Cell docking inside microwells within reversibly sealed microfluidic channels for fabricating multiphenotype cell arrays

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We present a soft lithographic method to fabricate multiphenotype cell arrays by capturing cells within an array of reversibly sealed microfluidic channels. The technique uses reversible sealing of elastomeric polydimethylsiloxane (PDMS) molds on surfaces to sequentially deliver various fluids or cells onto specific locations on a substrate. Microwells on the substrate were used to capture and immobilize cells within low shear stress regions inside channels. By using an array of channels it was possible to deposit multiple cell types, such as hepatocytes, fibroblasts, and embryonic stem cells, on the substrates. Upon formation of the cell arrays on the substrate, the PDMS mold could be removed, generating a multiphenotype array of cells. In addition, the orthogonal alignment and subsequent attachment of a secondary array of channels on the patterned substrates could be used to deliver fluids to the patterned cells. The ability to position many cell types on particular regions within a two dimensional substrate could potentially lead to improved high-throughput methods applicable to drug screening and tissue engineering.

1. Introduction

There has been great interest in testing the beneficial effects of both new and old drugs on multiple diseases. For example, aside from the ability of aspirin to relieve pain, it is currently being examined as a cancer preventative.[1] Also, drugs which treat erectile dysfunction, such as Viagra, are currently being tested to treat pulmonary hypertension.[2] This need to test existing drugs and the increasing ability to use combinatorial chemistry to synthesize large libraries of novel compounds have increased the demand for screening the effects of biochemical signals on multiple cell types in a highly parallel manner. Current methods to perform such experiments are expensive and limited in the number of tests that can be performed. For example, commonly used methods for high-throughput analysis involve the use of multi-well plates (*i.e.*, 384 or 96 well plates) that are operated using cumbersome manual or expensive robotics based operations.[3–5] Therefore, developing a technology that can perform such tasks in a cheaper, easier, and a higher throughput manner may be beneficial in a number of fields, ranging from drug discovery to tissue engineering.

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Microscale approaches such as cellular micropatterning[6,7] and microfluidics[8,9] hold great promise to perform high-throughput experimentation. Recently, methods to simultaneously test different extracellular matrix proteins and synthetic materials on the behavior of embryonic stem (ES) cells have been elegantly demonstrated through the use of robotic based surface deposition.[10,11] In these approaches, an array of adhesive regions, each containing a unique extracellular material, was tested for their ability to direct the differentiation of ES cells. Aside from testing various stimuli on the same cell type, it is potentially important to test the effect on multiple cell types. Previous approaches to fabricate multiphenotype arrays involved a number of techniques such as patterned co-cultures[12–15] and capturing cells within photocrosslinking[16] or natural[17] polymers. In patterned co-cultures, two cell types are positioned relative to each other, either by using selective adhesion of one cell type relative to the other to a patterned substrate[12,13,15] or by using the reversible adhesive properties of the substrate to position a cell type relative to the other cell type.[14] Patterned co-cultures are useful for controlling homotypic and heterotypic cell–cell interactions and enhancing the function of cell types that are hard to maintain *in vitro* (such as hepatocytes) through introduction of support cells that provide the signals to maintain these cells in culture. However, most patterned co-cultures to date only employ two cell types patterned relative to each other. Although micropatterning and microfluidics are useful for controlling the microenvironment and probing cellular interactions in cell culture, techniques for co-culture based on those two platforms are needed. One such approach involves immobilization of cells within photocrosslinkable hydrogels using an injection molding technique.[16] Such systems have been used to pattern multiple cell types on a two dimensional substrate. Despite the significant capability of this approach,

some potential challenges include the use of toxic photoinitiators and radiation to immobilize the cells inside the channels[18] and the need for expensive photolithographic patterning equipment. Also, by photocrosslinking the cells in a hydrogel, it is harder to retrieve the cells for subsequent analysis.

We have previously demonstrated that cells can be immobilized within microfluidic channels either on adhesive patterned regions[19] or within microstructures with regions of low shear stress.[20] In order to facilitate the fabrication of robust microdevices, our emphasis was to irreversibly seal microfluidic channels on the substrates. However, the reversible sealing of PDMS on microfluidic molds on a substrate is a powerful approach to sequentially pattern surfaces or to deliver fluids sequentially to various spots on a two-dimensional substrate.[21]

