwell on bottom glass layer, and iii) assembly by bonding the two layers. Both microchannel and PEG microwell can be fabricated by using a well-developed microfabrication method called soft lithography.

Soft lithography, invented by G.M. Whitesides and coworkers[13,51], refers to a series of patterning techniques using a soft mold as a stamp, mold or mask. Here, the soft mold is fabricated by molding on a pre-patterned hard mater, which is termed "replica molding"[13,51-53]. The original hard masters having micro or nano structures are fabricated by photolithography or electron beam lithography. By using the masters, replicas, which have an inverse pattern of the masters, are fabricated with a soft polymer such as PDMS. Because of the superior material properties of PDMS such as high softness[13,51-53] and low surface energy (21.6 mJ/m^2)[52], the PDMS replicas can be easily peeled off from the hard master without distortion of structures, and then can play a role as the new master in the following patterning process. Therefore, soft lithography provides simple and robust ways for constructing both polymeric microfluidic channel and PEG microwell structures.

In this section, we introduce the whole fabrication and integration processes for the PEG microwells in microchannels. The procedures can be tuned appropriately according to the purpose of research and given situation.

Fabrication of silicon masters by photolithography

In soft lithography, one needs two hard masters for PDMS channel layer and PEG microwell layer. Hard masters are generally fabricated by photolithography with silicon wafers. Then, PDMS channel and PEG microwells can be repeatedly replicated from each master. First, the floor plans for photomask are drawn up by using a CAD program such as AUTOCAD and photomask is manufactured. Two floor plans for photomask are prepared respectively for the fabrication of microchannel and microwell. When the size of feature is larger than approximately 20 μm, a transparency film mask is beneficial due to low costs, while, in contrast, a chrome photomask is preferred due to high resolution. After the preparation of photomask, the photoresist is spin-coated on the silicon wafer and soft baked. AZ positive photoresist or SU-8 negative photoresist is usually used. Then, the patterns of photomask are transferred to the coated photoresist, UV is irradiated for few tens of seconds, and then the exposed photoresist is developed. The exposed region of silicon wafer is dry-etched and remaining PR is removed. Finally the silicon master is treated by C_4F_8 gas or fluorosilane (e.g., TFOCS, Tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane) for easy demolding of PDMS. This final treatment process, in some cases, should be done for long-term use of the master.

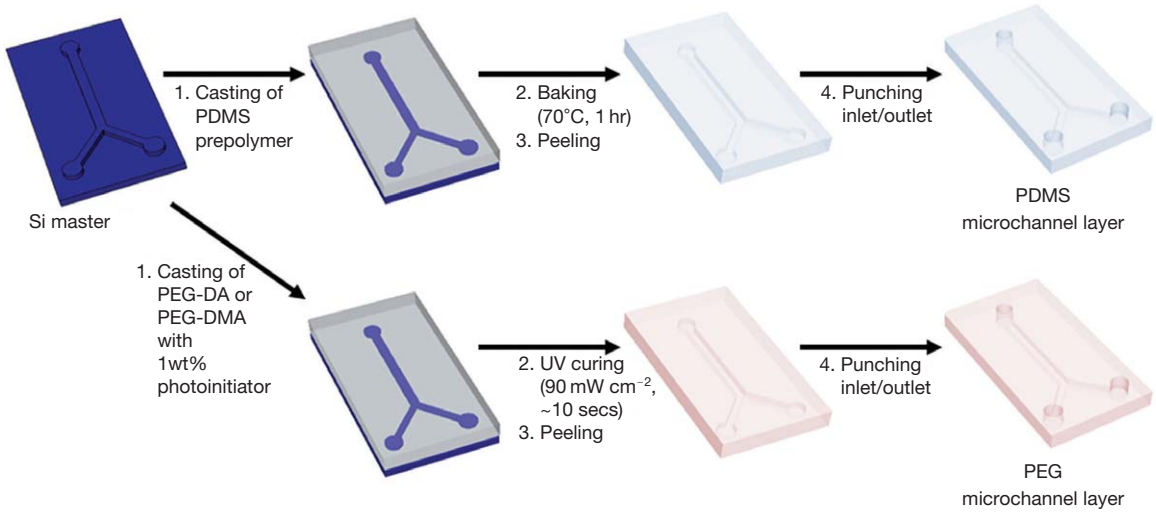

Figure 2. Fabrication process of PDMS and PEG microchannel layer by soft lithography.

