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Mini Review

# Soft Lithography for Microfluidics: a Review

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## Abstract

**Soft lithography has provided a low-expertise route toward micro/nanofabrication and is playing an important role in microfluidics, ranging from simple channel fabrication to the creation of micropatterns onto a surface or within a microfluidic channel. In this review, the materials, methods, and applications of soft lithography for microfluidics are briefly summarized with a particular emphasis on integrated microfluidic systems containing physical microstructures or a topographically patterned substrate. Relevant exemplary works based on the combination of various soft lithographic methods using microfluidics are introduced with some comments on their merits and weaknesses.**

**Keywords:** Soft lithography, Microfluidics, Micro/nanofabrication

## Introduction

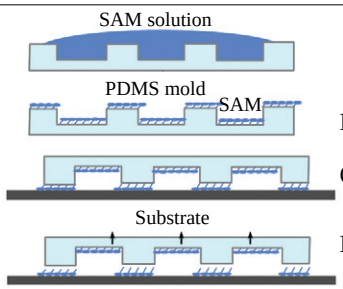
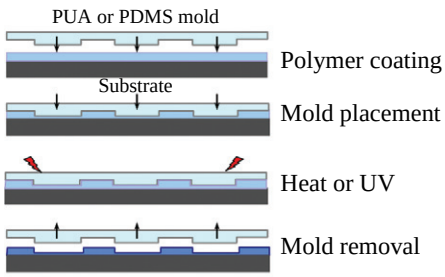
The development of biological micro-electromechanical (MEMS) devices comprised of microfluidic channels or a "Lab-on-a-chip", may revolutionize biological analysis and create new ways of analyzing cells *in vitro*[1]. Such microdevices are advantageous in that they use tiny volumes of reagents and can be scaled-up for a high-throughput analysis. Recently, microfluidic systems integrated with embedded physical microstructures or a topographically patterned substrate on the micro- or nanoscale (called an "integrated microfluidic system" in this paper, hereafter) have attracted significant attention. This is because the control of surface properties and the spatial presentation of functional molecules within a microfluidic channel is important for the development of diagnostic assays and microreactors, and for performing fundamental studies of cell biology and tissue engineering[2]. Also, precise control over material transport and manipulation has enabled the analysis of intracellular parameters and the detection of cell metabolites, even on a single-cell level[1,3-5].

Soft lithography is a valuable tool for an integrated microfluidic system. Soft lithography was first introduced by G.M. Whitesides *et al.*[6,7] and includes a family of techniques involving a soft polymeric mold such as a polydimethylsiloxane (PDMS) replica from an original hard master. Mold masters are typically fabricated by photolithography in order to define a stamp pattern. Stamps are made by curing a prepolymer of PDMS onto a mold master. Apart from "replica molding", a well-known technique for generating a polymer channel replicated from an original silicon master, soft lithography provides many simple yet robust routes toward the fabrication of micro/nanostructures onto a surface or within a channel. Although several review articles regarding integrated microfluidic systems for cell analysis are available in the literature[1,3,8-11], in this review we mainly focus on the microfluidic systems that are integrated with physical micro/nanostructures fabricated by soft lithography. Exemplary works of integrated microfluidic systems are introduced in the areas of cell docking/separation and protein or lipid membrane arrays.

## Soft Lithography Materials

An integrated microfluidic system is usually created by combining two layers: a substrate layer constructed with micro/nanostructures on the surface (a micro- or nanostructured layer), and a channel layer with a microchannel impression. For the channel layer, PDMS is widely used to fabricate the microfluidic channels because of its favorable mechanical/optical properties and its simple manufacturing by rapid prototyping[12]. To cure the PDMS prepolymer in general, a mixture of 10 : 1 silicon elastomer and a curing agent is poured onto the master and placed at 70-80°C for 1 h (see Figure 1A). In addition to the PDMS channels, other microfluidic devices have been introduced using different channel materials such as photocurable perfluoropolyethers, biodegradable polymers, photosensitive polymers, and polymerized hydrogels[13-21]. However, biofouling,

