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COMMUNICATION

Large Scale Patterning of Hydrogel Microarrays using Capillary Pinning

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Burcu Gumuscu, Johan G. Bomer, Albert van den Berg and Jan C.T. Eijkel

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Capillary barriers provide a simple and elegant means for autonomous fluid-flow control in microfluidic systems. In this work, we report on the fabrication of periodic hydrogel microarrays in closed microfluidic systems using non-fluorescent capillary barriers.

Selective hydrogel patterning offers a novel way to expand the capability of biological and clinical microarrays, gel-based lab on a chip bioassays, cell patterning techniques, and biomolecule separation technologies. A reduction in size to small volumes enables dramatic increases in number of analyses and throughput of hydrogel microarrays due to faster mass transport and increased surface to volume ratios. Despite the large promise of small volume hydrogel microarrays, their fabrication has remained challenging. Here we demonstrate that picoliter volume microarrays of photopolymerizing and thermo-gelling hydrogel types can be robustly and autonomously fabricated by capillary pinning in microfluidic devices. The method enables patterning in closed microfluidic systems entirely manufactured from non-fluorescent materials.

Hydrogels have found widespread use in microfluidic systems due to their unique material properties. They provide excellent sensitivity to chemical and physical stimuli such as pH,¹ ionic strength,¹ temperature,² electric field,³ and light⁴. Hydrogels have been used for electrokinetic biomolecule separations for over a century, as they provide a dense mesh of porous three dimensional matrix without a significant effect on electrolyte composition.⁵ These functionalities have brought hydrogel usage to the fore in wireless biomolecule measurements,⁶ two dimensional (bio)molecule separation,⁷ drug delivery,⁸ microdialysis,⁹ and biosensing^{10,11,12,13} applications with microfluidic devices. The incorporation of hydrogels in microfluidic systems is commonly accomplished by traditional methods such as optical and soft lithography.^{14,15} The majority of optical lithography techniques are based on masked photolithography¹⁵ and laser patterning.¹⁶ Optical lithography has proven to be a well-established and reliable method. However, patterning via conventional photomasks comes at the cost of poor structure resolution in closed platforms due to diffraction of UV light from the microchip walls and uncontrollable free radical diffusion during polymerization.¹⁷ Hence, working with

conventional lithography photomasks poses serious challenges when the aim is to fabricate hydrogel microarrays in tens of micrometers scale without any defects. Soft lithography techniques, including microcontact printing¹⁸ and micromolding,¹⁹ offer inexpensive, convenient, and scalable templates for patterning. However, these techniques require polymer (polydimethylsiloxane) molds for patterning and therefore are not suitable for hydrogel fabrication in closed microfluidic systems.²⁰

For the hydrogel array applications mentioned above, a high degree of control over the shape and the size distribution of hydrogels down to micrometer scale is needed. In the past, capillary valves ('phaseguides') have been successfully implemented in closed microchips to pattern hydrogel structures by local pinning of the hydrogel precursor in a scale range from ~100 of microns to tens of millimeters.^{21,22} Using this method, a maximum number of ~400 pinned liquid patches, each containing a few microliters of liquid, were patterned. In addition, phaseguide arrays presented in the literature were made of SU-8, a photopatternable polymer, that was chosen for its relatively less hydrophilic character ($65 < \theta < 85^\circ$, θ is the contact angle) in comparison with glass surfaces.²³ This approach hinders fluorescence-based biomedical applications because SU-8 is strongly autofluorescent, overlapping with the emission bands of many fluorescent tagging agents.²⁴ Both the large size and the fact that structures made of SU-8 present limitations to this approach. Further development of the phaseguide technique is thus warranted for applications where large scale patterning of picoliter volume hydrogel patches in large scale areas and/or the use of non-fluorescent polymers in fabrication are necessary.

In this work, we fabricated massively parallel hydrogel patterns by capillary pinning followed by photopolymerization or thermo-gelation in closed microfluidic platforms. Capillary pinning barriers were made of fused silica glass, which is a non-fluorescent material. Despite fused silica provides hydrophilic

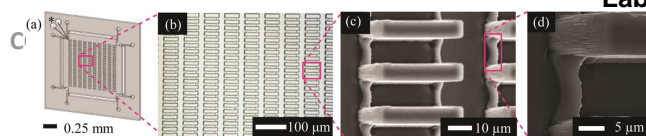


Figure 1. (a) 2D sketch of the microchip with sample inlets (*), microfluidic inlets and outlets, glass pillars and capillary barriers. (b) Optical microscopy image of glass pillar (rectangle) array, capillary barriers (structures between glass pillars) and air filled channels (light colored areas). (c) Tilted top view image of the glass capillary barriers between glass pillars under SEM; (d) a closer tilted top view of capillary barriers under SEM. The depth, width, and length of pillars and capillary barriers are $20 \times 4 \times 5 \mu\text{m}$ and $4 \times 1 \times 3 \mu\text{m}$, respectively.