Here, we introduce an approach to fabricate arrays of many different cell types that combines the ability to reversibly seal microfluidic channels on patterned substrates with the ability to capture cells in shear protected regions of microfluidic channels. The technique can be efficiently used to pattern multiphenotype cellular arrays on two dimensional substrates or within individual microchannels. This was accomplished by a multi-step method. Initially, two PDMS molds containing either the microchannels or the microwells were fabricated and subsequently reversibly sealed to each other so that the microwells were positioned within the channels. Each cell type was flowed through a unique microchannel, and deposited onto the microwells within that channel. After the cells filled the wells, the microfluidics mold was peeled from the substrate, while leaving the cells within the microwells, which generated a patterned array of multiple cell types. In order to facilitate high-throughput delivery of reagents to the various cell types, a secondary PDMS mold could be subsequently aligned orthogonal to the direction of the first array of channels. In this approach, each microwell on the substrate could potentially be used to perform a separate experiment. Microfluidic gradient generators upstream from the array of channels could be used to lower the number of independent inlets required and facilitate the delivery of a higher number of solutions to the various cell types.

2. Materials and methods

2.1. Fabrication of PDMS microfluidic molds and stamps

PDMS stamps and microfluidic molds were fabricated by casting PDMS (Sylgard 184 Silicon elastomer, Essex Chemical) against a complementary relief structure as previously described.[14,20,22] Two patterns were generated: a 5 × 5 array of circles of 75 μm in diameter, and an array of 5 channels with an individual channel width of 150 μm. These masks were subsequently used to generate a pattern of 80 μm high SU-8 photoresist on silicon wafers using contact photolithography to generate a negative 'master.' Positive replicas were fabricated by molding PDMS by curing the prepolymer (a mixture of 10 : 1 silicon elastomer and the curing agent) on the silicon masters at 70 °C for 2 h. The PDMS mold was then peeled from the silicon wafer and cut prior to use. The stamps had protruding (positive) features that were used to fabricate replicate microfluidic molds or patterned microwells.

For each array of microchannels, holes were drilled through the inlets and the outlet. For the inlets, independent reservoirs measuring about 3 mm in diameter were cut. Metal tubing was inserted into the outlet, sealed with epoxy, and connected to a piece of plastic tubing. This PDMS microchannel assembly was plasma cleaned for 30–45 s along with a 5 × 5 well patterned PDMS mold. The PDMS microchannels were manually aligned on the 5 × 5 PDMS substrates under a microscope, and were reversibly bound through bringing the two surfaces into contact and applying pressure. In some experiments, plasma treatment was limited to the regions of the PDMS molds immediately surrounding the microwell or microchannel arrays. This was done by covering the rest of the substrate with a reversibly sealed piece of PDMS. This process allowed the channels and the wells to be hydrophilic while allowing the rest of the substrate to remain hydrophobic.

2.2. Cell cultures

All cells were manipulated under sterile tissue culture hoods and maintained in a 5% CO_2 humidified incubator at 37 °C. Cell lines were purchased from Advanced Type Culture Collection (ATCC), and cell culture reagents were purchased from Gibco Invitrogen Corp. unless otherwise stated. Saos-2 and NIH-3T3 murine embryonic fibroblasts were maintained in Dulbecco's modified eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS). Once the cells were confluent, they were trypsinized (0.25% in EDTA, Sigma) and passaged at a 1 : 5 subculture ratio. AML12 murine hepatocytes were maintained in a medium comprised of 90% 1 : 1 (v/v) mixture of DMEM and Ham's F12 medium with 0.005 mg mL^{-1} insulin, 0.005 mg mL^{-1} transferrin, 5 ng mL^{-1} selenium, and 40 ng mL^{-1} dexamethasone, and 10% FBS. Confluent dishes of AML12 and NIH-3T3 cells were passaged and fed every 3–4 days. Murine embryonic stem (ES) cells (R1 strain) were maintained on gelatin treated dishes in 15% ES-qualified FBS in DMEM knockout medium. ES cells were fed daily and passaged every 3 days at a subculture ratio of 1 : 4. The prostate PC3 cell line was cultured in RPMI-1640 and Ham's F12K medium, respectively, supplemented with 100 U mL^{-1} aqueous Penicillin G, 100 μg mL^{-1} Streptomycin, and 10% fetal bovine serum. Confluent culture flasks were passaged every 4 days.

