IN-SITU PHOTOPATTERNING OF HYDROGEL MICROARRAYS IN POLISHED MICROCHIPS

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ABSTRACT

We present a fabrication method which enables simple and reproducible photopatterning of micron-sized hydrogel arrays inside closed microchips. To achieve this, the glass cover of the microchip is thinned by mechanical grinding and polishing. This procedure reduces the spacing between the photomask and hydrogel precursor and thereby the effects of UV diffraction. We demonstrate the effects of glass cover thickness and roughness, together with different optical lithography recipes on patterning success. The patterning method is tested with two different types of photopolymerizing hydrogels, polyacrylamide and polyethylene glycol diacrylate. The presented method enables in-situ fabrication of well-defined hydrogel patterns for microfluidic platforms.

KEYWORDS: Optical lithography, In-situ hydrogel patterning, Hydrogel microarray.

INTRODUCTION

Hydrogel arrays have attracted a great deal of attention in numerous microfluidic applications owing to their capacity to handle smaller sample volumes and perform in-parallel analyses [1]. Micron-sized hydrogel patterns enable faster mass transport and higher surface-to-volume ratios. These functionalities are essential to increase the sample throughput and number of analyses in clinical bioassays, biosensors, diagnostic assays, and fundamental biological applications [2]. Although the microfabrication of hydrogel arrays on rigid substrates has been extensively investigated, the ability to do the same in closed microchips has not been sufficiently examined. Previously, our group has used capillary pinning technique for autonomous fabrication of hydrogel microarrays in closed microchips [3,4]. Using optical lithography remained challenging for fabrication of micron-sized hydrogel patterns in closed microchips due to the thickness of the cover glass (typically 500 or 1000 μm), which maintains a large spacing between the photomask and hydrogel precursor [5]. Here, we present both a straightforward fabrication technique for a microchip with a thinned cover glass and a less cumbersome hydrogel patterning method enabled by optical lithography. Our approach greatly simplifies hydrogel integration into microchips as the microarrays are fabricated in-situ.

EXPERIMENTAL

Glass microchips containing pillar arrays to provide mechanical support for the hydrogel structures are fabricated using deep reactive ion etching (DRIE) (Figure 1, and Figure 2a-c). The depth, width, and height of pillars are 20 x 4 x 5 μm, respectively. Fluidic inlets and outlets are powder blasted, and the wafer is thermally bonded with a glass cover wafer. The cover glass is subsequently ground to reduce its thickness from ~525 μm to ~100 μm. The wafer is then polished to reduce the mean surface roughness from ~20 nm to ~3 nm and microchannels are silanized to allow covalent bonding of hydrogels to the glass surface [5]. After the microchannels are filled with 0.5 μm of hydrogel precursor, the microchip is carefully aligned and brought in direct contact with a chromium photomask containing periodic rectangular patterns (Figure 3). The photomask and the chip are held together using either EVG 620 optical mask alignment system or transparent tape. The hydrogel precursor is then exposed through the photomask using UV light at 365 nm. The power of the UV light source is 100 mW cm^{-2} and exposure duration is 10 min with intermittent illumination cycles (2 sec on followed by 4 sec off). Afterwards, the non-crosslinked hydrogel precursor is washed away. The patterning method is tested with

two different types of hydrogel precursors, polyacrylamide and polyethylene glycol diacrylate –PEG DA–. Polyacrylamide precursor preparation, injection, and UV exposure are performed under N_2 flow.

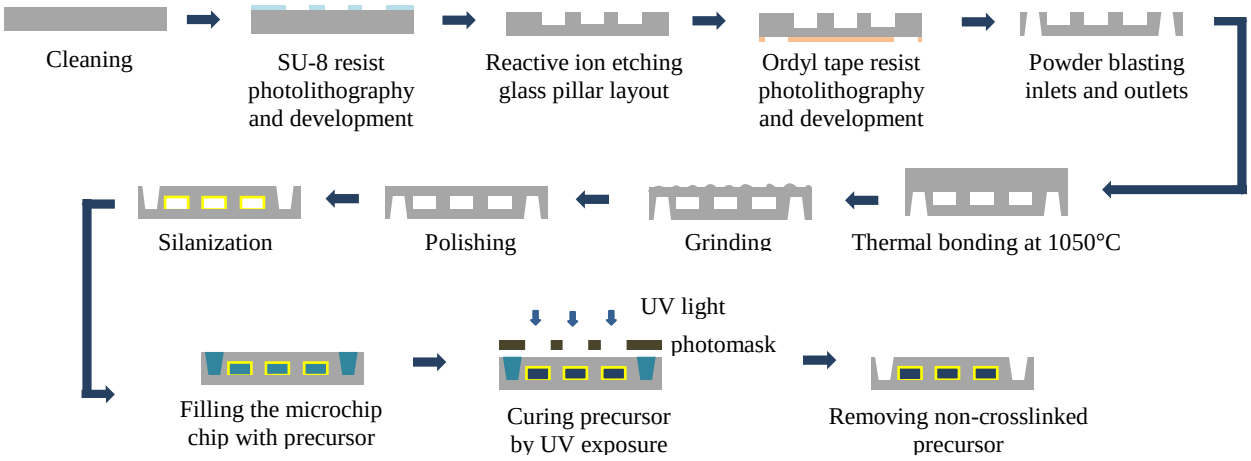

Figure 1. Schematic illustration of microchip fabrication and hydrogel patterning.

RESULTS AND DISCUSSION

Our approach consists of polishing the glass cover of the microchip, injecting hydrogel precursor into the microchannels, and applying optical lithography to pattern spatially defined hydrogel microstructures. Polishing and grinding processes minimize the diffraction of the light during photolithography. Figure 2a shows an assembled microchip containing glass pillars, figure 2d shows a microchip with patterned PEG DA (Figure 2d) and Figure 2e illustrates another microchip with patterned polyacrylamide hydrogels.

