Microfluidic contact printing: a versatile printing platform for patterning biomolecules on hydrogel substrates†

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This work describes a printing platform that utilizes components of two complementary techniques—microcontact printing and microfluidic patterning—to provide a high-throughput, reusable tool for patterning biomolecularly responsive chemical features on soft materials. Hydrogel substrates imprinted with biomolecular targets (*e.g.*, proteins or peptides) are of considerable interest because of their utility in diverse bioanalytical applications such as diagnostics and studies of cells in culture. In the system described here, a track-etched polycarbonate membrane is used to seal a PDMS microfluidic channel device to form a print head. Model 'inks'—solutions of biotin labeled biomolecular targets—are constantly replenished *via* perfusion of solution through the membrane and captured on a streptavidin-incorporating polyacrylamide hydrogel-coated substrate placed in conformal contact with the print head. The patterns obtained can be controlled through modifications of channel design and secondary programming *via* selective membrane wetting. Multiple channel designs have been used to pattern three model classes of biomolecular inks (*i.e.*, peptides, polysaccharides, and proteins). Hydrogels patterned with polylysine are used to illustrate a specific biological application for this soft-patterning method, in this case directing *in vitro* primary mammalian hippocampal neuronal growth. The short cycle time and reproducibility of this soft-patterning technique are highlighted.

Introduction

Methods for patterning biomolecules are of broad utility, with exemplary applications that include diagnostics,[1] chemical sensing,[2] and platforms for studying cellular behaviors.[3,4] The present report addresses this latter interest, highlighting the development of a protocol for patterning soft substrate materials in ways that complement those currently used to pattern hard substrates such as glass, silicon, or plastic. The patterning of the latter hard surfaces for use in biomolecular analysis has been intensively reported in the literature, with notable applications that include their use to direct neuronal growth,[5–7] study synaptogenesis,[8] and improve cellular attachment *via* modification with cell recognition molecules such as laminin.[9,10] Controlled biomolecular gradients have also been used to study important cellular processes, such as chemotaxis,[11] haptotaxis,[12] angiogenesis,[13] morphogenesis[14] and axon guidance.[15,16] Interest in performing similar cellular studies on soft materials is growing as it is now becoming well understood that substrate modulus can play a significant role in mediating the morphological and physiological development of cells.[17,18] In this context, soft materials, specifically hydrogels, are more suitable substrates for cellular studies because their mechanical properties can be easily tailored to better mimic *in vivo* conditions.[19,20]

Micropatterning technologies, including microcontact printing (μCP) and microfluidic patterning, are a logical choice for generating patterns and gradients of this type as they allow the manipulation of small volumes of solution with precise control over its temporal and spatial delivery.[4] Microcontact printing, first described by the Whitesides group in the 1990's,[21,22] can be used to create complex but useful patterns of alkanethiol (and alkyl silane) self-assembled monolayers (SAMs) on gold (and glass) surfaces, *via* a physical 'stamping'-based transfer technique. These SAMs can be used as both a resist and a platform that enables subsequent modification *via* covalent attachment. Proteins, for example, can be attached to an appropriately patterned SAM either *via* non-specific adsorption[23] or through specific functionalized end groups on the thiols.[24,25] James *et al.* later modified the technique to make it amenable to and compatible with the direct printing of proteins.[26] In the latter study, hydrophobic polydimethylsiloxane (PDMS) stamps made *via* replica molding were modified with an oxygen plasma to render them hydrophilic. This modification in turn allowed the direct printing of a positively charged protein (polylysine) over a 4 cm^2 glass substrate. A few recent studies have extended μCP to pattern biomolecules on soft substrates.[27,28] A study conducted by Hynd *et al.* utilized an acrylamide-based hydrogel substrate that was photopolymerized in the presence of a streptavidin-incorporating monomer. The resulting gel film was capable of binding biotinylated extracellular matrix (ECM) proteins transferred in patterned form *via* μCP.[27] A following study by the same authors examined cellular responses to these

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and found a preference for them to adhere in ECM modified regions.[29]

Microfluidic patterning is the most commonly used method to generate fluid-based gradients sustained by laminar flow,[30–32] but has also been used to create surface-bound patterns *via* adsorption.[9,33,34] In the latter case, a PDMS channel system is reversibly bound *via* a compliant soft-contact to a hard substrate such as glass or silicon. A solution containing a biomolecular adsorbate (*e.g.*, a protein) is pushed through the channels and bonded to the surface in spatial registration either through specific or non-specific interactions with the substrate. The channel system is then removed from the substrate and the resulting patterned surface, or even the PDMS stamp itself, used for biological studies. Applications made using this approach have been reported in the literature with notable examples including axon guidance,[9,34] haptotaxis,[35] and directed cellular migration.[36]