2.3 Reversible sealing and cell docking

To fabricate the device, the PDMS microfluidic mold was aligned under a microscope on the array of wells so that each row of wells was centered within the channels. Once the two PDMS pieces were reversibly sealed, cells were deposited in the five inlet reservoirs and flowed through the channels under negative pressure. The outlet was connected to a syringe pump (New Era Pump Systems Inc.) by polyethylene tubing, and the flow was regulated by operating the pump under either positive or negative pressures. To minimize the formation of bubbles within the microwells, ethanol was flowed through the channels at >10 μl min^{-1}, followed by a PBS wash. The channels were tested for leakage by flowing a visible dye such

as Trypan blue in alternating lanes under negative pressure at 5–10 μl min^{-1}. To seed the cells, 25–50 μl of each cell suspension (3×10^7 cells ml^{-1}) was deposited in the inlet reservoir of a unique microchannel and flowed through the channel at a flowrate of 2–5 μl min^{-1} under negative pressure. In most experiments, negative pressures applied at the outlet were used in order to eliminate leakage and the need for multiple syringe pumps. To sediment the cells into the microwells, the flow was stopped for 10 min. Excess cells were removed by aspirating the cells from the inlet reservoir with a pipette, and flowing medium through the channels at flowrate of 5 μl min^{-1}. Once the cells were captured in the wells, the PDMS molds were put into a PBS bath, and the microfluidic mold was gently peeled from the substrate. This step was required in most cases where the cells had not adhered within the channels to ensure that the cells remained in the wells.

To allow for the delivery of multiple fluids to the patterned cells, secondary microfluidic molds were placed orthogonally on the cell arrays. In this process, the PDMS mold containing the multiphenotype cell arrays was dried in regions around the microwell array. Subsequently another PDMS microfluidic mold containing an array of channels was aligned and pressed onto the substrate, forming a second set of microfluidic channels.

2.4 Cell tracking and viability

To stain with the cellular dye SYTO, cells were trypsinized and washed with DMEM medium without serum, and subsequently suspended at a concentration of 1×10^7 cells ml^{-1} and incubated for 4 min at room temperature. To stain with carboxyfluorescein diacetate succinimidyl ester (CFSE) dye, cells were suspended in 10 μg ml^{-1} CFSE in PBS solution at a concentration of 1×10^7 cells ml^{-1} and incubated for 10 min at room temperature. Both staining reactions were quenched with the addition of an equal volume of DMEM supplemented with 10% FBS and washed. To analyze cellular viability, a live/dead assay was performed by flowing ethidium homodimer and calcein AM dissolved at 2 μg mL^{-1} in DMEM containing 10% FBS through the channel for 20 min. PBS was then flowed through the channel to remove excess/non-specific staining.

2.5 Contact angle measurements

A Ramé-Hart goniometer (Mountain Lakes) equipped with a video camera was used to measure static contact angles. Reported values represent averages of at least 6 independent measurements.

3. Results and discussion

3.1 Approach

A novel technique to fabricate multiphenotype cell arrays is presented (Fig. 1). This approach utilizes three distinct concepts: (1) capturing cells within microstructures that contain low shear stress regions; (2) reversibly sealing elastomeric molds onto patterned substrates; and (3) orthogonally placing a series of microchannel arrays to deliver a unique set of fluids to particular 'spots' on a two-dimensional surface.

We have previously shown that cells can be captured within PEG microstructures and remain shear protected inside a variety of microstructures.[20] This approach provides a number of potential advantages for the fabrication of cell arrays, such as topographical heights as a barrier to localize cells in particular spots and the ability to pattern cells without the need for slow 'cell adhesive' processes. Also, the presence of microstructures allows for capturing multiple cells in the form of aggregates, which mimics the three-dimensional architecture of cell structures *in vivo*. These low shear stress microwells could be formed with protein coated or non-adhesive surfaces, making them useful for both anchorage dependent or anchorage independent cell types.

3.2 Reversible sealing on patterned arrays of microwells

Although irreversible sealing is desired for devices comprised of static microchannels, reversibly sealed microchannels have been shown to be useful for a number of applications such as surface patterning.[21,23,24] As well, the ability to use multiple sets of channels makes it possible to construct complex patterns. Here, the cells were flowed through channels that had been reversibly sealed onto a substrate containing microwells.

To test the reversible sealing properties of PDMS molds, both microwell patterned substrates and microfluidic molds were made from PDMS molded onto a master of SU8 patterned on silicon wafers. In most cases, unmodified PDMS substrates were used. Under these conditions PDMS–PDMS interactions were reversible. We used this property to immobilize multiple cell types onto microwell patterned surfaces and then removed the mold. We found that reversibly sealed microchannels frequently leaked even under low positive pressures and flow rates of $\sim$1 μl min^{-1} across the channel. To alleviate this problem, we used negative suction head by drawing the liquid from a common outlet on the microchannel arrays. As shown in Fig. 2, this setup eliminated leaking from the channels for both primary (Fig. 2a–b) and secondary PDMS channels (Fig. 2c). Furthermore, by having a common outlet, a single syringe and pump assembly was sufficient to regulate the fluid flow through many channels. Clamps could also be used to enhance channel sealing, but the use of negative suction head (*i.e.* syringe pump drawing fluid from a common outlet) minimized the need for clamping.