**Table 1.** Soft lithographic methods for the fabrication of micro/nanopatterns.

| | Contact printing (ref. 38, 39) | Capillary molding (ref. 40-43) |
|---|---|---|
| Mold | Soft mold<br>Poly (dimethylsiloxane) (PDMS)<br>Hard PDMS (h-PDMS) | Poly (dimethysiloxane) (PDMS)<br>Rigiflexible mold (PUA) |
| Resolution | ~500 nm<br>~50 nm with h-PDMS | ~50 nm with PUA mold |
| Application | 1. Channel fabrication<br>2. Direct printing of biological molecules (1D chemical modification) | 1. Channel fabrication<br>2. Fabrication of micro/nanostructures (2D structure) |
| Process |  |  |

weak mechanical properties, and the need for extensive expertise have potentially limited the versatile use of these devices.

Micropatterns can also be formed using PDMS molds of various patterns in a positive or negative sense. As with a PDMS channel, a PDMS replica is peeled off from a silicon master prepared using photolithography. For patterning materials, polyethylene glycol (PEG) is frequently used due to its easy processability and non-biofouling properties. To date, biofouling and a subsequent device malfunction have deteriorated device performance through hydrophobic interactions between the PDMS surface and biological samples[22]. When small sample quantities are involved, such as rare proteins, any sample loss through a non-specific binding may result in a critical error in the final analysis. To solve this challenge, silicon-based (e.g., silicon, glass, quartz, and PDMS) platforms have been surface modified using non-biofouling materials such as polyethylene glycol (PEG)[12,23-28]. It is believed that the resistant nature of PEG-based polymer may be attributed to a polymer chain mobility and steric stabilization force[29]. Surface modification of silicon-based devices with PEG can be performed using physical adsorption[23], covalent immobilization such as grafting and chemical coupling[24-26], or a gas phase treatment (plasma or deposition)[12,27,28]. These efforts have proven to be successful, but they might not be able to guarantee conformal coating and long-term stability, i.e., modified PDMS surfaces slowly recover their original hydrophobicity[30].

To overcome the above-mentioned problems, a technique has been developed to fabricate micro- and nanochannels comprised entirely of cross-linked polyethylene glycol (PEG) by using UV-assisted molding[31]. A flat or patterned PEG substrate was used for the fabrication in which a PEG channel was bonded to a patterned PEG substrate with microwells. Further details on the fabrication and analysis of a PEG channel, shown in Figure 1B, can be found in[31].

## Soft Lithography Fabrication Methods

To form an integrated microfluidic system, a micro /nanostructured layer is bonded to a microchannel layer[32-37]. Various micro/nanofabrication techniques can be used for manufacturing integrated microfluidic systems. Here, two soft lithographic methods are introduced to fabricate micro/nanopatterns onto a surface or within a microfluidic channel: contact printing[38,39] and capillary molding[40,41]. Contact printing generates a non-structured, chemically modified surface, while capillary molding fabricates a topographically modified physical micro/nanostructure.

Contact printing is a direct patterning method using an elastomeric stamp prepared by soft lithography[38,39]. After curing, PDMS stamps are soaked in a molecular “ink” and brought into conformal contact with a substrate in order to transfer the ink onto the substrate surface. Contact printing enables easy stamp replication, fast printing using parallelization, and low-cost batch production. The polymer stamps also