While powerful, microfluidic patterning and mCP techniques both have important limitations in particular regarding their capacities for efficiently and reproducibly generating multi-component biomolecular gradients or discrete chemical patterns imprinted on soft substrates. It is inherently difficult to use microfluidic methods to pattern soft materials, for example, due to the complexity involved in promoting close conformal contact with a hydrogel, which is necessary to preventleakage. Furthermore, the reusability of the microfluidic patterning technique is usually low because the flow is disrupted when the substrate is separated from the channel after each printing step. This disturbance necessitates an extended preparation time and can result in a loss of precious reagents (*e.g.*, a specific protein target for an assay). The limitations of mCP techniques in the latter context lie in the innate difficulty associated with rigorously controlling the amount of material transferred when using a complex macromolecular ink, which is essential for generating gradients of useful species such as proteins.[9,33,37] In addition, when patterning multiple biomolecular inks using mCP, difficult alignment steps are usually required. To address this limitation, Bernard *et al.* combined mCP and microfluidics to enable the printing of multiple biomolecules, without the need for a registration step.[38] In this study, a microfluidic channel was reversibly sealed to a planar PDMS stamp (*i.e.*, a slab without surface relief). Protein solution flowing through the microfluidic channel adsorbed to the surface of the PDMS slab and in this way defined the ''inking'' areas. This protocol was used to print 16 different proteins to a plastic substrate at the same time. The one drawback of this method, however, is that the stamp is not reusable and does not usefully enable the patterning of softsubstrates. There therefore remains a need and opportunity to provide further improvements to these techniques, especially in terms of the scope of both the inks and substrates that can be patterned.

The work described here draws inspiration from both microcontact printing and microfluidic patterning techniques and extends them further through utilization of a membrane-based microfluidic device design.[39] This system, presented schematically in Fig. 1 (steps I–IV), creates a 'microfluidic contact printing' tool by using microfluidic channels fabricated *via* typical soft lithography methods[37,40–42] that are irreversibly sealed to a modified polycarbonate track-etched (PCTE) membrane. PCTE membranes have been incorporated into microfluidic devices to make gateable nano- or micro-fluidic interconnects in multilayered separation systems[43–45] and in the

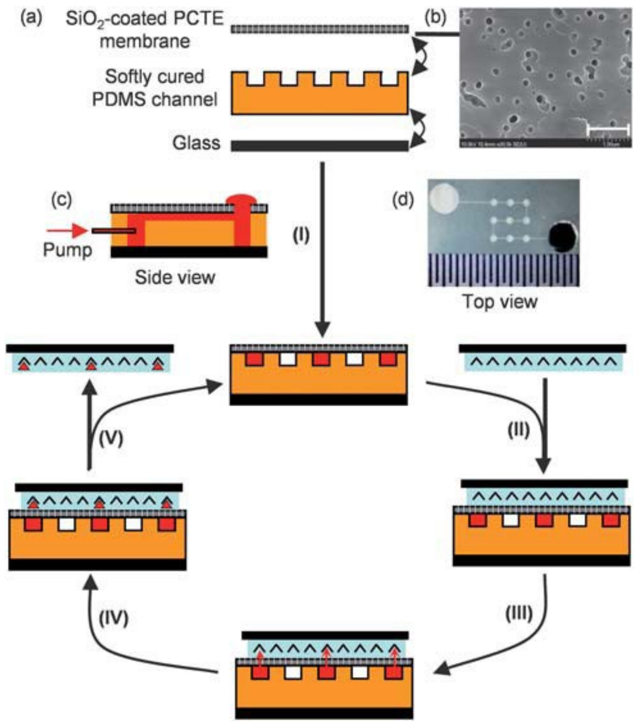

Fig. 1 Device fabrication (I) and printing process flow (II–V) are schematically represented in (a). The polyacrylamide gel (with incorporated streptavidin) is placed on top of the sealed microchannels (II) where the biotinylated biomolecule solution can diffuse through the PCTE membrane into the gel (III) and be captured by the streptavidin protein (IV), to form long term gradients/patterns (V). The pore morphology after Ti/SiO_2 deposition is shown in the SEM image in (b). A schematic representation of the side view of the device is presented in (c), while the top view of a completed device is shown in the bright-field image in (d). Scale bar in (b) is 1 mm and the smallest ruler mark in (d) is 500 mm.

fabrication of stacked microfluidic devices used to study the flux of platelet agonists into flowing blood.[46] Previous studies that incorporated PCTE membrane into their devices used adhesives (*e.g.*, epoxy or PDMS prepolymer) to interface the membranes with the PDMS microchannels.[45,47,48] Applying an ultra-thin layer of the adhesives is necessary and challenging because excess glue on the membrane can block membrane pores and, possibly, even neighboring microfluidic channels. The device reported here is fabricated using vapor deposited thin-film adhesion layers that obviate this latter requirement, thus simplifying fabrication.

In this study, the printing tool was used to pattern a single biomolecular ink solution *via* complex single channel designs; multiple biomolecular ink solutions were also patterned simultaneously using a multi-channel design. The system's capabilities for generating gradients using multiple channel designs also are demonstrated. We specifically demonstrate capabilities for patterning model biomolecular ink compositions including those based onsmall chain-length peptides, polysaccharides, and proteins.