In some instances, the surfaces of microchannels were modified using a variety of modification approaches. For example, surfaces were oxygen plasma treated to make the channels more hydrophilic or change the surface protein and cell adhesive properties. To make the PDMS surfaces cell and protein repellant, a silane-based anchoring PEG polymer (TMSMA-r-PEGMA) was flowed in the channels, spontaneously forming a polymeric monolayer on plasma treated PDMS surfaces.[22,25] The PEG surface modification could be performed in these channels without modifying the leaking properties and could reduce protein adhesion of up to 98% and cell adhesion of up to 99%.[26]

Alternatively, microwells were fabricated from photocrosslinkable PEGMA and the microfluidic molds were directly immobilized onto PEG cured surfaces. When

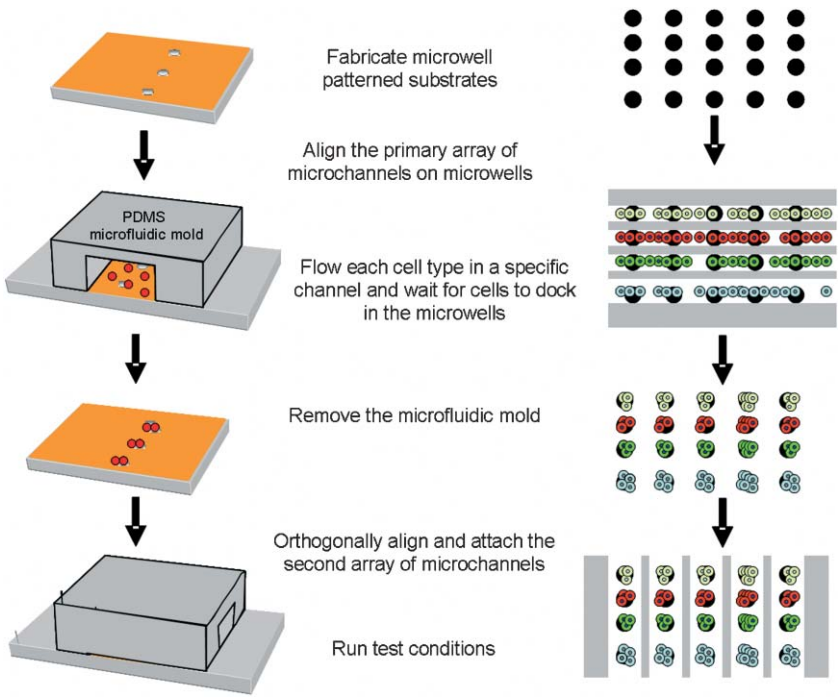

Fig. 1 Schematic diagram of reversible sealing of microfluidic arrays onto microwell patterned substrates to fabricate multiphenotype cell arrays. Initially, a PDMS microfluidic array was aligned on an array of microwells. As each cell type flowed through an independent channel, they docked onto the microwells, which resulted in a patterned array of cells. To deliver multiple solutions, PDMS microfluidic mold was removed and replaced with another mold which was placed orthogonally to create multiphenotype cell arrays inside each microchannel.

Fig. 2 Leak-proof reversibly sealed microfluidic channels: (a–b) represent the reversible sealing of a primary PDMS microfluidic mold on an array of wells while (c) represents the reversible sealing of a secondary array of channels on a substrate that was previously sealed. In (a) and (c) Trypan blue and PBS were flowed in alternating channels. In (b) red (rhodamine) and green (FITC) dyes in PBS (10 μg mL^{-1}) were flowed through the channels. The dye solutions did not leak, indicating that primary and secondary sealing of PDMS/PDMS can be performed. Note: (a) is a combined series of pictures to capture the entire microfluidic device.

photocrosslinkable PEG fabricated devices were used to create a reversible seal, significant leaking was observed. This was caused by the permeability of PEG microstructures to water, which allowed the water to penetrate the substrates and move from one channel into the surrounding channels (data not shown).