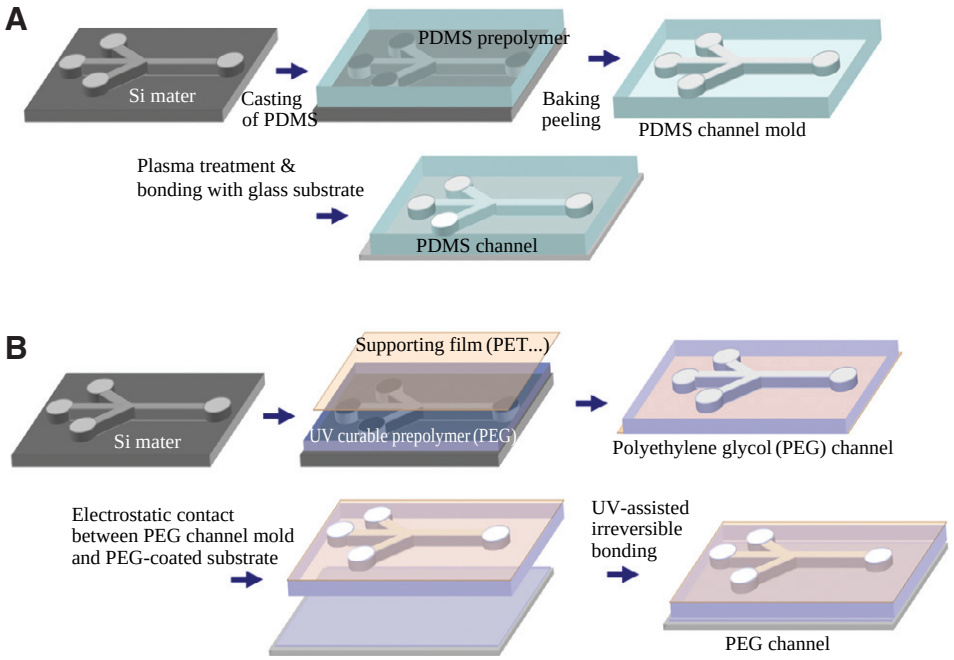



**Figure 1.** Schematic representation of microfluidic channel fabrication. (A) PDMS channels by using replica molding and (B) PEG channel by UV-assisted irreversible bonding.

minimize the problems of sample carry-over and cross contamination. However, contact printing has some limitations that are mainly caused by the use of a soft polymer stamp[38]. The swelling of a stamp during inking often results in an increase in the pattern size by diffusion of the excessive printed molecules on the substrate. Also, contact printing generates a one-dimensional pattern within a channel.

Capillary molding is an improved version of soft lithography by combining a nanoimprint and the use of an elastomeric mold[40,41]. When a patterned (positive or negative) PDMS mold is placed on a polymer surface and heated above the polymer's glass transition temperature ($T_g$), capillarity forces the polymer to melt into the void space of the channels formed between the mold and the polymer, thereby generating a negative replica of the mold. A pattern formation is also possible with a solvent-laden polymer or a UV-curable resin followed by solvent evaporation or exposure to UV light. Recently, a UV curable mold made from polyurethane functionalized with acrylate groups has been introduced to replace PDMS molds for sub-100-nm lithography, thus expanding the use of capillary molding to cell biology studies[42,43].

## Integraion of Soft Lithography with Microfluidics

### Arrays of Mammalian and Yeast Cells

In a microfluidic system, cells flow through a channel by a stable laminar flow, which is useful for highly effective and accurate cell manipulation[44]. Controlled transport, immobilization, and manipulation of biological molecules and cells are important functions to be incorporated into a microfluidic device in order to carry out on-chip biochemical and cell biological experiments[32].

Several approaches have been introduced to immobilize cells within particular regions of a microfluidic channel: laminar flow patterning[45], pre-patterning with adhesive ligands[46], and immobilization inside hydrogels[47]. However, there are potential limitations in these approaches. Laminar flow patterning can only pattern a limited shape of patterned regions, and hydrogel fabrication using UV radiation induces the exposure of cells to potentially toxic environments[48]. Also, the direct patterning of cells on a pre-patterned substrate of a channel could give rise to shear-driven modifications in cell behavior. To overcome these limitations, integrated microfluidic systems have been developed to capture and localize cells within particular regions of a channel with the aid of soft lithography[32,33,48-50].

Khademhosseini *et al*. introduced a simple soft lithographic technique for fabricating PEG microstructures within microfluidic channels that can immobilize cells within specific locations[48]. Microwells of various shapes were used to capture cells despite a shear flow within a channel. Immobilized cells within the microwells remained viable and were stained for cell surface receptors by a sequential flow of antibod-

ies and secondary fluorescent probes. Using soft lithography and reversible sealing, an advanced microwell system was also fabricated for multiphenotype cell patterning within an array of reversibly sealed microfluidic channels[49]. Microfluidic channels deliver various fluids or cells onto specific locations and microwells on a substrate in order to capture and immobilize cells within low shear stress regions (Figure 2A). Alternative orthogonal placing of reversibly sealed microchannel arrays can deliver a unique set of fluids or cell types. Multiphenotype cell patterning on specific regions within a two-dimensional channel system can be applied to high-throughput drug screening and tissue engineering.

