Video Article

A Versatile Method of Patterning Proteins and Cells

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Abstract

Substrate and cell patterning techniques are widely used in cell biology to study cell-to-cell and cell-to-substrate interactions. Conventional patterning techniques work well only with simple shapes, small areas and selected bio-materials. This article describes a method to distribute cell suspensions as well as substrate solutions into complex, long, closed (dead-end) polydimethylsiloxane (PDMS) microchannels using negative pressure. This method enables researchers to pattern multiple substrates including fibronectin, collagen, antibodies (Sal-1), poly-D-lysine (PDL), and laminin. Patterning of substrates allows one to indirectly pattern a variety of cells. We have tested C2C12 myoblasts, the PC12 neuronal cell line, embryonic rat cortical neurons, and amphibian retinal neurons. In addition, we demonstrate that this technique can directly pattern fibroblasts in microfluidic channels via brief application of a low vacuum on cell suspensions. The low vacuum does not significantly decrease cell viability as shown by cell viability assays. Modifications are discussed for application of the method to different cell and substrate types. This technique allows researchers to pattern cells and proteins in specific patterns without the need for exotic materials or equipment and can be done in any laboratory with a vacuum.

Video Link

The video component of this article can be found at <https://www.jove.com/video/55513/>

Introduction

In tissue engineering and biosensing, the ability to control the spatial organization of proteins and cells on a μm scale, has become increasingly important over the last four decades^{1,2,3}. Precise spatial organization of proteins and cells has allowed researchers to examine the interaction between cells and substrates containing similar or different types of cells, to guide cell growth, and to immobilize biomolecules for the fabrication of biosensors^{4,5,6,7,8,9}.

Current methods of patterning proteins include photopatterning and microcontact printing. Photopatterning utilizes light sensitive material which is crosslinked upon exposure to ultra violet (UV) light. UV light directed at a photomask (consisting of transparent areas with darker regions to prevent UV light transmission) causes crosslinking in specific regions which can then be used for subsequent attachment of biomaterials or cells^{10,11}. While this scheme is very accurate and allows for precise control of the topography of the culture surface, it is limited to UV-sensitive biomolecules that can be patterned by UV radiation¹². Microcontact printing is another popular method of patterning specific proteins^{13,14}. In this method, a poly-dimethyl siloxane (PDMS) stamp is treated with a variety of surface modification reagents before being soaked in a solution of the chosen biomolecular substrate. It is then gently pressed onto a glass coverslip or other surface thus "stamping" the biomolecule onto the culture surface. However, stamping is limited to the type of material that can be transferred as well as the wettability of biomolecules to the surface of the PDMS stamp¹⁵.

Direct patterning of cells can be more difficult and relies on complex methods such as switchable substrates, stencil based methods, or patterning with specific cell adhesion molecules^{16,17}. These methods are limited in their ability to pattern cells due to the lack of compatible cell adhesion substrates, incompatibility of the process to work with sensitive biological cells and constraints, inconsistency in reproducing the patterning, and complexity of the procedure. For example, with switchable substrates, custom substrates need to be designed for every cell type, to switch their adherence to specific cell types without degradation upon exposure to the UV light and heat used in process^{17,18,19,20}. Stencil based patterning methods are versatile in their ability to pattern cells; however, it is difficult to manufacture PDMS stencils at the appropriate thicknesses for use^{16,21}. Direct injection of cells into PDMS microfluidic channels have some advantages such as: 1) ease in fabrication of microfluidic channels and 2) suitability for many different cells and substrates. However, the prevalent issue of air bubble capture during the

injection process due to the hydrophobicity of PDMS without the use of plasma cleaning, or other methods to decrease air bubbles, makes it difficult to consistently create patterned cells on glass or plastic surfaces²¹.

This work expands upon capillary micromolding^{22,23,24,25,26} and reports a method to inject protein and cell suspensions into microchannels. The method used here demonstrates the patterning of substrates and both direct and indirect patterning of specific cell types. This technique overcomes the high hydrophobicity of PDMS and eliminates the presence of bubbles during injection of either substrates or cells by taking advantage of the gas permeability of PDMS²⁷. This paper demonstrates the use of the technique with several different substrates and cell types. The article also highlights the fabrication of molds for soft lithography using conventional photolithography as well as a simple and low-cost adhesive tape method useful in resource limited settings^{28,29}.