Results and discussion

Device design and general observations

The schematic presentation in Fig. 1 (steps I–IV) highlights the process flow used to fabricate the printing device. A significant feature of the design is the durable integration of a PCTE membrane with an open feature microfluidic device to create a flexible, reusable patterning tool. As noted above, this strategy addresses some important limitations of microfluidic patterning and μCP techniques, specifically in regards to patterning soft materials, simultaneous printing of multiple biomolecules, and long cycle time. The integration of the PCTE membrane into a microfluidic device not only facilitates conformal contact with the hydrogel substrates but also shields the laminar flow field from disturbance when the gel-bearing substrate is removed. A single device is therefore able to pattern multiple hydrogel coated slides with a very short cycle time. Despite recent efforts[38] to address registration issues related to μCP, there are at present no reports that describe its use to print biomolecules directly on hydrogels in either a gradient or registered multiple printing-level form. As we show in the data below, specific features of the method described here allow gradients and multiple biomolecular patterns to be printed in a single step.

The printing procedure involves two components of actuation: (a) transudation of a biomolecular 'ink' through a PCTE membrane; and (b) the subsequent capture of this 'ink' by an affinity modified hydrogel. The quantitative aspects of the transferred pattern (feature width, depth, shape, and contour; concentration of imprinted biomolecular ink; gradient profiles; and hierarchy) can be tailored by manipulation of simple mass-transfer responsive system features. For example, the location of the 'ink' supplied can be controlled through combination of the underlying channel design and the use of a secondary selective membrane wetting protocol (see below). The amount of 'ink' supplied also can be dynamically modulated by such factors as the flow rate/backing pressure and contact time between the hydrogel and the device. 'Ink' capture is the result of a competition between diffusive zone spreading within the hydrogel and the kinetics of the coupling reaction, which in the current model involve a non-covalent binding between the biotinylated 'ink' and streptavidin-incorporating gel. The printing resolution, therefore, is directly affected by intrinsic 'ink' characteristics (*e.g.*, the molecular weight or charge distribution) as well as the probe concentration in the hydrogel. These modifiable and controllable system parameters allow a versatile and dynamic patterning capability for soft substrates, as illustrated in the following sections.

Interfacing PCTE membranes with PDMS microchannels

Creating a strong bond between the PCTE membrane and PDMS microchannel support is a critical design requirement for this microfluidic device. To do so (Fig. 1a), the PCTE membranes are first modified with a thin layer of vapor-deposited titanium (an adhesion layer), followed by silicon dioxide, which substantially improves bonding between the PDMS microchannels and the membrane. This adhesion layer does not cause any obvious deformation of the PCTE membrane (Fig. 1b) but decreases the pore size by approximately 14 nm at these mass coverages (Fig. S1, ESI†). We believe that the SiO_2 coating gives the PCTE membrane a glass-like surface, which is able to form strong chemical bonds with PDMS after surface activation using a corona discharge.[49] The latter treatment in fact provides fully irreversible adhesive bonding.[50] We note that interfacing of the channel to the modified membrane is significantly improved when the cast PDMS channels are soft or slightly under-cured (procedure described in the ESI†).[51]

The PCTE membrane used was large enough to cover and seal the entire underlying channel system (including the inlet and outlet) to circumvent the possibility of leaking.[44,46,48] To accommodate this design, a syringe needle was inserted through the PDMS sidewall into the inlet reservoir and then sealed to the PDMS using epoxy, as is schematically depicted in Fig. 1c. The optical micrograph in Fig. 1d shows the underlying microchannel system of a completed printing device (white areas).

Channel designs and versatile patterning of biomolecular inks

Small chain peptide patterned using single-channel designs. We used the IKVAV (Ile-Lys-Val-Ala-Val) sequence as a model small chain peptide. The IKVAV sequence is a small peptide derived from the large glycoprotein laminin, which plays an important role in various cellular processes including migration, differentiation, attachment, and neurite extension.[52–54] It has more importantly been identified as the active recognition site in laminin and used in model form for controlling cellular adhesion and neurite extension.[55] To immobilize this sequence, the IKVAV was modified with biotin on one end for attachment to the streptavidin-incorporating polyacrylamide hydrogel and fluorescein isothiocyanate (FITC) on the other end for visualization purposes.