Park *et al*. proposed alternative techniques to fabricate microwells, either by soft lithographic capillary molding of UV curable PUA onto a glass substrate or by a direct replica molding of PDMS as shown in Figure 2B[51]. Cell docking within microwells inside a microfluidic channel was induced by receding meniscus in order to capture non-adherent yeast cells. First, cell suspension of the yeast cells was introduced into the microfluidic channel by a surface-tension-driven capillary flow. One to multiple yeast cells were then spontaneously captured onto microwells by lateral capillary force created at the bottom of the receding meniscus subsequently generated by natural evaporation.

Recently, Lee *et al*. presented a simple method for fabricating shear-protecting cell containers integrated within a microfluidic channel[52]. A capillary molding technique was used to generate hollow bottle-shaped structures by exploiting the partial capillary rise along the slanted walls with an acute wedge angle, as shown in Figure 2C. The molded hollow microstructure was used to capture the budding yeast cells within a microfluidic channel, and the shear-protecting ability was evaluated by measuring the fluorescent intensities of docked cells upon stimulation with a mating pheromone or high osmolarity over time. The unique shape of the hollow cell container offers superior shear-protecting ability compared with previous microwell-type structures[49,53]. In this approach, experimental and simulation results demonstrated that a higher microstructure with smaller neck dimension offer a more stable, non-invasive microenvironment for docked cells.

The integration of cell manipulation with a channel platform allows the measurement of biological responses of cells within a confined microscale feature.

