Silicon based solvent immersion imprint lithography for rapid polystyrene microfluidic chip prototyping

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Polystyrene (PS) is preferred over polydimethylsiloxane (PDMS) in microfluidics for applications in cell biology. However, PS has not found widespread use in microfluidics due mainly to the lack of rapid prototyping techniques. Here we address this issue by developing a silicon based solvent immersion imprint lithography (Si-SII) technique. Silicon is rigid, mechanically robust, and highly compatible with standard microfabrication processes, and therefore, is a promising candidate for molds. Various PS microfluidic channels as small as 20 μm in width with the aspect ratio as high as 5 were demonstrated using Si-SII. Bubbles and bending generated in the fabrication process were analyzed and eliminated. The surface roughness was about 27 nm (rms). Compared to the untreated PS, the molded PS retained almost the same surface properties, as characterized by contact angle measurement and X-ray photoelectron spectroscopy. Cell culture was tested to demonstrate the utility of Si-SII in cell biology applications. The results show that PS, with the aid of Si-SII, can be an alternative material to PDMS in building microfluidic chips.

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1. Introduction

In the past decade microfluidics in biological applications has experienced significant growth due to its advantages of small volume, low cost, short reaction time, and high throughput [1–5]. The materials for microfluidics usually include silicon, glass, and polymer [6]. While silicon and glass were commonly used in the earlier years of microfluidics development [7,8], polymer has become an increasingly attractive alternative [9–12]. Polymers encompass a large class of materials, including two major categories: elastomers and thermoplastics [13]. Since Whitesides et al. [14] fabricated complex microfluidic devices based on polydimethylsiloxane (PDMS), it has been widely employed in microfluidics due to its low cost, optical transparency, biocompatibility, and simple processing and prototyping [9,15,16]. However, despite all the afore mentioned beneficial properties, PDMS suffers from easy deformation, rapid liquid evaporation, absorption of molecules into the polymer, leaching of uncross-linked oligomers, and hydrophobic recovery [17,18], which significantly limit its adoption in microfluidics for biological research.

As an alternative microfluidic material, polystyrene (PS), one of the mostly used thermoplastics, has been studied and used for macroscopic cell culture and bioanalysis, thanks to its low cost, optical transparency, biocompatibility, chemical stability, and physical rigidity [19,20]. Furthermore, it can easily be transferred from hydrophobic to hydrophilic by plasma treatment and remains hydrophilic for 4 weeks, about 4 times longer than PDMS [21]. As such, PS is preferred over PDMS in microfluidics for cell biology applications. However, the fabrication of PS microfluidic chips is usually more difficult and expensive than PDMS. Therefore, it is crucial to develop simple and cost-effective processes with high resolution and repeatability for rapid PS microfluidic prototyping.

In the past, a number of PS microfabrication methods have been explored. Hot embossing relies on relatively high temperature (120°C, 20°C above the glass transition temperature of PS) and metal molds to create microfluidic devices [22,23]. However, metal molds are fabricated by a laser system, which process is time-consuming and of high cost, and therefore, may not be suitable for...
rapid lab prototyping. Laser cutting is another technology to fabricate microfluidic devices [24]. For example, Li et al. [25] used a CO₂ laser to create droplet microfluidic devices on a PS substrate. Laser cutting is a mask-free method, but its process is sequential and becomes very time-consuming and costly with a large number of devices. In contrast, injection molding is capable of fabricating multiple microfluidics simultaneously with low costs. However, it requires dedicated tools such as injection molding machines [26]. Johnson et al. [27] and Pentecost et al. [28] developed a method similar to the injection molding method but free of injection molding machines. In their method, PS powder was poured into an aluminum weighing dish and then heated to 250 °C for several hours. Unfortunately, this process does not allow for room temperature fabrication. To circumvent such an issue, Nargang et al. [29] used several toxic chemicals, e.g., toluene, isopropanol, and cyclohexanone, to dissolve PS before the use of a PDMS mold. A major drawback of this method is the distortion (such as swelling) in the PDMS mold in the presence of those chemical solutions [30]. Therefore, it is crucial to find a solvent that can dissolve PS, but does not distort the PDMS mold. Gamma-butyrolactone and delta-valerolactone were found to be appropriate solvents based on their ability to dissolve PS without swelling PDMS [30]. However, they need seven days to form a PS solution in a tube filled with PS solid and organic solvent [30]. Recently, solvent immersion imprint lithography (SiILL) has been developed that enables complete PS microfluidics prototyping in a single processing step [31,32]. In this method, the PS surface is first softened by acetone and then imprinted with a PDMS mold. SiILL is simple and rapid and does not require sophisticated tools or heating processes. However, PDMS has an elastic modulus of about 1–3 MPa, three orders of magnitude lower than that of thermostactics like PS (∼3 GPa), which makes PDMS easy to deform [17,31]. Consequently, it is difficult to transfer structures with high fidelity from a PDMS mold to PS, especially when a high aspect ratio is needed [31].