Examples of two single channel designs and the corresponding peptide patterns transferred to the hydrogel are presented in Fig. 2. As can be seen from the fluorescence micrographs, IKVAV is easily transferred to the hydrogel substrate in a registry directly corresponding to the underlying channel design. In these images, the concentration of streptavidin in the hydrogel was ~50 μg ml^{-1} which most likely explains the halo

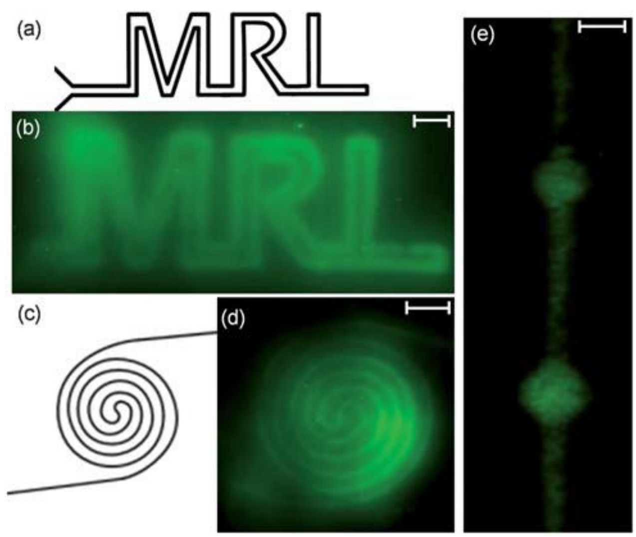

Fig. 2 Patterning results for the IKVAV peptide for different channel designs (width ¼ 50 μm) are shown in (a) and (c). The original channel design can be directly transferred to the gel (b) and (d). In (e) two 75 μm diameter dots, on a 20 μm wide channel, were transferred to the gel to highlight printing resolution capabilities. Scale bar is 250 μm in (b), 500 μm in (d) and 50 μm in (e).

effect seen around the transferred channel edges. When the streptavidin concentration was increased 20 times, however, a pattern that not only had improved resolution, but also smaller features was generated (Fig. 2e) as expected for a reaction-diffusion based capture system.[56] A specific quantitative comparison of the relationship between streptavidin concentration and printing resolution can be found in example presented in Fig. S2 of the ESI†.

It is worth noting that the peptide-patterned hydrogels, when stored at 4 ºC for several weeks, did not experience significant reduction in fluorescence intensity or diffusive spreading of the bound peptide. This affirms that the stability of the biotin–streptavidin affinity system permits the generation of patterned peptide substrates in advance, a feature we found to be useful in studies of guided cell growth mediated by immobilized peptide sequences.[57] This aspect of the work is discussed below.

Polysaccharides patterned using multi-channel designs. To further explore the printing capabilities of the system, two multi-channel designs were tested using fluorescein and rhodamine labeled biotinylated dextrans (M_w ¼ 10 kDa) as model polysaccharides. Dextran is a hydrophilic polysaccharide that has a high water solubility, is commonly used as both an anterograde and retrograde tracer in neurons,[58] and has been variously used as a drug delivery vehicle.[59] In the results presented in Fig. 3, fluorescein and rhodamine labeled biotinylated dextrans were injected into each individual channel. The printed pattern is directly related to the underlying channel design, as well as the specific ink moved through the corresponding microchannel. A proven channel design[30] (Fig. 3d) for generating complex gradients (a mixing tree), was incorporated into the system. The two labeled dextrans were injected into the channel system at points where the solutions are split, combined, and mixed, to create co-localized gradients which were then transferred to the hydrogel (Fig. 3e). These data demonstrate that parallel printing of multiple substances can be accomplished in a single patterning cycle. This provides a useful point of comparison to classical mCP methods, where the patterning of multiple biomolecular inks requires sequential inking and printing steps along with effective means of registration. The printing platform is for this reason one we believe could be useful for creating substrates for use in high-throughput screening methods.

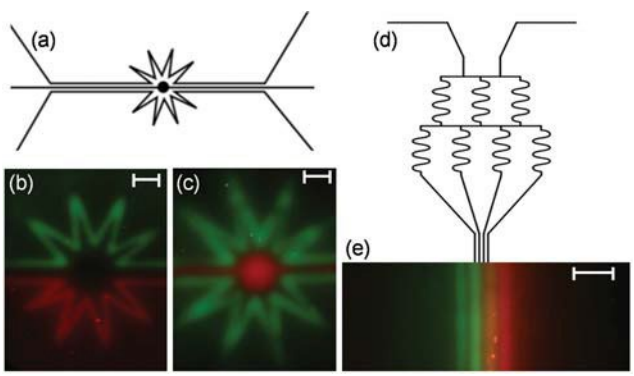

Fig. 3 Patterning results for the fluorescein and rhodamine labeled dextran solutions for the multi-channel designs highlighted in (a) and (d). The fluorescent micrographs in (b) and (c) show that multiple biomolecules can be patterned onto the gel in one printing attempt, directly related to the underlying channel design. The complex mixing scheme in (d) can be used to create gradients of biomolecules like the one pictured in (b). Scale bars are 500 mm.