Another issue regarding the formation of PDMS based microchannels is the retention of bubbles within the channels. Since PDMS is typically hydrophobic and even under plasma oxidation does not become fully hydrophilic, as measured by contact angles of $73^\circ \pm 1^\circ$ and $51^\circ \pm 2^\circ$ respectively, air bubbles can be captured within the microchannels. To solve this problem we devised a method of first flowing ethanol inside the channels to fill the microwells and then fill the channels with water. Ethanol has a lower PDMS surface contact angle either without ($33^\circ \pm 2^\circ$) or with ($15^\circ \pm 0.5^\circ$) oxygen plasma treatment, which allows it to wet the surface and fill the microwells. Once the initial surface wetting has occurred, the subsequent flow of medium and PBS will not cause bubble formation.

3.3 Delivery of reagents by serially placing microchannel arrays orthogonally on substrates

Here, we demonstrated that the sequential alignment and removal of two arrays of microchannels orthogonally placed relative to each other on microwell patterned substrates could be used to fabricate a high-throughput device. For example, if the first mold has M channels and the second mold has N channels, the ability to expose each region at their intersection

allows $M \times N$ unique testing conditions. Although more complicated channel designs using the sequential placement of microfluidic molds on patterned substrates could be envisioned, this linear microchannel array seems promising for its simplicity and ease of use in that the same microfluidic mold rotated 90° could be used as the secondary mold.

As a proof of concept, we designed a PDMS array of five parallel channels with independent inlets and a common outlet (Fig. 2a). Complementary to this design, we built an array of 25 microwells which we placed directly underneath the microchannels once the two molds were aligned.

3.4 Fabrication of multiphenotype cell arrays

To demonstrate that multiphenotype cell arrays could be generated, we used a variety of cell lines including murine embryonic stem (ES) cells, osteoblasts (Saos-2), hepatocytes (AML12), fibroblasts (NIH-3T3) and human prostate cells (PC3 cells). Once the device was fabricated, the cells were stained with cell membrane dyes CFSE and SYTO which shows up as green and red respectively under fluorescence. After prefilling with medium and reversibly sealing, each cell type was loaded into the reservoir at the inlet of one of the channels. The stream of flowing fluid carried the cells in the channels where they could be deposited onto the low shear stress confinements within the microwells. As seen in Fig. 3, cells were captured within the microwells. All cell types, independent of source (human or mouse) or organ of origin, could be deposited within the microchannels. Although in most experiments cells remained in suspension, the ability to capture cells and allow for their adhesion and spreading at the bottom of the microwell will permit the formation of multiphenotype arrays of adherent cell types. In order to seed the cells, they were either captured from the moving fluid[20] or the fluid was stopped for $<$10 min to facilitate cell docking within the microchannels.

Furthermore, the microfluidic channels were used to fabricate multiphenotype cell arrays (Fig. 4). These arrays were fabricated on two-dimensional surfaces (Fig. 4b) and within microfluidic channels (Fig. 4c). To fabricate the multiphenotype cell arrays on two-dimensional substrates, cells were flowed in the channels and allowed to dock.

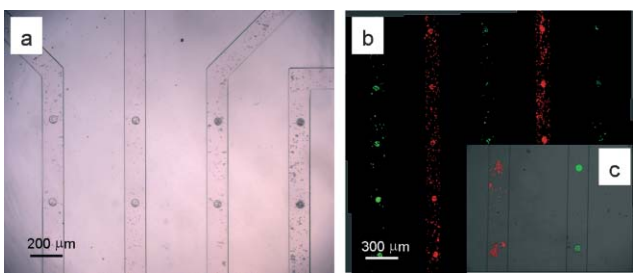

Fig. 3 Cell docking within microchannel arrays: (a) represents the light microscopy image of ES cells flowing within an array of microchannels; (b) is the fluorescent image of cells (right to left: ES/AML12/Saos-2/PC3/NIH-3T3 cells) labeled with membrane dyes CFSE (green) and SYTO (red) as they flow through the channels. (c) Once the cells had docked in the microwells, a cell-free solution was flowed through the channels to remove any remaining non-adhered cells.