Fabrication of microchannel layer

When constructing PEG microwells in microchannel, two kinds of materials have been representatively used for fabricating microchannels: PDMS and PEG. PDMS is a general material for fabricating the microchannels because of its rapid prototyping time and robust bonding chemistry to the silicon-based surfaces such as silicon, glass, quartz, and other PDMS[54]. The curing time for PDMS prepolymer is nearly an hour, and the bonding of PDMS is easily achieved by surface treatment through oxygen plasma. The plasma oxidation generates rich silanol groups on the PDMS surface, rendering the surface hydrophilic, and the activated surface can be bonded to other activated Si-based surfaces (e.g., glass, PDMS, silicon) through covalent bonding. In addition, the biocompatibility, transparency, and oxygen permeability of PDMS are advantages for the microchannel applications.

The PDMS microchannel layer can be easily generated through replica molding (Figure 2)[51]. In the first step, the PDMS prepolymer solution is poured onto Si microchannel master. The master having protruding features with the impression of microfluidic channels is generated by photolithography. PDMS prepolymer solution is prepared by thorough mixing of the silicone elastomer (Dow Corning, SYLGARD 184A) and the curing agent (Dow Corning, SYLGARD 184B) typically with 10 : 1 weight ratio. The mixing generates air bubbles in the PDMS solution. Therefore, after removing the bubbles by using a vacuum oven or a desiccator, the Si master covered with PDMS solution is placed in an oven horizontally for 1 h at 70°C for curing the mixed PDMS prepolymer[28,30]. The cured PDMS microchannel is peeled from the master carefully and cut into moderate pieces prior to use. Finally, the inlet and outlet of the microchannel are punched to introduce a liquid sample.

In contrast with PDMS microchannel, PEG microchannel can provide non-biofouling property, resulting to precise performance of bioanalysis. Kim *et al*. reported a PEG microchannel made from UV-curable PEG, PEG-diacrylate (DA) or PEG-dimethacrylate (DMA), through UV-assisted replica molding (Figure 2)[41,55,56]. Low molecular weight of PEG-DA (molecular weight: 258) and PEG-DMA (molecular weight: 330) is used for the channel fabrication due to its resistance to swelling by water. The PEG microchannel entirely consisting of crosslinked PEG chains were generated within ten minutes and irreversibly bonded to another PEG layer (including PEG microwell layer) through UV-assisted sealing.

Fabrication of PEG microwell layer

Herein, two soft lithographical methods are introduced for the fabrication of PEG microwell in the microchannel (Figure 3): contact printing[28,29] and capillary molding[28,29,32]. Both methods use a PDMS mold replicated from the silicon mater counterpart. Contact printing generates a chemically modified surface (i.e., microwell with low aspect ratio) while capillary molding constructs a topographically modified microwell (i.e., microwells with high aspect ratio). Here, a proper procedure needs to be chosen according to the individual purpose of research (Table 1).

PEG microwell fabrication by contact printing

Contact printing (in other words, stamping) is a kind of soft lithography, transferring patterns of materials call-