Protocol

NOTE: Please consult all relevant material safety data sheets (MSDS) before use. Some of the chemicals used in this protocol are toxic and carcinogenic. Please use all appropriate safety practices (fume hood, glovebox) and personal protective equipment (safety glasses, gloves, lab coat, full length pants, closed-toe shoes) when using toxic or acid/base materials.

1. Fabrication of Master Molds for Soft Lithography using Photolithography

1. Draw the layout of the microchannel using a computer-aided design (CAD) drawing tool.
2. Print the layout on a blank mask plate using laser mask writer.
3. Rinse a 4 inch silicon wafer with acetone followed by isopropanol and dry with a nitrogen air gun to ensure that no solvent is left on the wafer.
4. Dehydrate the wafer by baking it on a hot plate at 200 °C for 5 min.
5. Select a negative photoresist designed for the desired height of the microchannels. For example, use negative photoresist SU-8 50 to obtain microchannels with a height of 50 µm.
6. Allow the wafer to cool to room temperature, and then align the wafer on the chuck of a spin coater.
7. Dispense 4 mL (1 mL/inch diameter of the wafer) of negative photoresist onto the center of the wafer.
8. Spin coat the wafer with a two-step spin cycle using a spinner. First, apply a spread cycle of 500 rpm with an acceleration of 100 rpm/s for 10 s. Then apply a spin cycle of 2,000 rpm with an acceleration of 300 rpm/s² for 30 s to obtain a 50 µm coating of photoresist on a wafer.
9. Soft bake the wafer in two steps on a hot plate according to the manufacturer's directions. For a 50 µm coating of photoresist, first pre-bake the wafer at 65 °C for 6 min and then immediately ramp up the hot plate temperature to post-bake the wafer at 95 °C for 20 min.
10. Allow the wafer to cool to room temperature and then load the wafer onto the lithography stage.
11. Align the mask on the wafer using mask aligner and expose the wafer to UV light (as per the manufacturer's instructions) at an intensity of 1.5 mW/cm² for 146 s to apply the total UV dose of 220 mJ/cm² required for a 50 µm thick layer of photoresist.
12. Apply post exposure bake to the wafer in two steps on a hot plate. For a 50-µm coating of photoresist, first pre-bake the wafer at 65 °C for 1 min and immediately ramp up the hot plate temperature to post-bake it at 95 °C for 5 min.
13. Develop the wafer in by submerging the wafer in SU-8 developer and gently shaking until the features become clear on the wafer. Develop the wafer for ~6 min for the 50 µm thick photoresist.
14. Rinse off excess developer with isopropanol and ethanol, then dry the wafer with a nitrogen air gun.
15. Hard bake the silicon wafer on a hot plate at 150 °C for 15 min.
16. Place the wafer in a desiccator with 25 µL of the silanizing agent tridecafluoro-1,1,2,2-tetrahydrooctyl-1-trichlorosilane.
NOTE: The silanizing agent is corrosive, thus should be used in an acid/base fume hood, with appropriate personal protective equipment (safety glasses, gloves, lab coat, full length pants, closed-toe shoes).
17. Apply the vacuum for 5 min and expose the wafer to the vapors of silane for 30 min without releasing the vacuum.
18. Transfer the wafer into a suitably sized Petri dish.

2. Fabrication of Master Molds for Soft Lithography Using Adhesive Tape

1. Draw the layout of the microchannel using a CAD drawing tool and print the drawing on white paper.
2. Clean a glass slide large enough to accommodate the design with isopropanol and then dry the slide with an air gun.
3. Attach adhesive tape onto the cleaned glass slide. Be careful not to trap any air bubbles.
4. Tape the sides of the glass slide onto the paper with the design, with the tape side up.
5. Use a scalpel to cut the tape on the glass slide using the paper design as a reference and then peel off tape from unwanted areas of the glass slide.
6. Rinse the glass slide with isopropanol and then dry with an air gun. Bake the glass slide in an oven at 65 °C for 30 min.
7. Gently roll over the tape with a rubber roller to remove air bubbles and allow the slide to cool to room temperature.

3. Soft Lithography Fabrication of the PDMS Devices

1. Mix PDMS elastomer and its curing agent in a ratio of 10:1 (w:w), stir the mixture vigorously, and then degas the mixture by placing it into a vacuum chamber until no air bubbles appear in the mixture.
2. Pour the mixture onto a silanized silicon mold or adhesive tape mold in a petri dish to obtain a ~2 mm thick layer of PDMS and degas until all air bubbles disappear.
3. Cure the PDMS in an oven at 65 °C for 2 h.
4. Use a scalpel to cut the PDMS layer at least 5 mm away from the features and then peel the cured PDMS off the mold using tweezers.
5. Punch a single inlet hole with a 1 mm biopsy punch anywhere on the microchannel, preferably at the end of a network of microchannels as seen in **Figure 2a** and **4b**.