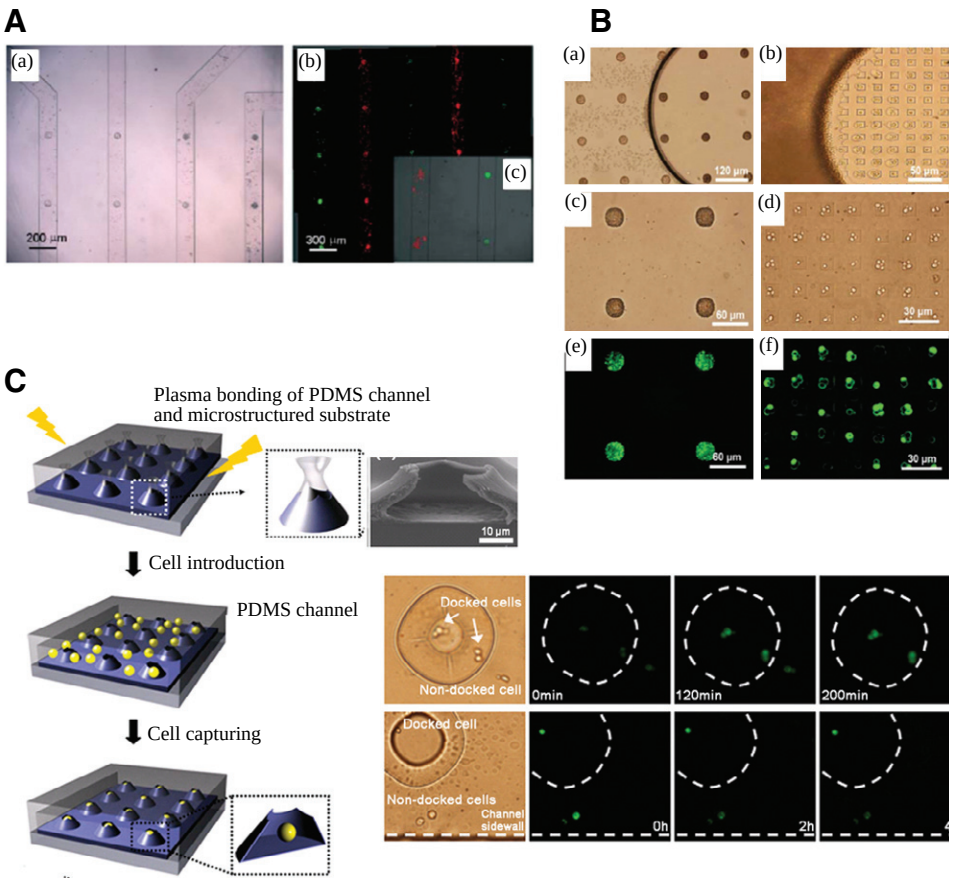



**Figure 2.** Integrated microfluidic devices for cell docking and manipulation. (A) Multiphenotype cell patterning on specific regions within a two-dimensional channel system using reversible sealing of PDMS channel with patterned surface. Reprinted with permission from[49]. (B) Docking of non-adherent yeast cells using receding meniscus inside a microfluidic chip with a microwell. Reprinted with permission from[51]. (C) Molded hollow microstructure having shear protecting ability within a microfluidic channel and capturing the budding yeast cells Reprinted with permission from[52].

This approach can provide a potential tool for high-throughput screening of single to multiple cells or optimization of cell-soluble signal interactions for biological research or tissue engineering. However, many methods developed so far lack control over surface chemistry or topography for anchorage-dependent cells within the captured microstructures, which would limit the widespread uses to most mammalian cells, in particular, for long-term cultures. The development of simple and direct techniques for fabricating microstructures within microchannels with precise control over their surface properties is of potential benefit.

## Protein and Lipid Bilayer Arrays

Integration of protein or lipid bilayer arrays with microfluidics is also important for a high-throughput analysis of diseases and cell-to-cell communications. The roles of proteins are enormously diverse and include mechanical support, signaling, and sensing. Beyond their central importance to biology, proteins are of great interest because these sub-microscale molecules have the potential to be integrated into microfluidic devices. Aiming toward this application, a micro/nanopatterning technique is required that is capable of accurately depositing proteins at predefined locations while retaining their native functionality.

To pattern microfluidic channels using soft lithography, surface patterning is usually performed prior to the attachment of the PDMS mold to the substrate. Non-specific adsorption of biomolecules should be suppressed for selective binding of proteins or lipid layers on a surface or inside a channel. Contact printing and capillary molding can be used to achieve this purpose via surface modification or a physical barrier with non-biofouling polysaccharide or PEG coatings[46,54,55].

Khademhosseini *et al*. introduced a soft lithographic technique to fabricate micropatterns of PEG or hyaluronic acid within a microfluidic channel[46]. In this approach, the patterned regions were protected from oxygen plasma by controlling the dimensions of the PDMS stamp and by leaving the stamp in place during the plasma treatment process. The PDMS stamp was then removed, and the microfluidic mold was irreversibly bonded to the substrate. The non-biofouling patterns were then used to fabricate arrays of fibronectin and bovine serum albumin. In addition, laminar flow patterning was used to control the adsorption of multiple proteins in various regions of an exposed substrate. It was demonstrated that the laminar flow of multiple proteins may be used to generate patterned protein arrays within the channels. The spatial patterning of multiple proteins within individual islands can be potentially useful in studying the effects of spatial organization of multiple extracellular matrix components on cell behavior such as asymmetric cell division[56].

Since the introduction of micropatterning of lipid bilayers by Boxer *et al*. in 1997[57], a number of methods have been developed for lipid membrane microarrays, such as deep-UV illumination through a photomask under aqueous conditions[58,59] and a polymer lift-off method[60-63]. Microcontact printing of the composition arrays of phospholipids bilayers was first accomplished by printing different sized bilayers of the same composition onto surface patterned corrals[64-66]. Also, micromolding in capillaries (MIMIC) was used to pattern lipid membranes by utilizing a laminarly flowing stream, as shown in Figures 3A and B[67-69]. The use of a laminar flow inside microfluidic channels is also an effective means of producing composition arrays of supported phospholipid bilayers in which two distinct chemical components can be varied simultaneously along a one-dimensional gradient[67,70].

Takeuchi *et al*. presented a method to form a planner lipid bilayer in a microfluidic chip by contacting two monolayers that are assembled at the interface between water and an organic solvent containing phospholipids[71]. Particularly, the bilayer was formed in a vertical direction, unlike in other approaches. The functionality of the bilayer membrane was proved by the insertion of a reconstituted antibiotic peptide.