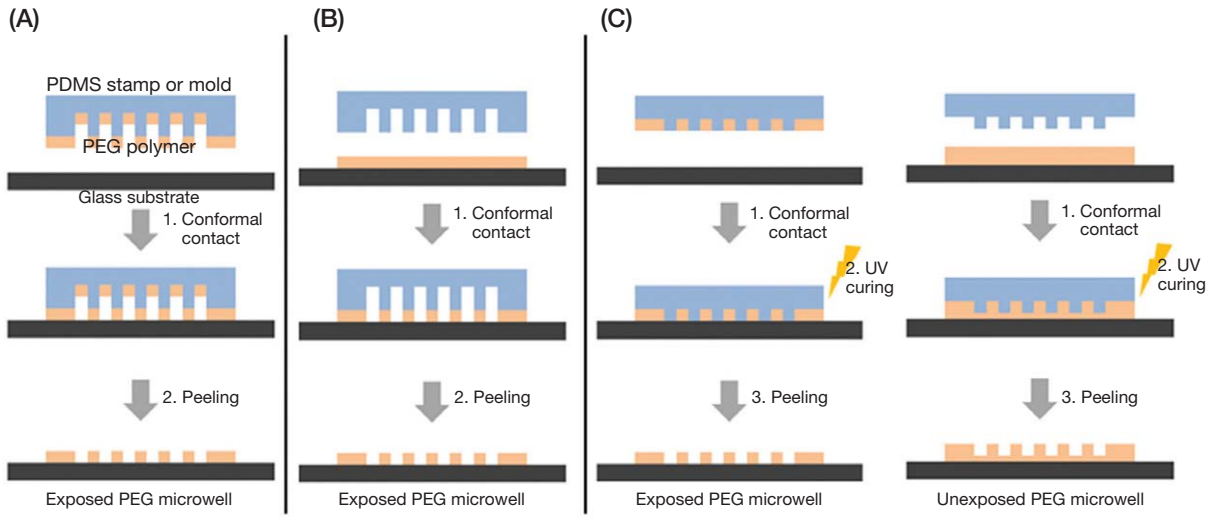

Figure 3. Fabrication processes of a PEG microwell layer by various soft lithographic techniques. (A) Contact printing. (B) Solvent-assisted capillary molding. (C) UV-assisted capillary molding.

Table 1. Classification of fabrication methods for PEG microwells. For each method, processable PEG polymer, resulting structure, and applications are summarized.

Fabrication Method		PEG Polymer	Resulting Structure	Applications	References
Contact Printing		Pure PEG, Poly(TMSMA-r-PEGMA)	- Exposed well - D[a] > 50 nm - H[b]: a few nanometers	- Protein, lipid membrane, cell array	26, 54
Capillary molding	Solvent-assisted	Poly(TMSMA-r-PEGMA)	- Exposed well - D > 70 nm - H > 70 nm	- Protein, lipid membrane array - Cell array with shear protection	26, 45
	UV-assisted	PEG-DA, PEG-DMA	- Exposed well - D > 100 nm - H > 100 nm	- Protein, lipid membrane array - Cell array with shear protection	29, 37
			- Unexposed well - D > 100 nm - H > 100 nm	- Spheroid cell culture	29, 86

[a]D: Diameter
[b]H: Height

ed "ink" from a master stamp onto a target surface[57,58]. In detail, after the soaking of a PDMS stamp in an ink solution, the molecules on protruding parts of the stamp are transferred onto the substrate surface via conformal contact and diffusion. The advantages of contact printing are easy stamp replication, short process time, and low-cost batch production. Contact printing, however, has some inherent limitations such as distortion of patterns due to PDMS swelling by ink solution and limited pattern heights of a few nanometers.

In the contact printing of PEG, pure PEG solutions[59] or comb polymer solutions (e.g., poly (TMSMA-r-PEGMA))[29] are generally used. Poly(TMSMA-r-PEGMA) developed by Jon *et al*. is comprised of a function part, PEG, and an anchor part trimethoxysilane[60]. The trimethoxysilane part of poly(TMSMA-r-PEGMA) makes binding to silicon oxide surfaces and crosslinks each polymer chains with enhancing the coating quality and resistance to swelling by water. Khademhosseini *et al*. have demonstrated the contact printing of poly (TMSMA-r-PEGMA) onto the glass substrate[29]. In their method, washed glass substrate is activated by plasma (1 min) for binding with the trimethoxysilane part of poly(TMSMA-r-PEGMA).

The patterned PDMS stamp is also treated by plasma (1 min) for proper cleaning and increasing wettability

of ink solution. Then, the PDMS stamp is inked with 1% (w/v) solution of poly (TMSMA-r-PEGMA) in 50 : 50 (v/v) water/ethanol mixture and placed directly onto the glass substrate during 30 s. The resulted PEG microwell has exposed glass substrate with the thickness of a few nanometers.

PEG microwell fabrication by capillary molding

Capillary molding is an improved type of lithography using a permeable elastomeric mold based on soft lithography and nanoimprint lithography[61,62]. When a patterned PDMS mold is placed on liquid phase of polymer such as melted polymer above glass transition temperature (Tg), solvent-laden polymer, or UV (ultraviolet)-curable prepolymer, capillary force makes the polymeric liquid fill the void space of the PDMS molds. After solidifying the polymeric liquid through methods such as cooling (at temperature-directed capillary molding), solvent evaporating (at solvent-assisted capillary molding), or UV curing (at UV-assisted capillary molding), PDMS mold can be subsequently peeled, which easily generates inverse polymeric patterns.