6. Clean the PDMS cast with adhesive tape to remove dust particles adsorbed onto the device. Sterilize the PDMS microchannel device (PDMS cast) by rinsing with a solution of 70% ethanol followed by sterile deionized (DI) water, and expose to UV for 30 min.
7. Keep the sterile PDMS device in a sterile petri dish until use.

4. Substrate Patterning

1. Place the sterile PDMS cast using tweezers onto a sterile glass coverslip or petri dish and apply gentle pressure using the tip of the tweezers. NOTE: The PDMS cast makes a conformal but reversible seal with the glass coverslip or petri dish forming microchannels without use of any adhesive.
2. Place a droplet (20 - 40 μ L) of the substrate solution on the inlet completely covering the inlet hole. Pattern Poly-D-Lysine (PDL) by placing a 20 μ L droplet of PDL in sodium tetraborate buffer on the inlet of the microchannels.
3. Put the petri dish in a vacuum of $\sim$ 254 mmHgA (equivalent to house vacuum in biological laboratories) for 10 min, and observe air coming out of the channel in the form of bubbles.
4. Release the vacuum and observe the substrate solution flowing into the microchannel.
5. Incubate the dish at the appropriate conditions for substrate adherence. See **Tables 1 and 2** for incubation conditions (these vary depending on the substrate). For PDL patterning, incubate for 1 h at 37 $^{\circ}$ C.
6. Carefully peel off the PDMS stamp using sterile tweezers and wash the pattern on glass coverslip thrice with DI water.
7. Add a 1% bovine serum albumin (BSA) solution to the petri dish to cover the dish or the glass coverslip and incubate overnight at 37 $^{\circ}$ C. NOTE: This will reduce non-specific adherence in uncoated regions.
8. Aspirate off the 1% BSA solution, and the patterned substrate is now ready to use.

5. Indirect Patterning of Cells

1. Prepare the cell suspension in serum-free culture medium. The cell type and cell density are listed in **Table 1**.
2. Add the cell suspension to the patterned Petri dish and completely submerge the patterned region in cell suspension.
3. Incubate the petri dish at 37 $^{\circ}$ C in an incubator for 15 min to allow cells to attach to the patterned substrate.
4. Aspirate the excess cell suspension from the Petri dish and wash the patterned cells three times with phosphate buffered saline (PBS) while gently shaking for 10 s to remove unattached cells.
5. Add the appropriate culture medium to the patterned cell cultures and incubate the patterned cells at 37 $^{\circ}$ C in a CO₂ incubator.

6. Direct Patterning of Cells

NOTE: This technique is an alternative to the indirect cell patterning described in step 5. However, unlike in step 5, in this technique cells are patterned on tissue culture surfaces with or without substrate coating.

1. Sterilize the microfluidic device by rinsing with a solution of 70% of ethanol followed by DI water.
2. Soak the device overnight in a solution of 1% BSA to prevent the cell adhesion to the PDMS.
3. Dry the device at room temperature and attach it to the bottom of the tissue culture-treated petri dish.
4. Prepare the cell suspension in serum-free culture media. The cell type and cell density are listed in **Table 2**.
5. Place a droplet (4 - 8 μ L) of the cell suspension, enough to fill the microchannels, on the inlet completely covering the inlet hole.
6. Put the petri dish in a vacuum for 10 min and observe air coming out of the channel in the form of bubbles. Release the vacuum and observe the cell suspension flowing into the microchannel.
7. Incubate the Petri dish in an incubator to promote cell adhesion as per the incubation conditions (depend on type of cell patterned) mentioned in **Table 2**. Add PBS to the petri dish submerging the PDMS device.
8. Peel the PDMS carefully off the petri dish with tweezers and wash the pattern with PBS. Add cell culture media to the Petri dish and place the cell culture in the incubator.