Recently, Kim *et al*. presented capillary molding and microcontact printing to create patterns of supported lipid bilayer (SLB) membranes onto a surface and inside a microchannel. Micro- or nanopatterns of a PEG random copolymer were fabricated on glass substrates by capillary molding to form a template layer against adsorption of lipid membranes[72]. As compared to microcontact printing, the molded structures provided a clean interface at the patterned boundary, and the adhesion on the PEG surface was strongly restricted. The functionality of the patterned SLBs was tested by measuring the binding interactions between the biotin-labeled lipid bilayer and streptavidin. SLB arrays were fabricated using a spatial resolution of down to ~500 nm on a flat substrate and ~1 μm inside the microfluidic channels.

To further elaborate on the performance of the lipid-based microfluidic device for analytical applications, monolithic PEG microchannels were fabricated by the same authors utilizing UV assisted molding, in which PEG microwells were located on the bottom of the channel, as shown in Figure 3C[31]. The

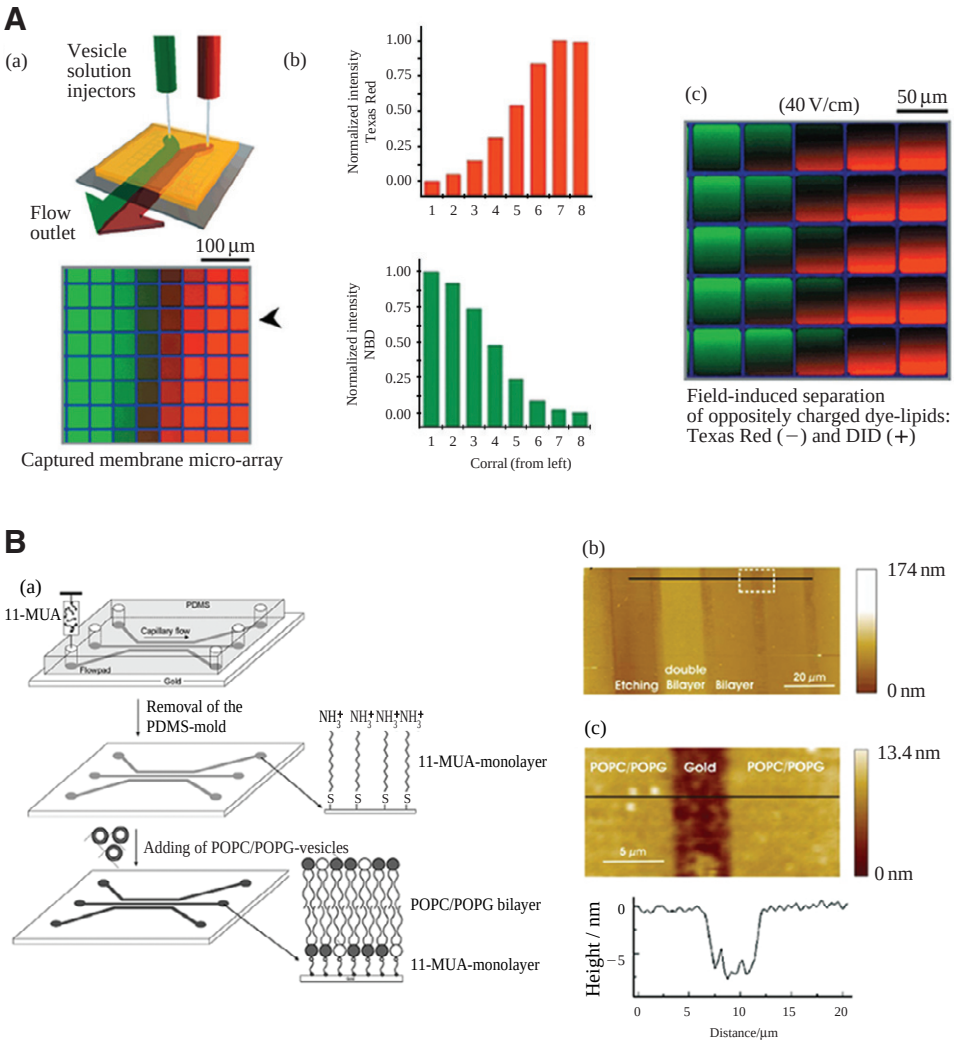



**Figure 3.** Microarrays of lipid membrane within a microfluidic channel. (A) Schematic illustration of the converging flow configuration used to produce the limited mixing of two types of vesicles in solution. Reprinted with permission from[67]. (B) Microstructuring of lipid bilayers on gold surfaces by MIMIC employing chemically modified PDMS. Reprinted with permission from[68]. (C) Supported bilayer membranes (SBMs) by using capillary molding of a PEGbased polymer within a PEG channel. Reprinted with permission from[31].