In comparison to contact printing, the generated patterns have a certain height ranging from few tens to hundreds of nanometers. The capillary molded structures also provide a clean interface at the patterned boundary, and the non-specific adhesion on the PEG surface is strongly restricted. Instead of PDMS mold, a UV curable mold made of PUA can be also used. Here, due to the rigid but flexible nature of PUA mold, it can be fabricated with sub-100-nm features in a simple yet robust manner[63-65].

PEG microwell fabrication by solvent-assisted capillary molding

In the solvent-assisted molding, the forming mechanism of structure is generally different according to the wetting property of the solvent. When the solvent effectively wets the PDMS mold, the prepolymer dissolved in the solvent fills the voids of mold by solvent absorption or capillarity at the time of contact[59]. Thus, an inverse structure of original mold is fabricated as a result of the micro molding. The ethanol is a representative good solvent with low contact angle ($<90^\circ$) on PDMS surface. In sharp contrast, in the case of a poor solvent (contact angle on PDMS surface $>90^\circ$) such as water, the prepolymer solution under the void region of mold dewets from the protruding structures of mold. As a result of dewetting, the underlying substrate is exposed[40,50]. Therefore, in the microwell fabrication with a poor solvent, the mold should have the concave void spaces for the desired microwell structure.

The use of poor solvent can successfully generate high-resolution PEG nanowells, which can be used to dock even single liposomes (~50 nm)[50]. The detailed procedure with a poor solvent is as follows: A few drops of 5% (w/v) poly(TMSMA-r-PEGMA) solutions in water are placed on a cleaned glass substrate. Then, PDMS mold is carefully placed onto the surface with conformal contact. The sample is stored overnight at room temperature to allow complete evaporation of the water and the mold is peeled off. The resulted PEG microwell has exposed glass substrate.

PEG microwell fabrication by UV-assisted capillary molding

UV-assisted molding with PEG oligomers having photo-polymerizable groups (e.g. acrylate and methacrylate) such as PEG-diacrylate (DA) or PEG-dimethacrylate (DMA) is a simple and robust method[32,41]. The method can be applied to forming microwells with unexposed bottom surface. Generally, a mixture of 99.5 wt.% PEG-DMA (MW 330, 550), or MW 1000 PEG-DMA (50 vol% in PBS) and 0.5 wt.% of a water-soluble photoinitiator 2-hydroxy-2-methylpropiophenoneis prepared. For fabricating exposed microwells, the polymer solution is spread on PDMS mold and the mold is place directly onto the cleaned glass substrate. For fabricating unexposed microwells, on the other hand, the polymer solution is spread on glass substrate and the PDMS mold is place directly onto the glass substrate. Then, the sample is exposed to UV light (365 nm, 10 mW cm^{-2}) for 30 s. The resulted PEG microwell has thickness corresponding to the height of PDMS pattern.

Integration of PEG microwells within microchannel

For the integration to the PEG Microwells in PDMS microchannel, the upper PDMS microchannel mold layer and the bottom PEG microwells layer should be bonded. The irreversible bonding by oxygen plasma is generally used for the purpose (Figure 4)[28,32,54]. In the first step, PDMS microchannel layer and PEG microwell layer are plasma cleaned without disturbing the PDMS mold used for PEG microwell patterning (i.e., in conformal contact with the substrate). Thus, the region of PEG microwells should be protected against oxygen plasma treatment in such a way that the covered mold for PEG microwells is unpeeled during the treatment. After the PDMS mold is peeled off from the PEG microwell layer, the microchannel layer is aligned on PEG microwell layer with conformal contact and firmly pressed to form an irreversible seal. The inlet and outlet of channel are connected with Teflon tubing and fluids with biosamples are driven through the channels using a syringe pump. Alternatively, reversible sealing of PDMS microchannel by negative pressures can simply used with eliminating leakage of flow in the channel[66].