Large-area protein gradients generated using a single-channel design. A serpentine single-channel gradient generator was used to transfer a FITC and biotin labeled polylsyine (M_w ¼ 15–30 kDa) to the hydrogel substrate (Fig. 4). Polylysine is commonly used to treat substrates for tissue culture to improve cell adhesion and viability, as well as in patterned form to guide neuronal growth on planar substrates.[60–62] A single channel design with an incrementally increasing channel spacing (Fig. 4a) was used to create the printed concentration gradient shown in Fig. 4b, one quantitatively analyzed in the data given in Fig. 4c. The chemical gradient formed results from both the incremented spacing as well as the pressure drop that occurs as the solution moves farther from the channel inlet.[63] Using a single channel to pattern a single component gradient in this way may be preferred for reasons of ease of implementation over other more complex designs, such as the mixing-tree[30] based device shown in Fig. 3d. While the latter is well suited to printing multicomponent gradients, the simpler single channel design serves as

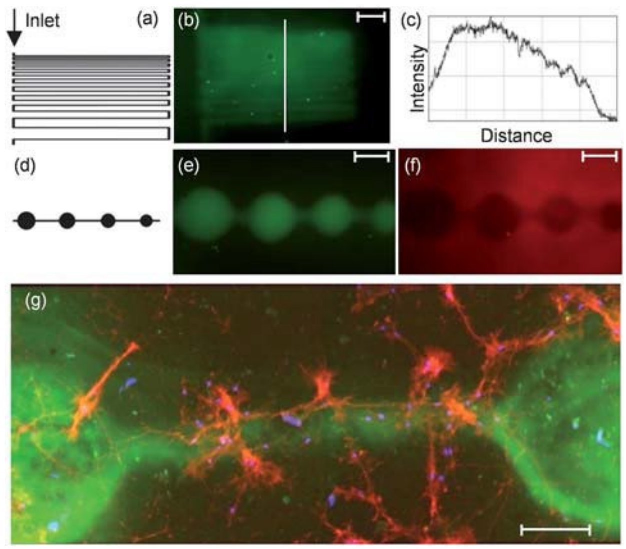

Fig. 4 Patterning results for the direct printing of FITC-labeled polylysine and indirect patterning of rhodamine labeled BSA. The channel design shown in (a) can be used to create gradients of biomolecules using a simple single channel design, which has a fixed channel width (50 mm) and range of spacing between loops from 40 to 160 mm. The transferred gradient shown in (b) is quantitatively represented by the line scan across the sample, presented in (c). The single channel design in (d) was used to create a pattern of fluorescently labeled polylysine (e) and then the substrate was exposed to a secondary patterning of rhodamine labeled BSA (f); the secondary patterning does not 'overwrite' the first. Finally, the response of primary hippocampal neurons to polylysine patterns created with the channel design in (d) is presented in (g). In this image, the biotin–polylysine–FITC pattern appears green, while the cell nuclei (stained with DAPI) appear blue and the cell processes (stained with rhodamine–phalloidin) appear red. Scale bars are 500 mm in (b), (e) and (f), and 100 mm in (g).

a convenient way to create well defined greyscale patterns for chemotactic[64] or haptotactic[65] cell migration assays.

Orthogonal patterning *via* secondary protein exposure. A secondary protein exposure does not overwrite the first protein pattern. The patterns are bound stably to the gel substrates *via* the biotin–streptavidin linkage, which allows a secondary patterning step to be carried out using a different biomolecular ink. This is illustrated by the example shown in the micrograph presented in Fig. 4e, which shows an initially printed pattern of FITC–polylysine–biotin. The image presented in Fig. 4f illustrates the results of a secondary exposure, an orthogonal patterning, of rhodamine–bovine serum albumin (BSA)–biotin on the same substrate. As is clearly seen in this latter image, the secondary exposure does not fully overwrite the initial patterning but in fact shows a significant orthogonality in the relative uptake of the BSA. We further quantify this phenomenon showing it in a plot that highlights the blockage (of BSA), Fig. S3, ESI†. This capability allows the substrate to be easily modified with a second biotinylated target to provide better control of the surface properties.

Protein patterned gel substrates for neuronal guidance. Hydrogels patterned with polylysine using the channel design presented in Fig. 4d were used to guide the growth of hippocampal neurons. The neurons can detect and respond in accordance (Fig. 4g) with the underlying polylysine pattern. As illustrated in the contrast enhanced image in Fig. 4g, the neuronal processes (red) are seen to follow along the edge of the protein pattern (green), connecting with other cell soma (blue) as they navigate the substrate. The images reveal a general tendency for the cell soma to attach near the transition boundary of the polylysine domain and propagate dense projections of processes into it. The highlighted phenomenon is similar to the findings presented in previous studies of neuronal guidance on polylysine patterned on hard substrates.[5,60–62] We do find that soft substrates based on polyacrylamide hydrogels are more challenging to use given the substantial cytotoxicity of residual monomer. We are currently developing significantly more biologically compliant gel chemistries that will remove this sensitivity (see below).