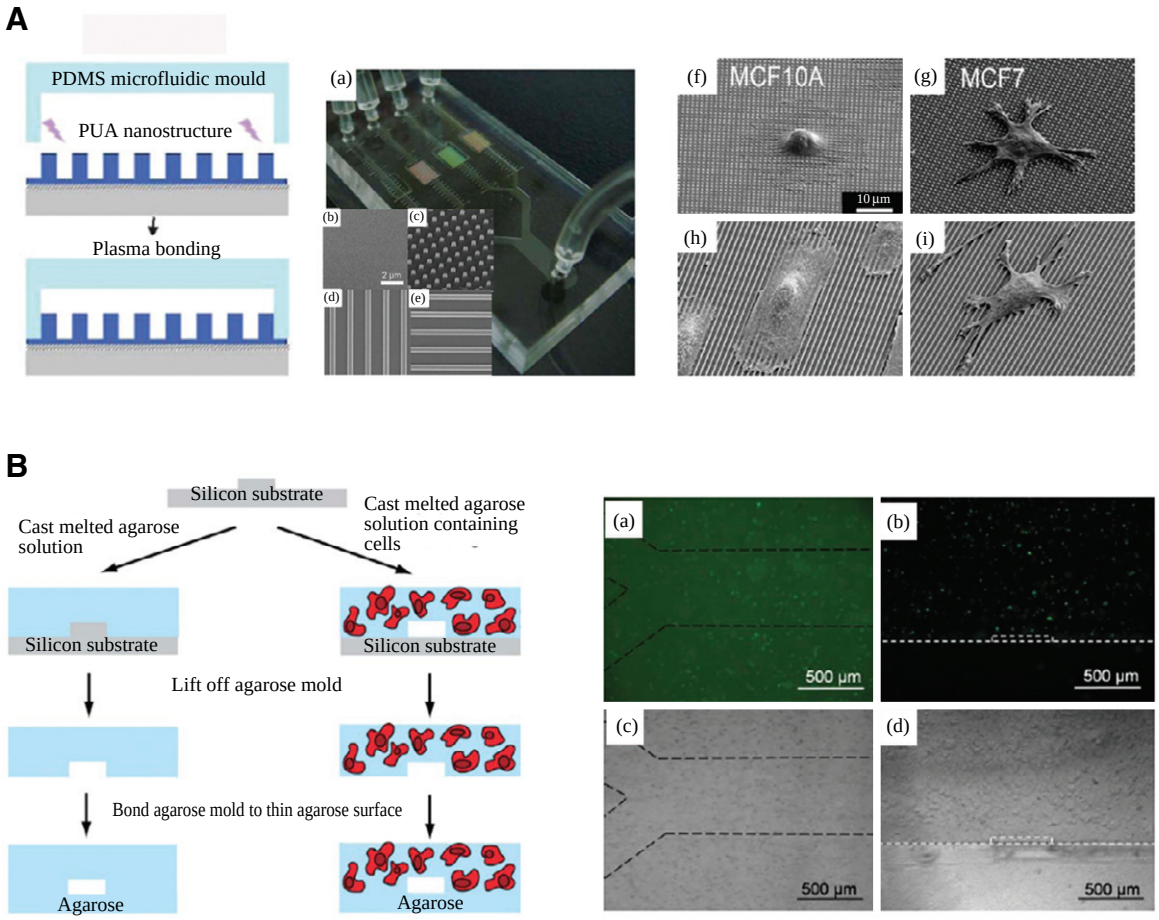



**Figure 4.** Other applications of integrated microfluidic devices. (A) PUA nanostructures included inside a microfluidic channel for study on cell separation by adhesion difference. Reprinted with permission from[83]. (B) Encapsulation of mammalian cells within the bulk material of microfluidic channels. Reprinted with permission from[84].

lipid bilayer membranes were neatly patterned onto the pre-defined regions of the substrate. Non-specific adsorption, which is frequently observed for most microfluidic devices, was not seen. Also, streptavidin was selectively bound to the biotinylated lipid bilayer membrane.