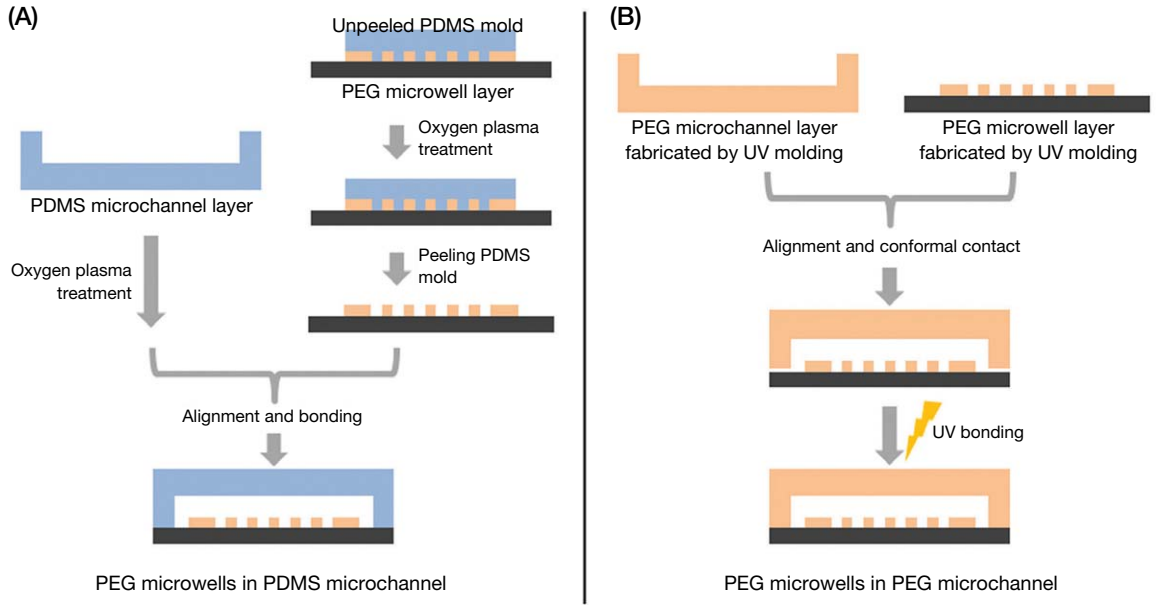

Figure 4. A schematic of bonding process for (A) PEG microwells in PDMS microchannel and (B) PEG microwells in PEG microchannel.

In case of PEG microwells in PEG microchannel, PEG microwell layer fabricated by UV-assisted capillary molding can be irreversibly bonded with the PEG microchannel layer by UV-assisted sealing[41]. The both surfaces of PEG microwells and PEG microchannels fabricated by UV-assisted molding have partially crosslinked PEG, thus have remaining unpolymerized acrylate or methacrylate groups. Therefore, by conformal contact and following UV exposure, the unpolymerized acrylate or methacrylate groups of the two surfaces are crosslinked to each other, with resulting the irreversible bonding of the two layers.

Applications of PEG microwells in microfluidics

PEG microwell is an attractive bioanalysis platform in which biosamples are arrayed on the surface of the microchannel and analyzed through multiple processes in microfluidics. The uses of PEG microwells in microfluidics for patterning protein, lipid membrane, and cell are introduced in this section.

Protein arrays

Protein microarrays that realize the biological functions of protein on selective regions of the solid substrate have been widely used in various biological assays such as cell/tissue engineering, pharmacology, and proteomics[1,4,5,9,10,17,67]. On the array, proteins are accurately patterned on predefined locations and play their native roles including sensing, signaling, and selective supports for other biosamples. Furthermore, the patterning of protein can be regarded as the initial process for patterning other biosamples such as cells[68-70], lipid membranes[71-74], and viruses[75-77] through selective binding between proteins and the biosamples. PEG microwells in PDMS microchannel is a valuable platform for constructing protein microarrays and performing the related assays, by utilizing the advantages of microfluidic system. In this system, the property of non-specific adsorption of PEG allows proteins to immobilize onto a substrate with high selectivity.