surfaces ($\theta < 25^\circ\text{C}$) unfavorable for pinning,²² we show that silanization can enable the production of periodic hydrogel patterns on this material. Capillary barriers allow for autonomous and precisely controlled trapping of the hydrogel precursor solution over large areas (cm^2), with high reliability and spatial resolution, and without any defects and distortions. For a proof-of-concept demonstration, we fabricated a closed microchip with an array of $\sim 400\text{k}$ hydrogel patterns, sandwiched between $\sim 400\text{k}$ glass pillars, and $\sim 800\text{k}$ capillary barriers using only $0.5 \mu\text{l}$ of hydrogel precursor. We showed that large scale microarray patterning by capillary barriers is applicable both to photopolymerized and to thermo gelling hydrogel types.

Microchip fabrication

The microfluidic devices were fabricated in the MESA+ cleanroom facility at the University of Twente. Microchips were structured in fused silica glass wafers and consisted of two plates. The top plate contained capillary barriers, channels, buffer reservoirs, and fluidic inlets and outlets, while the bottom plate remained unprocessed. Figure 1a and 1b show an assembled microchip with glass pillars and capillary barriers.

This design was fabricated using two consecutive reactive ion etching (RIE) steps to define glass pillar and capillary barrier height for hydrogel containment. Before each RIE process, an SU-8 layer was spin-coated on the wafer. The SU-8 layer served as a mask during the subsequent dry etching process to fabricate the structures in the underlying wafer. A photolithography step was performed to pattern the structure layout in SU-8. After the postbake step, the exposed layer was developed and dry etched. Subsequently, the SU-8 layer was stripped in a piranha bath. The etch depth of the structures was $15 \mu\text{m}$ measured after the first RIE step. After the second, the etch depth was $20 \mu\text{m}$ for the channels and $15 \mu\text{m}$ for the capillary barriers. Final capillary barrier structures were uniform, stable and reproducible. Figure 1c depicts glass pillars, capillary barriers, and channels after the second RIE step. Buffer reservoirs together with fluidic inlets and outlets were opened on the back side of the wafer by powder blasting. To pattern the holes, the wafer was laminated with an Ordyl tape resist (BF410; Tokyo Ohka Kogyo). The foil was then developed using 1% of NaHCO_3 , powder blasted with Al_2O_3 particles, and subsequently removed by acetone. Finally, the processed wafer was thermally bonded with a plain fused silica wafer. The minimum feature size obtained by the classical lithography and reactive ion etching processes are emerging as physical limitations in the hydrogel patterning by capillary barriers. This limitation can become an issue in the next

generation microfluidic devices where less than picoliter volume size hydrogel patterns are needed.

Surface silanization

Silanization allows the hydrogel to covalently bond to the glass surface, increases the mechanical stability of the hydrogel structures in the chip, and decreases the surface wettability of the fused silica.²⁶ The inner walls of the microchip were therefore silanized to enable the formation of covalent bonds between the glass plate and the hydrogel. To achieve this, the microchip was cleaned in 0.1 M NaOH for 30 min in an ultrasonic bath. The microchip was rinsed with DI water and placed in a solution of 2:3:5 (v/v/v) 3 trimethoxysilyl propyl methacrylate (Sigma): glacial acetic acid (Sigma): DI water for 60 min. After rinsing with DI water and acetone, the microchips were dried at 110°C to promote covalent siloxane bond formation to the glass surface.²⁶ Figure S1a, Supporting Information, shows a photopolymerized polyacrylamide hydrogel block firmly adhered to the silanized glass plate.