Here we developed a silicon based solvent immersion imprint lithography (Si-ILL) method for rapid PS microfluidics prototyping. Silicon is much more rigid than PDMS and highly compatible with standard microfabrication processes, and therefore, is a promising candidate for molds. In this article, we present the details of the Si-ILL and contrast it with PDMS-ILL whenever possible. Various microfluidic channels as small as 20 μm in width with the aspect ratio as high as 5 were demonstrated. Characterization of the Si-ILL molded PS chips using contact angle measurement, X-ray photoelectron spectroscopy (XPS), and cell cultivation is also discussed.

2. Material and methods

2.1. Fabrication of Si mold

The silicon mold with inverse structures was fabricated using standard microfabrication technology using the following steps. (1) A silicon wafer was cleaned using a mixture of H₂SO₄ and H₂O₂ solution. (2) A 300 nm SiO₂ layer was deposited on the wafer by plasma enhanced chemical vapor deposition (PECVD), which was used to form the mask layer for subsequent silicon etching. (3) The silicon wafer was spin-coated with a 5 μm AZ4620 photoresist layer, followed by baking at 95 °C for 120 s, exposure to UV light for 5.5 s, and development for 45 s. In order to increase the strength of the photoresist layer, the wafer was baked at 110 °C for 120 s. (4) The SiO₂ layer was etched by reactive ion etching [33] for about 40 min. (5) The Si wafer was etched for different amount of time using deep reactive ion etching [34] to obtain different depths. (6) Finally, the photoresist was removed using acetone.

2.2. Si-ILL protocol

The basic procedures of Si-ILL are summarized as follows. The PS surface was first softened using solvent, then the softened surface was imprinted with a Si mold, and finally the PS was bonded to a PS substrate. Acetone was used to soften PS according to the SiILL method [31]. During Si-ILL, a 1.48 mm thick PS slab was immersed in acetone solvent for 0.5–3 min at room temperature. Acetone diffuses into the PS to form a surface “gel” layer (Fig. 1(a)). A drop of acetone was dropped on the surface of a Si mold until acetone spread completely over the mold (Fig. 1(b)). The immersed PS was removed from the acetone solution and subsequently placed on the Si mold. A weight (about 1 kg) was placed on the other side of the PDMS slab via a 2 mm thick PDMS slab to provide pressure for structure transfer (Fig. 1(c)). The PS slab and the mold were placed in a small vacuum chamber for 1.5 h to let acetone evaporate and subsequently release the PS from the mold. Note that without the vacuum chamber, acetone evaporation and the PS release take 10 h. In contrast, in PDMS based SiII, the porous PDMS enables rapid solvent removal from the polymer and quick PS release [31]. Finally, another PS slab punched with inlets/outlets was immersed in acetone for about 5 s, and then bonded with the PS slab with structures (Fig. 1(d)).