Khademhosseini *et al*. reported a protein microarray using PEG microwells in PDMS microchannel (Figure 5)[29]. In this research, through micro contact printing or capillary molding, the PEG microwells were fabricated with exposing the substrate (glass or Si wafer) in the microwell and the PDMS microchannel was bonded to substrate through the oxygen plasma bonding. The exposed regions in the microwell (i.e., unmasked region by PEG) were used for selective adsorption of various proteins such as fibronectin and bovine serum albumin. In addition, the distinctive feature of laminar flow was used to inhibit lateral mixing of stream, thus parallel streams of multiple proteins gave rise to multiple protein arrays simultaneously. For example, two types of proteins were patterned on the bottom of channel or even in individual microwells (Figure 5(C)). This technique is potentially useful for high-throughput protein assay as well as research about cell behavior related to spatial organization of multiple extracel-

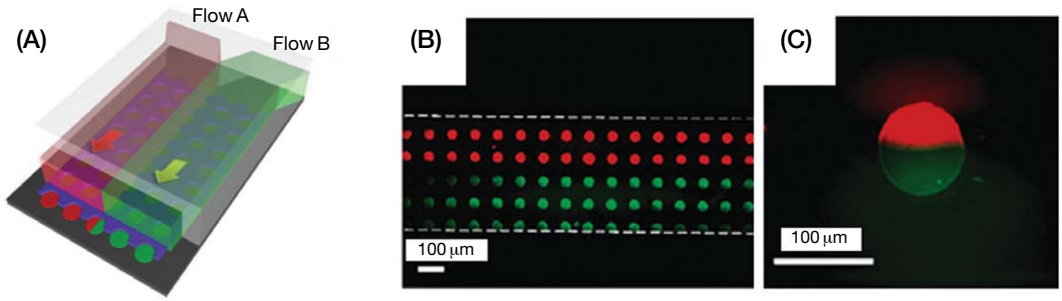

Figure 5. (A) A schematic illustration for patterning of proteins by laminar flow on PEG microwells in microchannel. (B) Patterning of two proteins (red-labeled BSA and green-labeled BSA) on the spatial region of the channel. (C) Patterning of two proteins in a single microwell. Reproduced from 29, copyright 2004, with permission from American Chemical Society.

lular matrix components.

Lee *et al*. demonstrated an improved protein nanoarray comprised of PEG nanowells and PEG self-assembled monolayers[78]. They fabricated exposed PEG nanowells (~100 nm) on the gold substrate by UV-assisted molding and modified the exposed gold substrate with PEG self-assembled monolayers consist of thiol-PEG and thiol-PEG-biotin. Subsequently, the protein nanoarray was constructed by stepwise self-assembly of streptavidin and biotinylated antihuman serum albumin through interaction between the biotin and streptavidin in the PEG nanowells. The combination of PEG microwell and PEG self-assembled monolayer provided highly selective assembly of the protein nanoarray, which was confirmed by atomic force microscopy. Based on the similar approach, Tuleuova *et al*. designed a microchip consist of PEG microwell in PDMS microchannel for detection of cytokine by fluorescence measurement[79].

Lipid membrane arrays

An artificial cell membrane structure (i.e. lipid bilayer and liposome) assembled from lipids is an attractive biomimetic platform for various biological applications such as bio-interfacial studies on cell-membranes, cell-to-cell communications, and lipid-assisted bioassays[71,73,80-85]. Similar to protein microarrays, the artificial cell membrane or lipid vesicles can obtain the benefits of microarrays integrated with microwell system[28,41,50,86].

Kim *et al*. presented supported lipid bilayer (SLB) membrane arrays in PEG microwells inside a PDMS or PEG microchannel having exposed microwells fabricated by capillary molding or micro contact printing (Figure 6)[28,41]. The liposomes in flow were captured in PEG microwells and fused onto the exposed substrate, forming lipid bilayer structure. The functionality of the patterned SLBs was tested by confirming the specific binding interactions between the biotin-labeled lipid bilayer and streptavidin. Furthermore, docking of single liposome onto PEG nanowells constructed on exposed gold electrode was also demonstrated for fabricating an electrochemical biosensor[50]. The single liposome-based biosensor has enhanced sensitivity resulting from the functional affinity by minimizing nonspecific binding. These patterning methods for lipid membrane structures provide attractive platforms for high-performance lipid-based immunoassay and observing cell membrane interactions.