Hydrogel preparation and patterning

Polyacrylamide hydrogel precursor was prepared by blending 20% acrylamide/bis solution (19:1) (BioRad), 2% 2,2 dimethoxy 2 phenylacetophenone (Invitrogen) and 2% ammonium persulfate (Invitrogen) solutions. A pore size range between 10 and 50 nm was obtained, as shown in Supporting Information, Figure S1b. Preparation, patterning and polymerization of precursor solution were performed under N_2 flow due to the oxygen sensitivity of polyacrylamide. Polyethylene glycol diacrylate (PEG DA) (MW 3400) (Laysan Bio, Inc.) was dissolved in PBS at 15% w/v and combined with 10% 2-hydroxy-1-[4-(2-hydroxyethoxy)phenyl]-2-methyl-1-propane (Irgacure 2959) (Sigma) solution to prepare PEG DA hydrogel precursor. No special equipment was used in preparation, patterning and polymerization of PEG DA precursor solution. Collagen hydrogel precursor was prepared by blending purified collagen solution (Advanced Biomatrix, Inc.) with 10X PBS, and 1 M NaOH (Collagen: PBS: NaOH ratio of 8:1:1). Agarose hydrogel precursor was prepared by dissolving ultrapure

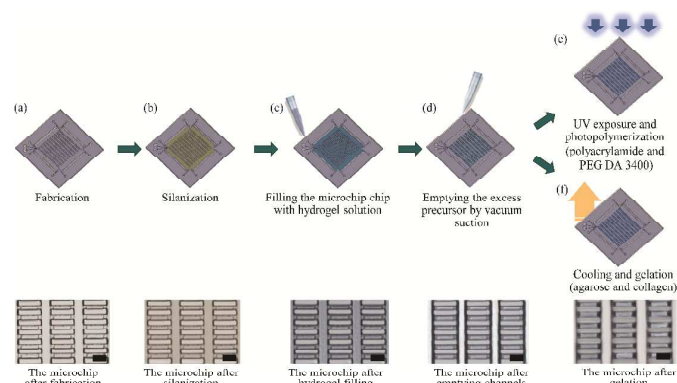


Figure 2. Fabrication process flow for custom hydrogel patterning in a closed microfluidic chip. Scale bars indicate $15 \mu\text{m}$ for all images.

agarose powder (Invitrogen) in 1X TBE buffer at 0.5% w/v in a hot water bath. Preparation and gelation of collagen and agarose precursor solutions were performed in ambient conditions.

Patterning of agarose hydrogel was performed on a hot plate adjusted to 45°C.

Figures 2a-2f outline the hydrogel fabrication process. Immediately after the silanization (Figure 2b), the precursor solutions were injected into the closed microchip. The entire void space in the chip was then filled by capillary action owing to the positive pressure applied through pipetting (Figure 2c). Excess precursor solution was subsequently removed by vacuum suction through the outlets (Figure 2d). In this stage, the hydrogel precursor remained contained between the pillars due to capillary pinning at the barriers. No air bubbles were trapped in the microchip during filling and emptying. Patterned polyacrylamide and PEG DA hydrogel precursors were then cured by UV light at 400 mW cm⁻¹ for 3 min (Figure 2e), while patterned collagen hydrogel precursor was placed in an incubator at 37°C and patterned agarose hydrogel precursor was cooled to room temperature for gelation (Figure 2f). Processed microchips were kept in DI water at room temperature. In this process, ~1.25 pL hydrogel structures with 4 x 20 x 20 μm dimensions were produced for all of the photopolymerized and thermo gelling hydrogel types, confirming the versatility of the method. The final hydrogel patterns were robust with a patterning consistency of more than 99% as seen in Supporting Information, Table S1, Figure S2 and S3.

Contact angles of hydrogels on fused silica surface were measured using the Dataphysics OCA-20 contact angle software.