## Other Applications

Cells are inherently sensitive to local mesoscale, microscale, and nanoscale topographic and molecular patterns in an extracellular matrix (ECM) environment[73-75]. Integration of microfluidics and micro/nanofabrication methods can thus be employed to precisely control the composition and topography of the ECM adhesion proteins on a topographically patterned substrate within a fluidic channel.

After the pioneering work of Chen *et al*., micro/nanoscale topographic features have been incorporated into an *in vitro* experimental platform to mimic various *in vivo* 3D ECM environments with structural and mechanical similarity by using advanced fabrication methods[76,77]. To improve the design of the biomaterial interface within a channel, Zaari *et al*. created substrates with variations in mechanical compliance by combining microfluidics and photopolymerization[78]. In this integrated platform, a well-controlled gradient-compliance profile on the microscale was used to study cell migration guided by substrate rigidity (called "durotaxis"). In addition to this chemical tuning by controlling the crosslinking density of hydrogels, one can control the wettability, adhesion, or contact guidance of cells by incorporating various physical micro/nanostructures[79,80]. For example, a recent study demonstrated that superhydrophobic surfaces are generated inside a microfluidic channel by forming high-density arrays of tall and sharp nanoposts ("nanoturfs") with a submicron pitch on the top and bottom substrates[81]. Martines *et al*.

presented a microfluidic device having nanopits in a microchannel in order to investigate their response to cell adhesion under dynamic conditions by means of a shear flow[82]. Dynamic cell adhesion was quantified and compared on flat and nanopitted polymethyl methacrylate (PMMA) substrates with a cell suspension flow.

Kwon *et al*. recently developed a label-free microfluidic method for the separation and enrichment of human breast cancer cells using controlled cell adhesion as a physical marker[83]. As shown in Figure 4A, the nanostructured polymer surfaces (400 nm pillars, 400 nm perpendicular, or 400 nm parallel lines) were constructed on the bottom of polydimethylsiloxane (PDMS) microfluidic channels in a parallel fashion using a UV-assisted capillary molding technique to maximize the adhesion difference between human breast epithelial cells (MCF10A) and cancer cells (MCF7). The normal cells showed higher adhesion than the cancer cells regardless of culture time and surface nanotopography at all flow rates, resulting in label-free separation and an enrichment of the cancer cells. The separation efficiency was increased on the 400 nm perpendicular line pattern followed by flow-induced detachment.

Ling *et al*. demonstrated the encapsulation of mammalian cells within a bulk material of microfluidic channels for applications ranging from tissue engineering to cell-based diagnostic assays, shown in (Figure 4B)[84]. Channels of different dimensions were generated, and it was shown that agarose, though highly porous, is a suitable material for performing microfluidics. Cells embedded within the microfluidic molds were well distributed, and media pumped through the channels allowed the exchange of nutrients and waste products.

## Conclusions

Soft lithography has been proven useful in microfluidics under a wide range of applications from channel fabrication to pattern generation. In particular, the fabrication of precise micro/nanostructures on a surface or within a fluidic channel can not only offer simple routes toward cell docking and manipulation, but can also serve as a template for protein or lipid bilayer arrays. In addition, controlled adhesion using micro/nanostructures in a microfluidic device can be used as a label-free method for the separation and enrichment of cancer cells from a body fluid containing a mixed population of normal and cancerous cells. To fabricate physical structures for mechanical topography and surface patterning, the materials used need to be biocompatible and compatible with fluidic applications, and they should provide rigid, smooth surfaces with dimensions relevant to the biological sample, such as a cell, bacteria, and yeast. It is envisioned that along with other advanced fabrication methods, soft lithography will continuously find uses in integrated microfluidic systems due to its simple, cheap, and low-expertise route toward micro/nanofabrication.

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