Cell arrays

Cell culture and analysis in microfluidic systems have attracted much attention in various fields including cell biology, neurobiology, pharmacology, and tissue engineering with new discoveries[14,87-90]. The microfluidic system provides several advantages for cell study, such as precisely control of flows having nutrients, reduced time and cost for cell culture experimentation, and *in vivo*-like environments in a spatio-temporal manner. The PEG microwells in microchannel can be also applied to manipulation of cells such as mammalian cells[32,66,91], cell aggregates[92-96], and bacteria[97]. Especially, tall microwell structure provides physical confinement and barriers against the fluidic shear stress to cells docked in the wells.

Khademhosseini *et al*. introduced PEG microwells in PDMS microchannels for culturing mammalian cells within specific locations in the microchannel (Figure 7)[32]. Various shapes of PEG microwells having exposed or unexposed substrate were used to dock cells in fluidic environments. In the case of substrate-exposed microwells, fibronectin proteins can be pretreated onto the surface, so that cells can be biochemically attached to the surface with specific interactions. Such immobilized cells in the microwells remained stable and viable and were able to manipulate the sequential pro-

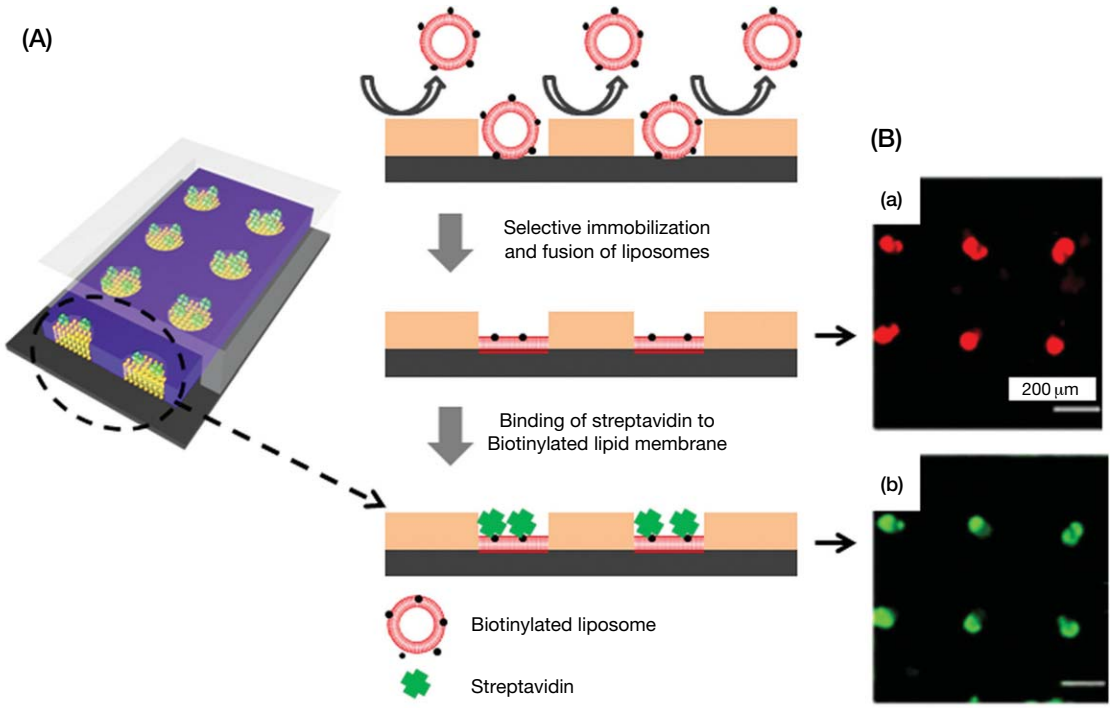

Figure 6. (A) A schematic illustration about patterning of supported lipid bilayers (SLBs) on PEG microwells in microchannel, and binding assay between biotinylated SLBs and streptavidin. (B) Red fluorescent images of the patterned biotinylated SLBs (a) and green fluorescent images of streptavidin after the conjugation (b). Reproduced from 28, copyright 2006, with permission from Royal Society of Chemistry.