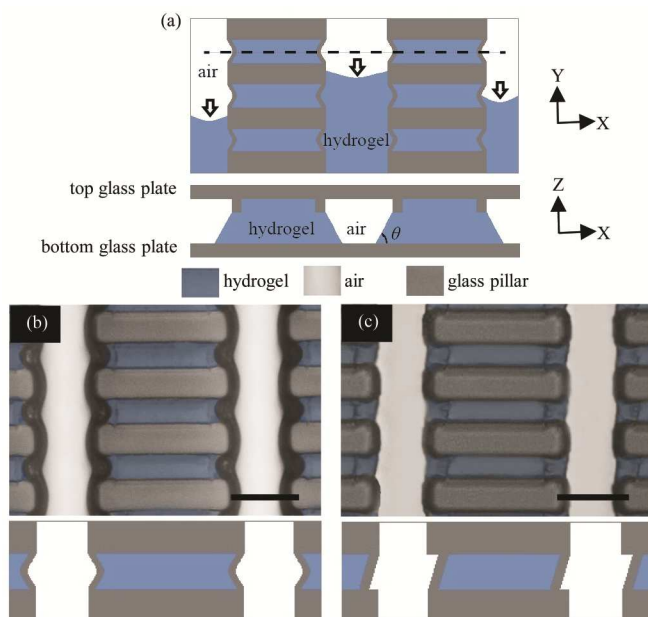


Figure 3. (a) Top and cross sectional schematic views of meniscus pinning at capillary barriers. Direction of hydrogel flow in channels during vacuum suction is indicated by arrows and cross sectional planes are shown by dashed lines. Phase contrast microscopy image of custom patterned polyacrylamide trapped in between glass pillars after photopolymerization using (b) parenthesis-shaped capillary barriers and (c) slash-shaped capillary barriers (the images are artificially colored on the basis of gray-scale differences). Scale bars indicate 10 μm for all images.

Scanning electron microscopy (SEM) images were taken using a JEOL JSM 5610 field emission SEM.

Capillary barrier operating principle

Hydrogel precursor was pinned by the capillary barriers in the channels during the vacuum suction. Pinning is attributed to the abrupt expansion in the cross sectional geometry of the channel at the barriers, hydrogel meniscus alteration and the local Laplace pressure change (Figure 3a).²⁴ As described in literature, the Laplace pressure is determined by the angle of expansion and constriction at the abrupt expansion point, channel height, capillary barrier height, and the contact angle, θ , between the channel wall and hydrogel, requiring $45^\circ < \theta < 90^\circ$ (Figure 3a).^{23,27,28,29,30} The latter requirement is consistent with our measurements of the contact angle at capillary barriers, which was found to be $62.9^\circ \pm 8.4^\circ$ after silanization. Figure 3a illustrates the top view of the channel structure and hydrogel pinning during the vacuum suction. Hydrogel overflow does not occur at the capillary barriers despite the fact that the hydrogel did not recede with the same speed from neighboring channels during vacuum suction.

The pinning performance theoretically improves with increasing capillary barrier to channel height ratio. Barriers with one fourth of the channel height were chosen as optimal in this study.²³

We also studied the effect of the capillary barrier shape on hydrogel pinning. Both slash- and parenthesis shaped capillary barriers were tested for their pinning performance. As depicted in Figure 3b and 3c, the barriers in top-view made an acute angle to the pillar walls. A capillary barrier glass pillar wall interface angle of 60° was found to facilitate microchip filling for both parenthesis- and slash shaped barriers.

Pinning performances of parenthesis shaped barrier, slash shaped barrier and no barrier geometries were evaluated on a pass/fail basis. For this evaluation, three representative images were collected from two opposite corners and the middle part of the arrays. In case of microchips with barriers, full hydrogel occupation between both barriers was counted as pass. In case of microchips without barriers, hydrogel patches covering the entire gap between glass pillars were counted as pass. All other configurations were counted as fail. The pinning performance of the parenthesis shaped barriers during hydrogel emptying was slightly superior to slash-shaped ones ($P > 0.9$). Both parenthesis- and slash shaped capillary barriers demonstrate statistically significant superiority ($P < 0.0001$) when compared to the no capillary barrier geometry (Supporting Information, Figure S2, Table S1). These findings are in accordance with previous reports, describing that an acute angle at both ends of the barrier provides a minimal meniscus stretching and maximal surface wetting area for the hydrogel precursor.²³

Conclusions

In summary, non-fluorescent capillary barriers were used to fabricate high resolution hydrogel structures over large areas with neither defects nor distortions in closed microchips. Small volumes of different hydrogel types, fabricated with photopolymerization and thermo gelation, were uniformly patterned using this method. This design strategy allows for robust processing of elaborate microfluidic networks consisting of ~400k periodic picoliter-volume hydrogel patterns. Capillary barrier technology is a highly reliable technique, enabling full control over selective hydrogel patterning and allowing for fluorescence based analyses. Picoliter volume hydrogel

microarray patterning is promising as a unique method with great potential for biological and clinical applications, such as subdivided-hydrogel microarrays for bacteria cultures^{31,32} and 3D cell cultures,³³ and enclosed hydrogel microarrays for electrokinetic biomolecule separation purposes.³⁴ Although our demonstration is limited to hydrogel formation, patterning of aqueous solutions would be equally possible, for example enabling massively-parallel liquid³⁵ and droplet³⁶ patterning in microfluidic devices, and liquid compartmentalization for sensing and bioassays.³⁷

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BIOS Lab-on-a-Chip Group, MESA+ Institute for Nanotechnology, MIRA Institute for Biomedical Technology and Technical Medicine, University of Twente, Enschede, the Netherlands.

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