MICRO-PATTERNED POLYSTYRENE SUBSTRATES FOR HIGHLY INTEGRATED MICROFLUIDIC CELL CULTURE

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ABSTRACT

Adherent mammalian cells dynamically interact with their extracellular matrix (ECM) and culture substrate. To accommodate this sensitivity, standard culture techniques typically utilize *tissue culture polystyrene* (TCPS), a treated polystyrene substrate that promotes cell attachment. However, TCPS cannot be easily integrated into microfluidic devices as it is incompatible with conventional fabrication techniques. We have developed a process that integrates micro-patterned polystyrene (MPPS) onto glass substrates, combining the cell-culture compatibility of polystyrene with the fabrication compatibility of glass. Importantly, this process integrates cell culture surfaces directly within a device and preserves the standard microfluidic assembly process of plasma bonding.

KEYWORDS: polystyrene patterning, microfluidic cell culture, stem cells

INTRODUCTION

Although it is well known that varying the ECM composition can alter cell phenotype [1], the culture substrate itself also affects the physiological state of the cell [2]. In particular, different substrates adsorb ECM differently, which in turn affects cell attachment and function [3]. Standard culture techniques typically utilize *tissue culture polystyrene* (TCPS), a treated polystyrene substrate that promotes cell attachment. Microfluidic devices, which are increasingly used for studying cell biology, typically use *glass* substrates, in part because of the compatibility of glass with standard microfabrication techniques, in particular polydimethylsiloxane (PDMS) bonding. However, glass substrates can alter both cell morphology and function for sensitive cells types such as embryonic stem cells (ESCs). Although TCPS substrates can be clamped to PDMS channels to create devices [4], clamping is low-throughput, prone to failure, and difficult to scale with increasing device complexity.

EXPERIMENTAL

We have developed a process that successfully combines the fabrication compatibility of glass with the culture compatibility of polystyrene (PS) substrates by using micro-patterned elastomeric stencils to pattern dissolved polystyrene on glass substrates (Figure 1). We have integrated polystyrene patterns within previously demonstrated microfluidic perfusion devices [4]. In this platform (Figure 2A) the polystyrene patterns serve a dual purpose – (1) as cell culture surfaces (Figure 2B) and (2) as valve seats for normally closed valves (Figure 2C). This illustrates a second ad-

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vantage of the MPPS; since it does not bond to PDMS, we can use it to avoid irreversible bonding of the valve seat to the glass substrate [5].

RESULTS AND DISCUSSION

Because adherent cells are sensitive to both the topography and composition of the culture substrate, we characterized the surface roughness and elemental composition of MPPS by Atomic Force Microscopy (AFM) and X-ray photoelectron spectroscopy analysis (XPS), respectively, and determined that they are comparable to TCPS. Assessment of the surface contact angle before and 3hrs after plasma treatment showed contact angles comparable to those of TCPS, indicating that plasma bonding does not alter the surface properties. Next, we assessed adsorption of gelatin (a common matrix molecule for mouse ESC culture) to MPPS, glass, and TCPS. MPPS adsorbed significantly more protein than glass and resulted in cell morphologies improved over glass and similar to TCPS. (Figure 3B).

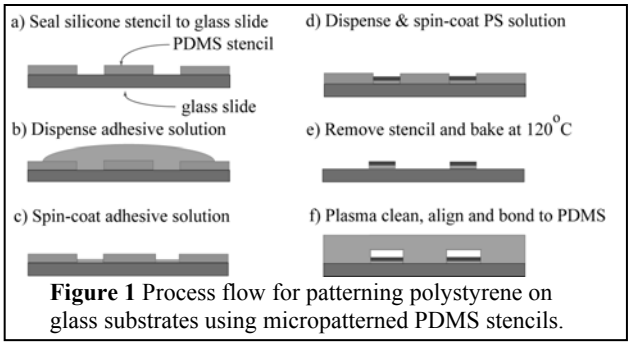

Figure 1 Process flow for patterning polystyrene on glass substrates using micropatterned PDMS stencils.