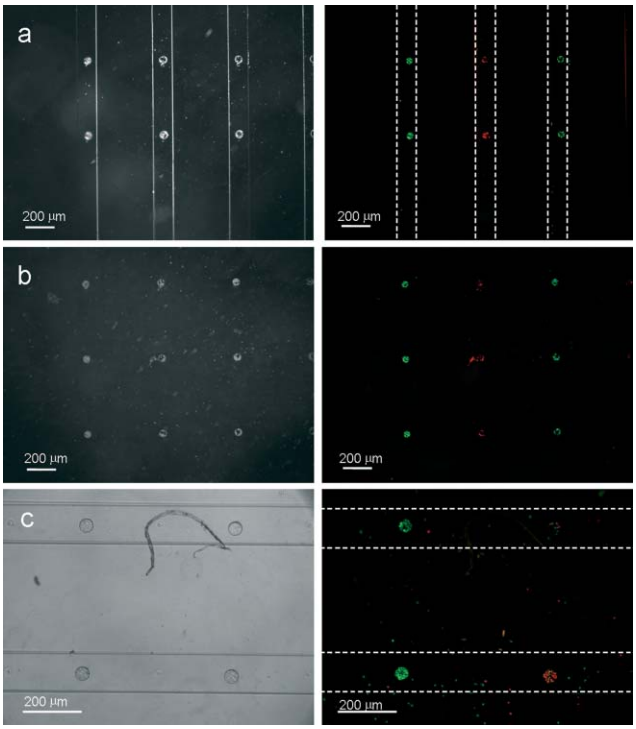

Fig. 4 Formation of multi-phenotype cell arrays on two-dimensional substrates or within microfluidic channels: (a–c) show the light and fluorescent microscope images of the steps required in fabricating multiphenotype arrays. The fluorescent images are those of various cell types stained with two membrane dyes, CFSE (green) and SYTO (red) (right to left: ES/AML12/NIH-3T3 cells). (a) Each cell type was allowed to dock within microwells inside a microchannel. (b) The cells remained stable inside the microwells even after the PDMS mold was removed, giving rise to multiphenotype cell arrays. (c) Secondary microchannel molds were aligned orthogonally and reversibly sealed on the patterned substrates, resulting in wells that contained multiple cell types.

Afterwards, the microfluidic PDMS mold was removed. To ensure that the cells remained within the microstructures, the PDMS mold was carefully and slowly removed. The removal and the placement of subsequent molds is a delicate process that requires a balance between keeping the cells in a wet environment while allowing for the two PDMS pieces to adhere to each other under normal conditions.

Differences in the number of cells that settled in each well may introduce artifacts in subsequent (high-throughput) analyses. As shown in Fig. 4, the initial number of cells and their subsequent stability within each well was cell type dependent. This is due to cell specific characteristics such as rate of aggregation and adhesion to the substrate. Cell types that aggregated faster and did not adhere strongly to the surfaces of the microwells detached more easily from the microwells and led to more rapid deterioration of the patterns. We anticipate that by changing process conditions such as depth of the wells, fluid flow rates and surface characteristics, cell specific variability between wells and different cell types may be reduced.

Cells that underwent the docking process and the subsequent sealing of secondary microfluidic molds remained viable

as measured by their ability to exclude ethidium homodimer or metabolize calcein AM. A live/dead stain was flowed through the channels of unstained cells. The cells remained alive as marked by the green color (data not shown). In addition, many cells adhered to the wells, further indicating that they were alive.

Using the reversible adhesion between the PDMS microchannels and the underlying PDMS patterned microstructured substrate, multiple cell types were simultaneously probed. After the first set of channels was successfully removed, the cells remained immobilized within the wells. A secondary set of channels was aligned perpendicular to the first microfluidic channel set, and test solutions were flowed through each channel. As visualized in Fig. 3, the new channels did not leak either and maintained isolated channel environments.

An interesting question arises here about the level of miniaturization that can be achieved using this approach in comparison to the standard multi-well (*i.e.* 384 and 1536) plate assays. We anticipate that by using standard soft lithography, it is possible to position microwells 100 μm apart with sufficient space to align individual channels on each pattern. Using these dimensions, it is anticipated that a space of 200 μm × 200 μm would be sufficient for each experimental condition. Therefore, theoretically 2500 tests can be performed in a 1 cm^2 area, which is much greater than the densities achieved using existing multi-well plate technologies. The ability to perform experiments in a high-throughput manner and to integrate microfluidic components such as valves, pumps and gradient generators can be used to eliminate expensive robotics and labor costs associated with current technologies. Also, the reduction in the volume of the samples and reagents is another advantage of this technique relative to multi-well plates.

3.5 Potential problems and future directions

Docking cells within microstructures has a number of advantages for capturing cells inside microdevices. Although it is possible to flow cells through an array of channels and wait for them to adhere to a patterned substrate, the approach presented here is faster, easier to employ and more practical. Another potential advantage of using microwells to immobilize cells is that negative features (*i.e.* 'microwells that are sticking into the substrate') allow the microfluidic mold to be realigned and moved without disturbing the cells, which is not possible if the cells were adhered onto a flat surface or immobilized inside hydrogels.