2.3. Gel layer thickness and transmittance

The immersed PS slab after acetone evaporation was used for the ultraviolet-visible (UV-vis) measurement. Then the PS slab was broken into two pieces to measure the gel layer thickness.

2.4. Water contact angle measurement

To study the effect of immersion time in acetone on the PS surface properties, the static water contact angle was measured using droplets of water (about 1 μl) applied to the free surface of PS slabs that underwent acetone immersion, acetone immersion followed by O₂ plasma treatment, and no treatment.

2.5. Cell culture

The biocompatibility of the PS after Si-ILL was assessed by 24 h cultivation of transduced ATDC5 cells, which are derived from mouse teratocarcinoma. The responsiveness and proliferation of the cells on the chip were determined by drug-induced luminescence, while cell morphology and death rate were visualized by
fluorescence imaging [19]. The PS chip was rinsed with DI water and air dried before sterilized in a UV hood overnight. Collagen coating was performed before UV sterilization for improvement of cell adhesion. Then, cells were seeded on the PS surface within the Si-SiIL defined fluidic channels. Cells were incubated in a 37 °C, 5% CO2 incubator. 6 h after seeding, doxycycline was added to the medium, which can stimulate luciferase expression inside the transduced cells. The substrate D-luciferin was added 11 h after seeding. Luminescence was measured 12 h and 24 h after seeding. Then cells were exposed to acridine orange and propidium iodide to visualize live and dead cells simultaneously. Propidium iodide can only enter and stain dead cells by interacting with DNA, generating red fluorescence (>600 nm) upon excitation around 570 nm. Acridine orange, on the other hand, can diffuse freely through live cell membrane and interact with DNA, resulting in a green fluorescence (~520 nm) signal upon excitation around 500 nm.

3. Results and discussion

3.1. Characterization

PS microfluidic chips with various channel widths and heights (20 μm × 25 μm, 30 μm × 150 μm, 100 μm × 100 μm, and 150 μm × 100 μm) were fabricated successfully using Si-SiIL. It is shown that structures with an aspect ratio (height:width) as high as 5 (30 μm in width, 150 μm in height) can be imprinted, much higher than 0.24 aspect ratio achieved previously with PDMS-SiIL [31] (Note: an aspect ratio of 2 was achieved with PDMS-SiIL, but in a channel of only about 3 μm long). In order to test the bonding performance, we injected blue ink into the chip and no leakage was observed from the channel, as shown in Fig. 2(a). The images of scanning electron microscope (SEM) and a channel cross section are shown in Figs. 2(b) and (c), respectively. The wall of the

![Fig. 2](image-url) (a) A prototype of a PS microfluidic chip with an aspect ratio of 5 (30 μm wide, 150 μm deep). Blue ink was used for visualization of the channel. (b) SEM image of the circled part in (a). (c) Image of a channel cross section.

![Fig. 3](image-url) (a) AFM images of (a) Si mold, (b) original PS, (c) PS after immersion, and (d) PS after imprint, showing an average surface roughness (rms) of about 1.7 nm, 1.8 nm, 4.9 nm, and 27 nm, respectively, over an area of 400 μm².
channel is not completely vertical and the angle between the wall and the bottom of channel is about 85°, which might be caused by shrinkage of the gel layer during the drying process. Fig. 3 shows the images of roughness distribution of the Si mold, the original PS, the PS after immersion, and the PS after imprint measured by atomic force microscopy (AFM), giving an average surface roughness rms of about 1.7 nm, 1.8 nm, 4.9 nm, and 27 nm, respectively, over an area of 400 μm². We note that the surface roughness is different from between the channel wall and the place of without structure. As compared to the plane part of without structure, the protrusion structure of the mold may cause in-homogeneities in imprint and drying process, which may further increase the roughness.