Discrete features patterned using selective membrane wetting

A selective membrane wetting method can be used to advantageously extend the patterning of discrete features using the microfluidic contact printing tool. As received or post-modification with Ti/SiO_2, PCTE membranes are hydrophobic so pores in the membrane must be 'wetted' with a low surface tension solvent,[25] such as isopropyl alcohol (IPA), to initiate solution penetration. In the previous sections, the membrane covering the channel system was wet extensively by IPA using a cotton swab (Fig. S4, ESI†), which in turn created patterns determined explicitly by the channel design. The 'writing' method described here uses a capillary tube to precisely deliver the IPA wetting agent (Fig. S5–S6, ESI†) to distinct membrane regions to create a secondary degree of control on the pattern generation that operates in hierarchy with the channel design (Fig. 5a and b). We found that it is only in these selectively

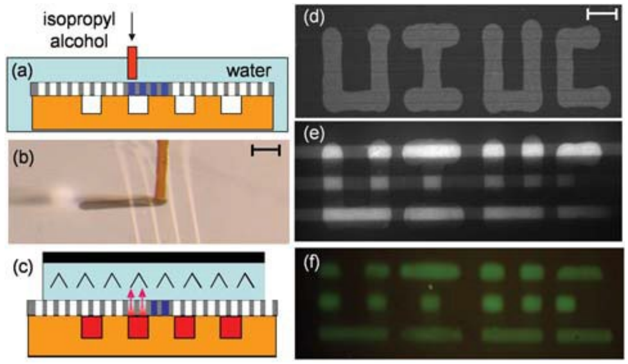

Fig. 5 The secondary membrane wetting technique is schematically represented in (a), while an optical micrograph of the 'writing' process is presented in (b). Solution can diffuse out through the underlying microchannels only in the regions that have been wetted with solvent (c). The optical micrograph in (d) shows a pattern that was 'written' on the membrane, while the fluorescent image in (e) shows the diffusion of the FITC–biotin molecule through the wetted regions. The resulting pattern that was transferred to the gel is shown in (f). Scale bars are 300 mm.

wetted regions where biotinylated solutions transude through the membrane to be captured subsequently by the streptavidin-incorporating hydrogel (Fig. 5c). The micrograph presented in Fig. 5d shows a 'UIUC' pattern that was 'drawn' on the PCTE membrane-sealed channel system, while Fig. 5e and f show the resulting transudation of a biotinylated FITC solution through these wetted regions along with the corresponding pattern of the FITC–biotin captured in the gel. These exemplary results suggest this method is a versatile way to pattern substrates in the form factor of multiplexed arrays without the requirement for a 3D integrated microfluidic delivery system.

Reusability of the printing platform and reproducibility of the pattern transfer

The plot in Fig. 6 illustrates the recycle capability of the system and the pattern transfer reproducibility between consecutively printed gels. The analysis was done using fluorescein-labeled dextran with the channel design shown in Fig. 1d. In order to determine an appropriate testing time, the fluorescence intensity and diameter of the transferred patterns in relation to printing time were also investigated and found to increase linearly as the stamping time was incremented from 0.5 to 10 minutes (Fig. S7, ESI†). This follows in accordance with a diffusive broadening of the target flux in the gel over time. Based on these data, a 5 minute stamping time was adopted to test the reusability and reproducibility of the printing technique. In this case, the fluorescence intensity of the transferred patterns was analyzed and compared, as described in the ESI†. As seen from the plot given in Fig. 6, with inset micrographs displaying printing results from the same device at different time points over several days, the intensity of the transferred patterns was essentially constant over multiple printing cycles. These data affirm a broader capability for the generation of uniformly patterned substrates with short cycle time and useful copy numbers.

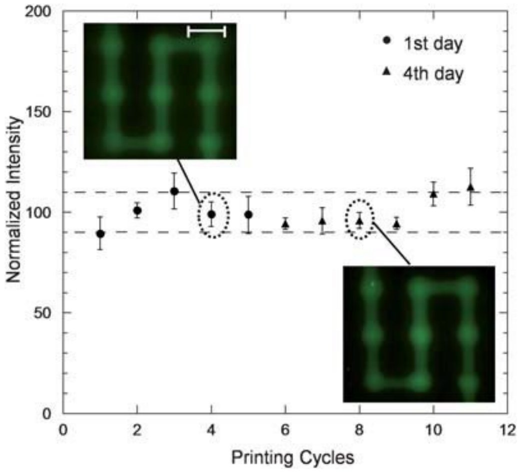

Fig. 6 The plot presented here shows the system's high pattern transfer reproducibility over consecutive printing cycles. The first 5 data points represent printing attempts on day 1, while the last 6 data points represent printing attempts completed on day 4. The inset micrographs visually highlight the similarity in fluorescence intensity between individual printing cycles. Scale bar is 500 μm.