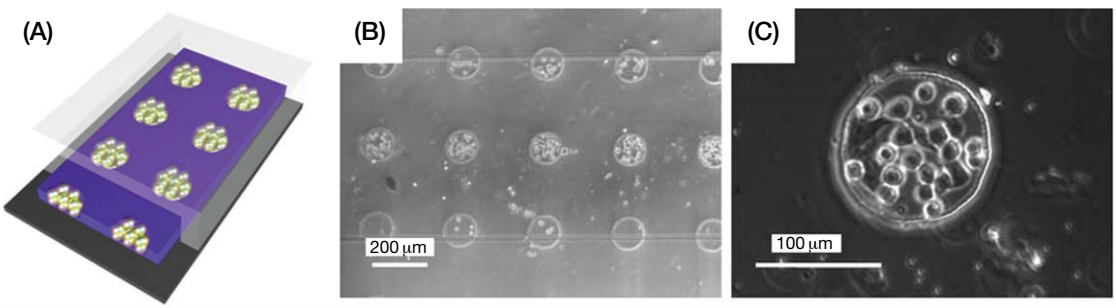

Figure 7. (A) A schematic illustration of patterned cells on PEG microwells in microchannel. (B) Immobilized cells (NIH-3T3) in exposed microwells. (C) Enlarged image of (B). Reproduced from 32, copyright 2004, with permission from Royal Society of Chemistry.

cesses such as immunostaining or live-cell imaging with biomarkers. Furthermore, utilizing reversible sealing of PDMS microchannel, multi-phenotype cell patterning within a microchannel was achieved[66]. In particular, the multi-phenotype cell patterning is a necessary process for high-throughput drug screening and tissue engineering. Serial orthogonal placing of reversibly sealed microchannel arrays can deliver a unique set of fluids or cell types to the specific locations of microwells patterned with multiple cell types.

PEG microwells for cell array are also useful for applying a microfluidic cell sorting technique, dielectrophoresis. Tsutsui et al demonstrated pattering of the embryonic stem cells in the exposed PEG microwells within PDMS microchannel, by using dielectrophoresis[98]. Through such active method combined with non-biofouling property of PEG, highly efficient cell patterning was achieved.

An important contribution from patterned PEG microwells has been reported recently. Here, unexposed

microwells lead to form cell aggregates when the cells are anchorage dependent due to the low adhesion affinity of PEG[92-96]. By taking advantage of this property, embryonic stem cells were initially docked in the PEG microwells, formed uniform spheroids, and then were retrieved with a small disturbance for directed differentiation[92,94]. In addition, hepatocyte spheroids were spontaneously formed with high viability and maintaining their activity related to the metabolism[93,95]. This novel approach would find many uses in various applications such as tissue engineering, cell biology, and drug discovery.

As well as array of mammalian cells and its aggregates, the PEG microwells have been applied to the array of bacteria[97]. The pattering of bacteria through the PEG microwell is useful for detection of toxic bacteria and high-throughput single-cell quantification of bacteria.

Summary and outlook

In this review, PEG microwells in microfluidic platform have been overviewed with their detailed fabrication methods and a wide range of applications for patterning biosamples in an array format. The fabrication of PEG microwells in microchannels consists of three steps, i) forming top microchannel layer, ii) bottom PEG microwell layer, iii) irreversible bonding microchannel and PEG microwell layer. With soft lithographical approaches, the PDMS or PEG microchannel layer is fabricated by molding of PDMS or PEG against a hard master. Unexposed or exposed PEG microwells are constructed on glass substrate either by contact printing or capillary molding. In an integrated microfluidic system, the microchannel structure fully exploits the advantages of microfluidic systems such as small volume analysis, and high-throughput screening with laminar flow. In addition, PEG microwell structures not only offer a simple patterning method of various biosamples such as protein, lipid membrane, and cells, but also serve as a robust physical barrier to minimize chemical noises through its non-specific binding property.

Despite of the above-mentioned advantages, several limitations should be addressed. First, controlling transport of biosamples to an individual PEG microwell is great benefit for high-throughput screening, but is challenging. In this sense, constructing stimuli-responsive valve-like structure on PEG microwell can be one of the potential solutions. Second, escaping of docked biosamples from microwells by flow can lower the accuracy of the assay. Clever design of shape and size of PEG microwells would help solve this issue. PEG microwells in microchannels would present great potential and strength for assaying and handling various biosamples in diverse biological fields such as cell/tissue engineering, pharmacology, and proteomics.

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