Acetone diffuses into the PS to generate a surface gel layer. The gel layer after acetone evaporation is visually different from the bare PS. Fig. 4 shows the gel layer growth and the transmittance decrease over the immersion time of PS in acetone. The gel layer thickness increases with the immersion time but the progress slows down gradually, which reflects the acetone diffusion process inside the PS. In about 2.5 min, the gel layer reaches 150 μm, which meets the need for imprint a channel of 150 μm in depth.

The visible light (wavelength: 400–760 nm) transmittance of a 1.48 mm thick bare PS is about 89%. According to Lambert-Beer law, the transmittance T of a sample is related to its absorbance A by:

\[ T = 10^{-A} e^{-bx} \]

where b is the extinction coefficient, x is the thickness of the sample. The extinction coefficient of the bare PS is about 0.08 mm⁻¹. The decrease in the transmittance after the PS immersed in acetone is due to the formation of the gel layer, which has an extinction coefficient of about 2 mm⁻¹ based on the results in Fig. 4.

The Si mold can be re-used. The number of reuses depending on the features on the mold. For example, the Si mold with high aspect ratios (e.g., \( L \times W \times H = 1 \text{ cm} \times 30 \text{ μm} \times 150 \text{ μm} \)) could be reused about 4–10 times on average due to high aspect ratios and long channel lengths. Any damage along the 1 cm thin protrusion on the mold will make it unsuitable for re-use. In contrast, a Si mold with low aspect ratios (e.g., \( L \times W \times H = 1 \text{ cm} \times 20 \text{ μm} \times 25 \text{ μm} \)) had been re-used more than 50 times without any damage to the mold or failure in the channels.

Although a minimum resolution of 20 μm was presented, we believe that 20 μm is not the resolution limit of the method and features smaller than 20 μm can be imprinted. However, with smaller features, the Si mold becomes easier to get damaged due to a large aspect ratio, making the Si-SIIL practically challenging.

Fig. 4. The gel layer thickness and the transmittance related to the immersion time of PS in acetone. The gel layer thickness increases and the transmittance decreases with the immersion time of PS in acetone.

3.2. Bubble formation and elimination

It is important to create a PS structure with a smooth inner surface. Previously, in the PDMS-SIIL method, since the gas generated at the interface of PDMS and PS can be vented out via the PDMS mold due to its high gas permeability [35–37], the PS surface quality could be maintained and no bubbles were observed. However, this is not the case for a silicon mold, which is impermeable to gases. Gas trapped between the silicon mold and the PS slab resulted in bubbles and patches on the PS surface, as shown in Fig. 5(a). To solve this issue, a drop of acetone was dropped on the silicon mold surface. Acetone spread completely over the mold to remove the gas near the mold surface. Therefore, when the PS slab was subsequently placed on the mold, they were in contact via a layer of acetone and there was no gas between the PS slab and the mold. The resultant clean PS slab is shown in Fig. 5(b).

3.3. PS bending and elimination

Ideally, the side of a PS slab that does not have any structures should not be treated with acetone in order to maintain a smooth and clean surface. However, the PS slab with a single side (the side with structures) treated with acetone bends (Fig. 6(a)) due to the different stresses between the side with and without acetone treatment. To mitigate and even eliminate PS bending, in our studies both sides of the PS slab were acetone treated. To avoid bubbles generated at the interface between the flat side of the PS slab (the side without structures) and the weight. A 2 mm thick PDMS slab was placed between the PS and the weight to vent out residual gases. As a result, a PS slab free of bending and bubbles on both sides can be fabricated (Fig. 6(b)). Note that the PDMS slab used here had no structures and was employed simply to avoid PS bending.