Representative Results

This method allows the patterning of proteins and indirect patterning of cells using dead-end microfluidic channels with dimensions as small as 10 μ m and equipment available in almost all biological laboratories once the master mold is made. This technique can be utilized with PDMS microfluidic channels created using traditional soft photolithography, or with PDMS microfluidic channels created with adhesive tape fabrication (**Figure 1**)^{28,29}. We have previously demonstrated the use of this procedure in indirect patterning of neuronal cells for evaluation of axon guidance in photoreceptors^{4,30}, and have now demonstrated its use in several additional cell types of varying origins. Embryonic rat cortical neurons patterned on the poly-D-Lysine (PDL) and laminin surfaces demonstrate general adherence to the patterned areas and growth along the PDL and laminin stripes (**Figure 3A**). C2C12 myoblast cell lines also adhere to the patterned area and demonstrate fusion into multinucleated myotubes (**Figure 3B**). We have also adapted this technique for direct cell patterning as seen with fibroblasts in **Figure 4**. As previously reported, this direct cell patterning method showed no adverse effect on cell survival³⁰. The variables that lead to successful direct or indirect patterning of stated cell types are listed in **Table 1 and 2**. Thus in the future, researchers may utilize this technique to pattern cells and discover phenomena related to their specific patterning.

Spin coat SU-8 Apply Adhesive tape to glass

Expose to UV

Fix the glass on drawing
& Cut the tape

Develop SU-8

Remove Excess Tape

Coat with PDMS

Coat with PDMS

Cut out device

Cut out device

PHOTOLITHOGRAPHY PROCESS ADHESIVE TAPE FABRICATION

Figure 1: Fabrication of Mold by Soft Lithography. Left: Photolithography process. SU-8 is spin coated onto a silicon wafer, exposed to UV radiation, and developed to remove excess SU-8. Right: Adhesive tape fabrication process. Adhesive tape is attached to a clean glass slide, cut with a scalpel, and excess tape is carefully removed. PDMS cast is then fabricated from each mold using soft lithography. [Please click here to view a larger version of this figure.](#)

Figure 2: Example of a PDMS Device for Substrate Patterning. (a) PDMS microfluidic cast with inlet used for microfluidic patterning of substrates. Inlet was punched out at one end of microchannels. (b) Microfluidic channel as seen under phase contrast microscopy. Microfluidic channels are 15 mm in length, 20 μm wide, and separated by 200 μm . Figure 2 is reproduced with permission from IOP Publishing. All rights reserved³⁰. [Please click here to view a larger version of this figure.](#)

Figure 3: Indirect Cell Patterning. (a) Vacuum assisted protein patterning process. PDMS microfluidic channel device is attached to a petri dish, substrate solution is loaded into inlet, vacuum is briefly applied to chamber and released, PDMS device is removed, pattern is washed, and ready to use. (b) Embryonic cortical rat neurons seeded onto a coverslip with a patterned PDL and laminin substrate. (c) C2C12 myoblast cell lines grown on patterned PDL and laminin. Figures 3a and 3c are reproduced with permission from IOP Publishing. All rights reserved³⁰. [Please click here to view a larger version of this figure.](#)

Figure 4: Direct Cell Patterning. (a-c) Fibroblasts patterned via direct cell patterning. (a) Microfluidic channel device fabricated using adhesive tape lithography. (b) Microfluidic channels after filling with fibroblast cell suspension using vacuum assisted patterning technique. (c) Fibroblasts after removal of microfluidic device. (d) Calcein-AM staining of fibroblasts after microfluidic patterning. (e) Phase contrast image of fibroblasts after microfluidic patterning. (f-g) Fibroblasts stained with phalloidin-TRITC (red) and DAPI (blue). Figure 4 is reproduced with permission from IOP Publishing. All rights reserved³⁰. [Please click here to view a larger version of this figure.](#)

Indirect Cell Patterning	Substrate	Protein Incubation Conditions	Cell Density	Cell Adhesion Duration
PC12	Collagen-1	1 h at 50 °C	5 x 10 ⁶ / mL	1 h at 37 °C
Fibroblast	Collagen-1	1 h at 50 °C	5 x 10 ⁶ / mL	1 h at 37 °C
C2C12	PDL + Laminin	1 h at 37 °C, wash PBS, overnight at 37 °C	2 x 10 ⁶ / mL	15 min at 37 °C
Embryonic Rat Cortical Neurons	PDL + Laminin	1 h at 37 °C, wash PBS, overnight at 37 °C	2 x 10 ⁶ / mL	15 min at 37 °C
Salamander Retinal Neurons	Sal-1	overnight at 10 °C	N/A	1 h at 10 °C

Table 1: Indirect Cell Patterning Conditions. List of incubation conditions such as substrate type, time, temperature, and cell density for typical cell types. Researchers should optimize the incubation conditions for their own model substrates and cell type. Sal-1 is the antibody substrate used to grow Salamander retinal neurons.