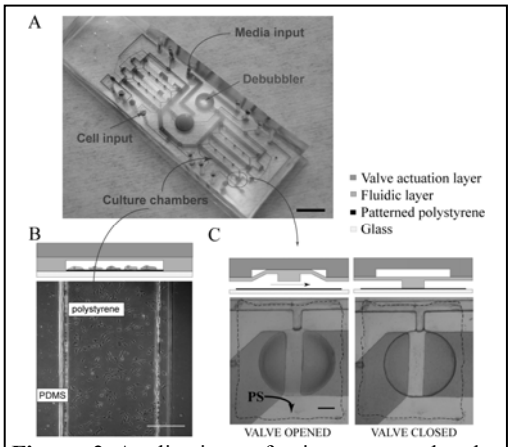

Figure 2 Applications of micro-patterned polystrene. (A) Image of a two-layer microfluidic device that incorporates cell culture chambers and normally closed valves. Scale bar 5 mm. (B) Top view of a MPPS culture chamber with NIH-3T3 mouse fibroblasts. Scale bar 150 μm. (C) Schematic of valve cross section (C, top). PS patterned valve seat prevents PDMS valve membrane from bonding (C, bottom). Scale bar 200μm.

Because cell adhesion is a complex process [3], functional assays are ultimately needed to test any material system used as a potential culture substrate. We evaluated growth of mESCs on various substrates (glass, MPPS, and TCPS), and found that proliferation and morphology of mESCs on MPPS in static cultures was both qualitatively and quantitatively similar to that of TCPS and significantly improved over glass (Figure 3A-B). Finally, to demonstrate the ability to integrate MPPS into microfluidic devices, we performed multi-day perfusion culture of mESCs on our microfluidic platforms with integrated MPPS culture surfaces. Cells cultured on the MPPS surfaces showed improved morphologies over cells grown in glass chambers (Figure 3C).

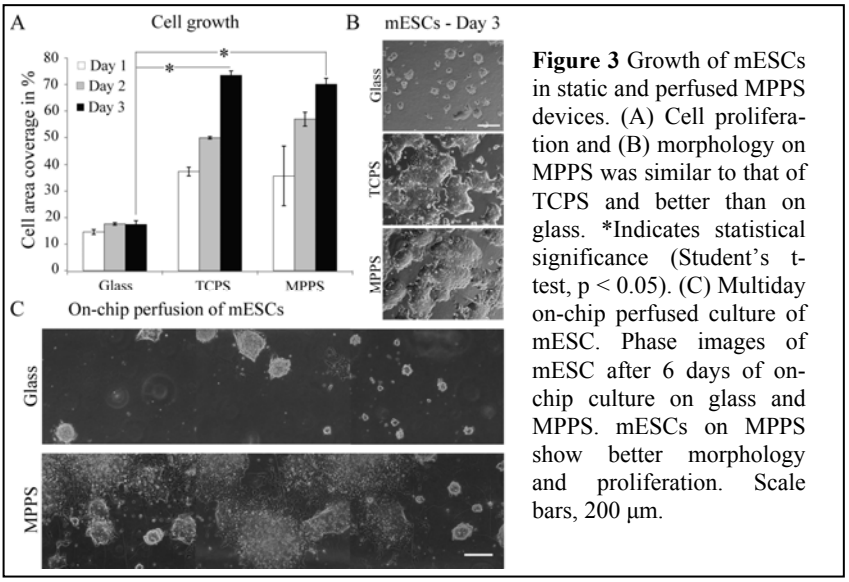

Figure 3 Growth of mESCs in static and perfused MPPS devices. (A) Cell proliferation and (B) morphology on MPPS was similar to that of TCPS and better than on glass. *Indicates statistical significance (Student's t-test, $p < 0.05$). (C) Multiday on-chip perfused culture of mESC. Phase images of mESC after 6 days of on-chip culture on glass and MPPS. mESCs on MPPS show better morphology and proliferation. Scale bars, 200 μm.

CONCLUSIONS

We have demonstrated a simple technique for realizing multi-functional polystyrene patterns for the fabrication of complex, highly integrated microfluidic cell culture platforms. The functionality and ease of fabrication of MPPS should facilitate the application of microfluidics to the study of substrate sensitive cell types.

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REFERENCES

[1] C. Flaim, S. Chien, and S. N. Bhatia, Nature Methods, vol. 2, pp. 119-125, (2005).
[2] J. G. Steele, A. Dalton, G. Johnson, and A. P. Underwood, JBMR, vol. 27, pp. 927-940, (1993).
[3] C. J. Wilson, G. Stephanopoulos, and D. Wang, Tissue Eng., vol. 11, pp. 1-18, (2005).
[4] K. Blagović, L. Y. Kim, A. M. Skelley, and J. Voldman, Proc. Micro Total Analysis Systems '08, pp. 677-679, (2008).
[5] D. Irimia, and M. Toner, Lab on a Chip, vol. 6, pp. 345-352, (2006).