One potential limitation of using microfluidic arrays for high-throughput experimentation is the connection between the array of microchannels and its macroscopic inputs. To reduce the number of independent inlets we integrated a variety of approaches such as the use of an orthogonally placed array of channels. To further reduce such limitations we integrated microfluidic mixers that have been previously used to generate concentration gradients[27,28] upstream from the array of channels. In these gradient generators a series of mixing and merging steps create various combinations of the inlet streams. As seen in Fig. 5, by using a gradient generator, two independent inlets could give rise to a number of channels

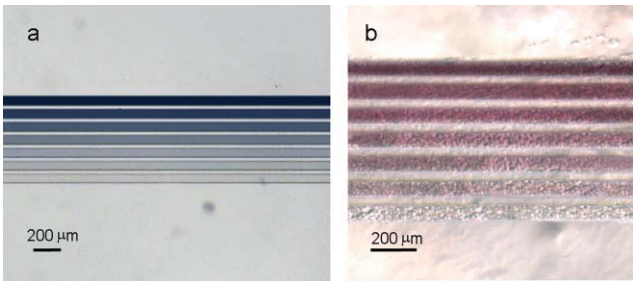

Fig. 5 Microfluidic arrays with upstream microfluidic mixers. To lower the number of independent inlets into the device, micromixers can be incorporated upstream from the microchannel arrays: (a) represents experiments in which a concentration gradient was generated in an array of channels, and (b) is a monolayer of NIH-3T3 cells immobilized in such a microfluidic array.

with a linear mixture of the two streams. These and other types of upstream modifications, such as the integration of fluidic valves, could further enhance the throughput of these devices by minimizing the number of independent inlets that are required to perform a large number of experiments.

The reversible sealing of PDMS molds is a potentially powerful tool for high-throughput technology because it allows the integration of microchannels on patterned substrates. Although the PDMS–substrate seal is sufficient for low flow rates, research in making systems that can be operated under a variety of flow regimes may be beneficial. Future approaches for creating robust, reversibly sealed PDMS–substrate bonding may include making the surfaces around the microfluidic channels hydrophobic. The hydrophobic surfaces can be used to minimize fluid retention during the conformal contact between the substrate and the PDMS mold. Alternatively, negative pressure (*i.e.* vacuum) systems could be used to hold the PDMS onto the substrate. These approaches could dramatically increase the bonding strength of channels onto the substrate.

In this paper, the first and the second PDMS molds were aligned manually under an optical microscope. Clearly, this is a crude method to achieve the accuracy and consistency desired for high-throughput technology. We envision that future versions of this technology will incorporate more robust alignment methods such as micromanipulators and lock-and-key systems for precisely fitting a PDMS mold on a patterned substrate. Other features of the system that may benefit from further refinement include the peeling and the reversible sealing of the microfluidic molds onto the substrate. Also, while the microfluidic devices remain stable and leak-free for at least a few hours, the use of external forces (*e.g.* clamps, *etc.*) to reinforce the PDMS–substrate sealing may further enhance the stability and lifetime of these devices. Once optimized, this system shows the potential of providing users with maximal process reproducibility and controllability.

4. Conclusions

In summary, a technique was developed based on reversible sealing of PDMS molds onto microwell patterned substrates to form multiphenotype cell arrays. Arrays of various mouse and

human cell types were prepared by flowing a distinct cell type inside each microchannel. By allowing the cells to dock onto low shear stress regions and by subsequently removing the PDMS microfluidic mold, multiphenotype cell arrays were formed on a two-dimensional surface. The subsequent alignment and reversible attachment of an orthogonally oriented array of channels facilitated the formation of multiphenotype cell arrays inside microchannels. This technique may enable a whole new class of investigations in which cell–cell interactions can be probed. In addition, this technique could have potential applications in high-throughput screening or optimization of cell-soluble signal interactions for biological research or tissue engineering.