Further system modifications

The system reported in this work has significant potential to be modified, whether by altering the channel design, membrane material selection, method of membrane wetting, the incorporated affinity binding system, or hydrogel system used as a receiving substrate. In our continuing work, for example, we are exploring printing platforms that are modified to use binding schemes exploiting complementary strands of ss-DNA as well as antibody/antigen recognition methods. The more important modification, however, centers on new gel chemistries to replace the benchmark polyacrylamide system used here, ones that will significantly enhance biocompatibility in cell-based studies. We will report on progress in this area shortly. Finally, we would like to stress the challenges related to printing large biomolecules like proteins, which are known to have biofouling issues caused by denaturization and agglomeration. These challenges are closely related to the intrinsic characteristics of the inks, which in turn need to be reflected in the optimization of system parameters like membrane selection, modification of the pore surface chemistry, sample preparation, and flow rate control.

Conclusions

Microfluidic contact printing, a new patterning technique described here, combines several of the desirable aspects of μCP and microfluidic patterning techniques, and addresses some of their important limitations through the integration of a PCTE membrane. Using this technique, biomolecules (*e.g.*, peptides, polysaccharides, and proteins) were printed in high fidelity on a receptor modified polyacrylamide hydrogel substrate. The transferred pattern is primarily dictated by the design rules of the underlying microfluidic channel system, coupled with diffusive zone spreading. The application of a selective membrane wetting protocol, allows a versatile secondary patterning capability. The protocols support the printing of multiple reagents without registration steps and fast recycle times.

Experimental

Device fabrication

Polyvinylpyrrolidone (PVP) or PVP-free (PVPF) PCTE membranes (0.22 μm pore, GE Osmonics Labstore, Minnetonka, MN) were coated with 5 nm of titanium, followed by 10 nm of SiO_2 using an electron beam evaporator to improve binding to the PDMS devices. Please see ESI† for a detailed description of the membrane modification protocol and a comparison of pore sizes before and after modification (Fig. S1†).

Silicon masters were generated using standard photolithography techniques (described in detail in the ESI†),[66,67] and covered with degassed PDMS (10 : 1 ratio, Sylgard® 184). Samples were heated at 70 °C until they were softly cured (~20 minutes, see ESI† for a detailed description). The softly cured PDMS mold replicas were removed from the masters, and holes were punched at the channel inlets and outlets using biopsy punches. The backside (or unpatterned side) of the PDMS stamp and a microscope slide were permanently bonded together, after both surfaces were treated by Corona discharge (BD-20AC with power line filter, Electro-Mechanics, Ravenswood, IL, starting voltage 45 KV) for 40 seconds.[49] The previously Ti/SiO_2 modified PCTE membranes were exposed for 2 minutes to Corona discharge, while the open PDMS channels (previously attached to the supporting glass microscope slide) were exposed to Corona discharge for 40 seconds. The two activated components were carefully placed into contact and then placed in a 70 °C oven to cure for at least one hour. Once curing was complete, a syringe needle (26 gauge) that had been removed from its plastic housing, was inserted into the inlet hole (from the sidewall of the device) and sealed with 5 minute epoxy. The device was then placed in 70 °C oven for at least one hour to ensure that the epoxy was fully cured.

Hydrogel preparation

Microscope slides were cut into 25 mm × 10 mm pieces to serve as supports for the hydrogel substrates. The glass pieces were cleaned with piranha solution for 20 minutes, rinsed with Milli-Q water and dried with nitrogen. To promote adhesion between the cleaned glass slides and the hydrogel substrate, each slide was treated with 100 μl of 3-(trimethoxysilyl)-propyl acrylate : glacial acetic acid : H_2O (1 : 2 : 2, v/v) for 1 h and then rinsed with water and then ethanol.[29] The silane treated glass slides were generally used within a few days.

Prepolymer solution was prepared containing 10% acrylamide, 0.26% *N*,*N*-methylene-bis-acrylamide (Bis) and 0.05% *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TEMED). A 5 mg ml^{-1} solution of streptavidin acrylamide (Invitrogen, Carlsbad, CA) in PBS was added to the prepolymer solution in a 1 : 100 ratio for regular patterning experiments or in a 1 : 5 ratio for biological studies and small feature size/high resolution patterning. A 10 wt% aqueous solution of ammonium peroxydisulfate initiator was added in a 1 : 50 ratio. The pre-polymer solution was quickly distributed to the previously silane-treated glass slides, which were placed on a PDMS slab to prevent liquid overflow. The pre-polymer solution was covered by a glass microscope slide, with one or three #1 coverslips used as spacers, to allow for full gelation. As prepared, the hydrogel slabs were approximately 50 μm thick (one cover slip as a spacer) or 150 μm thick (three coverslips as a spacer). Thicker gels were used for general patterning experiments, while thinner gels were used for cell culture studies. Once gelation was complete, typically 20 minutes, the glass supported gels were removed from the PDMS slab, separated from the microscope slides and stored at 4 °C in deionized water until needed.