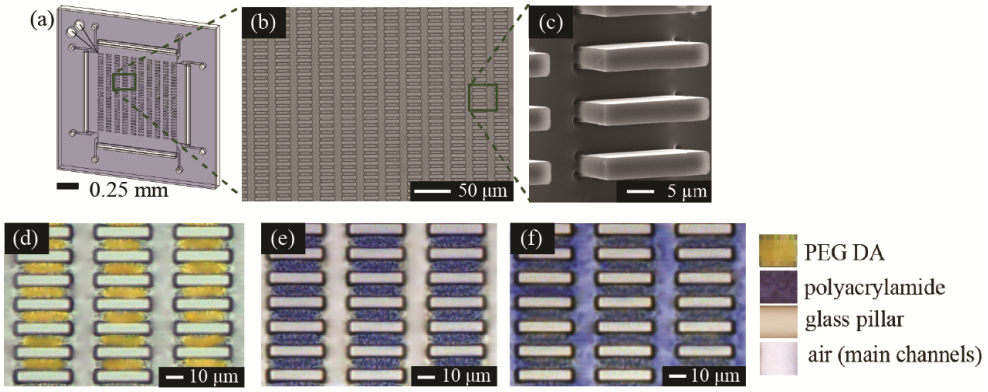

Figure 2. (a) Sketch of the polished microchip. (b) Optical image of the glass pillar array. (c) SEM image of glass pillars. Optical images of hydrogels between glass pillars after photopatterning of (d) PEG DA and (e) polyacrylamide using photomask and intermittent illumination for 10 min. (f) Photopatterning of polyacrylamide using photomask and continuous illumination for 10 min. The images are artificially colored on the basis of gray scale differences.

During UV light exposure, free radicals are generated in non-crosslinked hydrogel precursor. These free radicals together with monomers start the polymerization process and grow polymer chains in the exposed region. Diffusion of free radicals and monomers between the exposed region and the masked region, increased by the heat generation, lead to polymerization near the illumination interface, making it difficult to accurately control the shape and resolution of the final structures [6] (Figure 2f). We achieved to control the diffusion process by using a 12 mW cm^{-2} UV source and applying periodic illumination with on/off cycles. We found that this illumination power, in combination with intermittent illumination, allows monomers and

free radicals to diffuse for shorter distance before being crosslinked in microchannels. Here the intermittent illumination eliminates excessive heat generation by the crosslinking reaction and light adsorption [6].

Patterning success is evaluated based on the similarity between the hydrogel patterns in the microchannels and structures on the photomask. Hydrogel structures with a similar width as the patterns on the photomask were counted as success, while structures with increased width and indistinct edges were counted as failure. Patterning success improves with a thinner glass cover thickness for both polyacrylamide and PEG DA hydrogels as shown in Table 1. The data indicate a clear relationship between glass cover thickness and patterning success.

Table 1. Patterning success versus upper glass layer thickness. The results present the mean and standard error deviations (STD), n=2.

	Polyacrylamide		**PEG DA**	
Upper layer thickness (μm)	Patterning success (%)	STD (%)	Patterning success (%)	STD (%)
100	98.5	0.8	98.7	1.0
115	94.9	4.5	95.5	4.7
250	37.7	10.6	20.0	10.3
400	0	0	0	0
525	0	0	0	0

CONCLUSION

In this work, conventional optical lithography has been used to pattern well-defined hydrogel structures in closed microchips over large areas with high reliability and without any defects. Using this technique, patterning of ~400k hydrogel structures with a minimum feature size of 4 μm and a volume of 1 pL is achieved. This method enables in-situ construction of well-defined hydrogel patterns, and presents an alternative approach to fabricate 3-D hydrogel matrices for biomolecule separation, biosensing, tissue engineering, and immobilized protein microarrays.

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REFERENCES

[1] D. Dendukuri, P. Panda, R. Haghgooie, J.M. Kim, T.A. Hatton, and P.S. Doyle, “Modeling of Oxygen-inhibited Free Radical Photopolymerization in a PDMS Microfluidic Device,” *Macromolecules*, 41, 8547-8556, 2008.

[2] T.G. Fernandes, M.M. Diogo, D.S. Clark, J.S. Dordick, and J.M. Cabral, “High-throughput Cellular Microarray Platforms: Applications in Drug Discovery, Toxicology and Stem Cell Research,” *Trends Biotechnol.*, 27, 342-349, 2009.

[3] B. Gumuscu, A. van den Berg, and J.C.T. Eijkel, “Custom Micropatterning of Hydrogels in Closed Microfluidic Platforms Fabricated by Capillary Pinning,”, The 18th International Conference on Miniaturized Systems for Chemistry and Life Sciences, 2014.

[4] B. Gumuscu, J.G. Bomer, A. van den Berg, and J.C.T. Eijkel, “Large Scale Patterning of Hydrogel Microarrays Using Capillary Pinning,” *Lab Chip*, 15, 664-667, 2015.

[5] M.E. Helgeson, S.C. Chapin, and P.S. Doyle, “Hydrogel Microparticles from Lithographic Processes: Novel Materials for Fundamental and Applied Colloid Science,”. *Curr. Opin. Colloid Interface Sci.*, 16, 106-117, 2011.

[6] C. Hou, and A.E. Herr, “Ultrashort Separation Length Homogeneous Electrophoretic Immunoassays Using On-chip Discontinuous Polyacrylamide Gels,” *Anal. Chem.*, 8, 3343-335, 2010.

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