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References

1 R. S. Sandler, S. Halabi, J. A. Baron, S. Budinger, E. Paskett, R. Keresztes, N. Petrelli, J. M. Pipas, D. D. Karp, C. L. Loprinzi, G. Steinbach and R. Schilsky, *N. Engl. J. Med.*, 2003, **348**, 883–890.
2 J. Alaeddini, P. A. Uber, M. H. Park, R. L. Scott, H. O. Ventura and M. R. Mehra, *Am. J. Cardiol.*, 2004, **94**, 1475–1477.
3 S. B. Chen, Q. S. Zhang, X. Wu, P. G. Schultz and S. Ding, *J. Am. Chem. Soc.*, 2004, **126**, 410–411.
4 X. Wu, S. Ding, G. Ding, N. S. Gray and P. G. Schultz, *J. Am. Chem. Soc.*, 2004, **126**, 1590–1591.
5 X. Wu, S. Ding, Q. Ding, N. S. Gray and P. G. Schultz, *J. Am. Chem. Soc.*, 2002, **124**, 14520–14521.
6 J. Cheng, P. Fortina, S. Surrey, L. J. Kricka and P. Wilding, *Mol. Diagn.*, 1996, **1**, 183–200.
7 S. N. Bhatia, M. Toner, R. G. Tompkins and M. L. Yarmush, *Ann. N. Y. Acad. Sci.*, 1994, **745**, 187–209.
8 M. A. McClain, C. T. Culbertson, S. C. Jacobson, N. L. Allbritton, C. E. Sims and J. M. Ramsey, *Anal. Chem.*, 2003, **75**, 5646–5655.
9 K. R. King, C. C. J. Wang, M. R. Kaazempur-Mofrad, J. P. Vacanti and J. T. Borenstein, *Adv. Mater.*, 2004, **16**, 2007–2012.
10 D. G. Anderson, S. Levenberg and R. Langer, *Nat. Biotechnol.*, 2004, **22**, 863–866.
11 C. J. Flaim, S. Chien and S. Bhatia, *Nat. Methods*, 2005, **2**, 119–125.
12 S. N. Bhatia, U. J. Balis, M. L. Yarmush and M. Toner, *J. Biomater. Sci., Polym. Ed.*, 1998, **9**, 1137–1160.
13 S. N. Bhatia, U. J. Balis, M. L. Yarmush and M. Toner, *Biotechnol. Prog.*, 1998, **14**, 378–387.
14 A. Khademhosseini, K. Y. Suh, J. M. Yang, G. Eng, J. Yeh, S. Levenberg and R. Langer, *Biomaterials*, 2004, **25**, 3583–3592.
15 S. N. Bhatia, M. L. Yarmush and M. Toner, *J. Biomed. Mater. Res.*, 1997, **34**, 189–199.
16 W. G. Koh, L. J. Itle and M. V. Pishko, *Anal. Chem.*, 2003, **75**, 5783–5789.
17 M. D. Tang, A. P. Golden and J. Tien, *J. Am. Chem. Soc.*, 2003, **125**, 12988–12989.
18 V. A. Liu and S. N. Bhatia, *Biomed. Microdevices*, 2002, **4**, 257–266.
19 A. Khademhosseini, K. Y. Suh, S. Jon, G. Chen, G. Eng, J. Yeh and R. Langer, *Anal. Chem.*, 2004, **76**, 3675–3681.
20 A. Khademhosseini, J. Yeh, S. Jon, G. Eng, K. Y. Suh, J. A. Burdick and R. Langer, *Lab Chip*, 2004, **4**, 425–430.
21 E. Delamarche, A. Bernard, H. Schmid, A. Bietsch, B. Michel and H. Biebuyck, *J. Am. Chem. Soc.*, 1998, **120**, 500–508.
22 A. Khademhosseini, S. Jon, K. Y. Suh, T. N. T. Tran, G. Eng, J. Yeh, J. Seong and R. Langer, *Adv. Mater.*, 2003, **15**, 1995–2000.
23 D. T. Chiu, N. L. Jeon, S. Huang, R. S. Kane, C. J. Wargo, I. S. Choi, D. E. Ingber and G. M. Whitesides, *Proc. Natl. Acad. Sci. U. S. A.*, 2000, **97**, 2408–2413.
24 G. M. Whitesides, E. Ostuni, S. Takayama, X. Jiang and D. E. Ingber, *Annu. Rev. Biomed. Eng.*, 2001, **3**, 335–373.
25 K. Y. Suh, A. Khademhosseini, G. Eng and R. Langer, *Langmuir*, 2004, **20**, 6080–6084.
26 S. Y. Jon, J. H. Seong, A. Khademhosseini, T. N. T. Tran, P. E. Laibinis and R. Langer, *Langmuir*, 2003, **19**, 9989–9993.
27 N. L. Jeon, H. Baskaran, S. K. W. Dertinger, G. M. Whitesides, L. Van de Water and M. Toner, *Nat. Biotechnol.*, 2002, **20**, 826–830.
28 J. A. Burdick, A. Khademhosseini and R. Langer, *Langmuir*, 2004, **20**, 5153–5156.