Membrane wetting

As received and following surface modification, the PCTE membranes are hydrophobic so must be exposed to IPA to allow solution to penetrate through the pores. The microfluidic contact printing device was for this reason exposed to solvent in two ways. In the first method, a cotton swab soaked in IPA was used to wet channel regions where solution perfusion was desired (Fig. S4, ESI†) and then was immediately submerged in water to prevent solvent evaporation. This technique was most commonly used to wet devices used in patterning experiments. We note that, when using this wetting technique, the best printing results were attained using PVP modified membranes.

In the second method, a 'pen' was used to 'write' the pore wetting solvent in discrete places along the PCTE membrane covering the channel system. An image of the experimental set-up (Fig. S5†) and a detailed description of this procedure are given in the ESI†. When using this selective pore-wetting technique, the resulting printing attempts were most successful using PVP-free membranes.

Prior to use, and after being 'wet' with IPA, the microfluidic devices were degassed for at least 20 minutes in a vacuum oven, submerged in deionized water, using the channel outgas technique (COT) described previously.[68] After degassing, devices were stored submerged in deionized water until needed.

Peptide patterning

Biotin–Ile-Lys-Val-Ala-Val-Lys (FITC)–NH_2 (Biotin–IKVAV–FITC) was synthesized by the Biotechnology Center at the University of Illinois at Urbana-Champaign. A 100 μM stock solution of this peptide was prepared using Milli-Q water and stored at 4 °C until needed. The peptide solution was loaded into a syringe, connected to a single-channel microfluidic device *via* PE-20 polyethylene tubing, and then loaded into a syringe pump (1 μl min^{-1}, Harvard Apparatus, Holliston, MA). To prevent solvent evaporation, a drop of water was placed on the membrane and covered with a cover slip. The progression of the fluorescently labeled peptide through the microchannel system was tracked using a fluorescence microscope (Epifluorescent Microscope Olympus, AX-70 with a CCD camera Optronic MagnaFire, Melville, NY). Once flow of the labeled peptide could be observed through the wetted channel regions (typically 5–10 minutes), the cover slip was removed, the membrane surface was rinsed briefly with water and then blotted dry. A streptavidin-incorporating hydrogel was placed on the channel system, after excess water was removed, and held in place with a small weight to ensure conformal contact. Care should be taken to avoid trapping air at the interface as this will detract from successful patterning. Peptide pattern transfer was typically complete after 2.5 minutes. Once the patterning was complete, the gel was removed from the device, rinsed with deionized water and then imaged *via* fluorescent microscopy. All fluorescent images were background corrected using the method described in the ESI†.

Dextran patterning

Biotinylated dextran (M_w = 10 kDa) labeled with either a tetramethylrhodamine (mini-ruby) or fluorescein (mini-emerald) (Invitrogen) was used. A 20 μM stock solution of each labeled dextran was prepared using Milli-Q water and stored at room temperature until needed. The dextran solutions were interfaced with the microfluidic devices and visualized in the same way as described previously for the peptide solution.

Contact times of ~5 minutes typically gave high fidelity pattern transfer. Individual images of the mini-ruby and mini-emerald components of the pattern were captured using appropriate filter sets and then combined using Adobe PhotoShop (Adobe, San Jose, CA).

Protein patterning

Biotin–polylysine (M_w = 15–30 kDa) was purchased from Nanocs (New York, NY) and labeled with FITC using the EZ-Label Fluorescein Isothiocynate Protein Labeling Kit (Pierce Biotechnology, Rockford, IL) according to the included instructions. Typical dialysis methods were used to remove the excess FITC reagent. Biotinylated Bovine Serum Albumin (BSA) was purchased from Pierce Biotechnology and functionalized with NHS-rhodamine (Pierce Biotechnology) as per the included instructions. Once the labeling was complete and the excess dye had been removed, the protein solutions were stored at 4 °C until needed.

Polylysine solution, 2 mg ml^{-1} in Milli-Q water, was prepared and then degassed in a vacuum oven. After the solution had been degassed, it was interfaced with the microfluidic device in the same way as described above. For these patterning attempts, the flow rate was set to either 0.5 or 1 μl min^{-1} depending on the channel design. It was found that higher flow rates were better for creating gradients as the printed pattern resolution decreased with an increasing flow rate. For printing discrete features, lower flow rates are desirable (less than or equal to 0.5 μl min^{-1}). Printing protein patterns typically require longer contact time (15–30 minutes) depending on channel design. Patterned gels were stored in PBS buffer overnight at 37 °C to remove any unbound protein and then transferred to 4 °C for further storage.

For the secondary patterning of BSA, approximately 100 μl of rhodamine and biotin labeled BSA (1mg ml^{-1} in Milli-Q water) was placed on top of the polylysine patterned gel and allowed to adsorb for 1 hour. The gel slab was rinsed with deionized water, placed in fresh deionized water at 37 °C for several hours and then imaged.

Primary hippocampal neurons were used to highlight a potential application for patterned gels created using this platform—guided cell growth. The cell plating procedure and immunocytochemistry protocol used are similar to those published previously.[69]

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