Direct Cell Patterning	Substrate	Cell Density	Cell Adhesion time
PC12	Collagen-1	6 x 10 ⁶ / mL	1 h at 37 °C
Fibroblast	Collagen-1	6 x 10 ⁶ / mL	1 h at 37 °C
C2C12	PDL + Laminin	2 x 10 ⁶ / mL	15 min at 37 °C
Embryonic Rat Cortical Neurons	PDL + Laminin	2 x 10 ⁶ / mL	15 min at 37 °C
Salamander Retinal Neurons	Sal-1	N/A	1 h at 10 °C

Table 2: Direct Cell Patterning Conditions. List of incubation conditions such as temperature, time, and cell density for typical cell types. Researchers should optimize the incubation conditions for their own model cell type.

Discussion

While conventional photolithography is a well-established technique for the creation of molds for soft lithography, the equipment, materials, and skills necessary to use conventional photolithography are not readily available to most laboratories. For laboratories without access to these resources, we have presented adhesive tape fabrication as a method of creating molds with relatively simple features for microfluidic devices. This method allows any laboratory to create and utilize microfluidic devices for research purposes with readily available tools. The adhesive tape method may be improved with a low-cost desktop vinyl cutter³¹. Desktop vinyl cutters may increase reproducibility, resolution, as well as layout complexity. Each of these options, conventional photolithography, desktop cutters, or adhesive tape fabrication have different capabilities and limitations and researchers must carefully consider which method would be sufficient for their needs.

In cured PDMS, the long polymeric chains of dimethyl siloxane form a lattice, nanoporous structure which creates empty regions that can be filled with air molecules²⁷. This gas permeable property is key to our method of filling microfluidic channels. When placed into a low pressure environment, air molecules are removed from the PDMS bulk material as well as the microchannels themselves²⁵. When the PDMS is then exposed to atmospheric pressure, the PDMS bulk material and microchannels retain a negative pressure for some time^{25,30}. This negative pressure draws liquid into the microchannels automatically filling the microfluidic channels. This negative pressure continues to draw liquid into the channel until the bulk PDMS equilibrates with atmospheric pressure.

This vacuum-assisted technique allows for patterning of specific proteins and substrates onto a growth substrate. In this study, we were able to pattern several different substrates and cell types both directly and indirectly (**Table 1** and **2**). However, the experimenter may need to test different incubation times, cell densities, or seeding media for their particular cell type. These variables likely relate to the innate ability of either the cell or substrate to adhere to the surface it is being placed upon. For example, here C2C12 cells required the use of serum-free media for the seeding media before culturing the cells in DMEM with 2% horse serum. Additionally, conditions may vary based upon whether a glass coverslip or plastic petri dish is being patterned. Here, both worked well to allow for specific patterning of various cell types.

One of the initial concerns we had while utilizing this technique was how exposure to vacuum might affect cell viability. This study and previous studies, however, should alleviate those concerns as we have shown that vacuum has little to no effect on a variety of cells³⁰. Wang *et al.* previously utilized degassed irreversibly sealed microfluidic chambers to load HeLa cells³². Other studies have used vacuum-assisted cell seeding for a variety of cell types including human stem cells, adherent and non-adherent cells, and human-hamster hybrid cell line (A_L) cells to load into microfluidic channels³³. In addition, other researchers have subjected cells to much greater vacuum pressures in comparison to this current study with little to no discernible effect on the cells³³. Bubbles formed during the removal of air from the microchannel tend to congregate on the surface of the droplet of the suspension at the inlet. Often these bubbles do not rupture due to the surface tension of the suspension. We have not observed a noticeable decrease in cell viability due to bubbles. In addition, the experiment with Calcein-AM does not suggest a significant decrease in cell viability. Because of this, we have not thoroughly examined cell death specifically due to bubbles.

Previous attempts to pattern substrates or cells were often plagued by problems such as the formation of air bubbles in the microfluidic devices. The formation of air bubbles made it difficult to inject liquids easily and efficiently without the use of equipment or materials that are not available in most laboratories. For example, previous methods utilized the use of plasma treatment or corona treatment to decrease the hydrophobicity of PDMS microchannels^{15,34}. While effective, plasma and corona treaters are not readily available in most laboratories. In this protocol, we

demonstrate the ability to pattern cells and substrates simply using common laboratory vacuums. Using the adhesive tape fabrication technique, the methodology can be utilized to create PDMS microfluidic channels to pattern substrates or cells in nearly any laboratory.

In order to pattern substrates or cells with the vacuum patterning protocol, several steps are critical for successful patterning. First, to fabricate the adhesive tape master, the adhesive tape must be completely attached to the glass slide and free of air bubbles. Air bubbles weaken the bond between the tape and the glass slide and may lead to the tape being peeled off when cured PDMS is peeled off the adhesive tape mold. In addition, bubbles will distort the geometry of the microfluidic channel causing the surface of the channels to be non-planar. To make the PDMS cast from SU-8 mold, the SU-8 mold must be silanized before casting with PDMS. This step is critical in preventing the permanent bonding of PDMS to the SU-8 mold after curing of PDMS. Prior to conformal sealing of the PDMS microfluidic channel to glass coverslips or plastic petri dishes, the PDMS must be cleaned of all dust particles. These particles may prevent the formation of a conformal seal between PDMS and glass and thus prevent the injection of cells or substrates into the microfluidic channel. As stated in the above protocol, cleaning the PDMS channels with adhesive tape is critical prior to adhering the microchannel to glass coverslips or plastic petri dishes. Finally, after placing the device into the vacuum chamber, the vacuum must be gently released to prevent displacement of the droplet of substrate or cell solution from the inlet hole.

If no fluid is observed flowing into the microfluidic channel, there may be a leak in the microfluidic channel which would prevent liquid from flowing into the channel. Remove the PDMS stamp, dry and clean it and then repeat step 4, 5, or 6 of the technique. If the solution does not flow into the microchannel after release of vacuum, ensure that the solution is completely covering the entrance of the microchannel. This can be done by pipetting solution up and down several times while putting it in the inlet hole. If the substrate, after injecting into the microchannel, does not attach to the glass, evaluate and determine the optimal incubation conditions necessary for the substrate used. If removing the PDMS cast from the glass surface or plastic petri dish causes the patterned cells or substrates to be destroyed, use a thinner PDMS cast, submerge the PDMS cast in PBS or media, and slowly and gently peel off the PDMS cast from the glass or plastic surface with tweezers.

Finally, BSA may be critical in decreasing nonspecific adhesion of cells to either the unpatterned glass or plastic surface. BSA is typically used as a blocking reagent in western blotting, immunostaining, and other molecular biology techniques. Other researchers have also utilized it for indirect or direct patterning protocols to decrease nonspecific cell adhesion^{35,36,37}. We found that incubation of the substrate with BSA was necessary for most cell types except for salamander retinal cells. This may be because of the more specialized nature of the substrate (an antibody called Sal-1) that was used as well as the general lack of cell adhesion by this cell type³⁸. In our hands, BSA also did not noticeably decrease the adherence of cells to the patterned areas and mainly served to decrease nonspecific adherence.

While this method is designed to be versatile and flexible in its application to different substrate and cell types, there are several limitations to this protocol and the compatible cells and substrate. Because of the very nature of patterning cells onto a surface, the cell types that this technique can be applied to are limited to those which are amenable to any patterning protocol such as those cells that are able to survive with more limited cell to cell contact as well as those that can survive at lower cell seeding concentrations. For example, one potential application is the patterning of yeast cells for atomic force microscopy (AFM)³⁹. While we have tested many different cell substrates, other substrates may not work with this particular technique such as those that form three-dimensional gels. At this time, we have not examined if gels properly form and stay on the surface of the glass coverslip or plastic petri dish after patterning. This technique is capable of patterning complex shapes and large areas. However, it cannot pattern an array of individual separated shapes without interconnecting microchannels. In the absence of interconnected microchannels, each individual shape would require its own inlet making the technique cumbersome. While we did not notice any non-uniformity in patterning protein, the distribution of cells patterned inside the microchannel may be non-uniform. This non-uniformity may be due to the random distribution of cells in cell suspensions, geometry and dimensions of the microchannels, and viscosity of the cell suspension.

In the future, this technique may be applied to other types of substrates and cells. For example, three-dimensional gels such as basement membrane matrix or collagen scaffolds may potentially be patterned and formed using PDMS microfluidic channels. By injecting a hydrogel into the microfluidic device, the hydrogel may take on the shape of the microfluidic device and be able to form complex patterns without the use of 3-D printers or other technologies. These would allow for rapid creation of structured scaffolds with readily available tools and microfluidic devices.

Disclosures

The authors declare no competing financial interests.

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