3.4. Water contact angle measurement

The water contact angle of the original PS prior to acetone immersion was 93°, which agrees well with values from the literature [29]. The contact angle for the PS with 0.5 min, 1 min, and 2 min immersion in acetone was 90°, 92°, and 91°, respectively, as shown in Fig. 7, suggesting that acetone (and the immersion time) has little effect on surface hydrophobicity. To reduce adsorption of hydrophobic molecules, the PS surface can be made more

Fig. 5. (a) Image of the rough PS surface caused by the gas generated from the contact of Si mold and PS; (b) Image of the smooth PS surface using a drop of acetone dropped on the silicon mold surface.
hydrophilic using oxygen plasma treatment \cite{38-40}. The water contact angle for the PS with 5 min of O$_2$ plasma treatment was 25° (without prior acetone immersion) and 27° (with 2 min of prior acetone immersion), as shown in Fig. 7, indicative of a more hydrophilic surface. The surface can remain hydrophilic for 4 weeks \cite{19,21}. The hydrophobic recovery can be further delayed by storing PS chips in water after treatment \cite{19}.

3.5. X-ray photoelectron spectroscopy

In order to better understand the PS surface chemistry change after Si-SIIIL, XPS was carried out to characterize the surface element composition. Fig. 8 compares the relative atom contents of C 1s (C-C or C-H bonds, 99.67% vs. 69.79%), O 1s (C-O bonds, 0.25% vs. 17.96%), and Si 2p (Si-O bonds 0.08% vs. 12.26%) in the original PS and after acetone treatment and imprint on a Si mold. Significant O 1s increase is expected, as acetone is an oxidation agent. The increase of Si 2p is due to the use of the Si mold. Those changes have little effect on water contact angles, as previously shown in Fig. 7.

Fig. 6. (a) A bent PS slab caused by the different stresses on both sides due to acetone treatment of only single side. (b) A straight PS slab when both sides of a PS is treated with acetone. To further removes bubbles on the flat side (the side without structures), a 2 mm thick PDMS slab is placed between the PS and the heavy object to vent out gases at the interface.

Fig. 7. Water contact angles of PS at different immersion time. (a) no immersion, (b) 0.5 min immersion, (c) 1 min immersion, (d) 2 min immersion, (e) no immersion and 5 min O$_2$ treatment, (f) 2 min immersion and 5 min O$_2$ treatment.

Fig. 8. XPS analysis of the surface element of original PS and PS after acetone immersion and imprint on Si mold.

3.6. Cell culture

50,000 cells were seeded on a 6 cm$^2$ Si-SIIIL treated PS chip. As control, 50,000 cells were also seeded on the bottom of a conventional culture plate well. Luminescence measurement shows that cells on the PS chip had a similar increase trend (around 3 fold) in 12 h (from 12 h after seeding to 24 h after seeding) as those on the cell culture plate (Table 1). This suggests that cells on the PS chip have similar growth rate and luciferase expression level as control. Difference in absolute luminescence counts between the control well and PS chips results mainly from different optical characteristics of the chip and the well plate. For fluorescence imaging, culture medium was removed and the chip was rinsed with PBS buffer, then stained for 4 min with 5 μg/mL propidium iodide and 2 μg/mL acridine orange. Then cells were rinsed with PBS and imaged immediately using an Olympus fluorescence microscope. As shown in Fig. 9, cells on a Si-SIIIL treated PS chip had normal morphology and no dead cells were visible on the chip. We also performed the same test with a PS chip coated with collagen. No significant difference was observed in luminescence tests, compared to the non-coated chip, in 24-hour incubation (Table 1), suggesting that cells adhered to and proliferated normally on the PS chip either with or without the collagen coating. These experiment results suggest that the Si-SIIIL treatment on PS surface does not affect the biocompatibility of PS. Cells can adhere on the treated PS surface

\begin{table}[h]
\centering
\begin{tabular}{|c|c|c|}
\hline
 & 12 h luminescence & 24 h luminescence & Growth ratio \\
\hline
Control well & 95 & 290 & 3.05 \\
PS chip w/o collagen & 848 & 2984 & 3.52 \\
PS chip w/collagen & 910 & 2717 & 2.99 \\
\hline
\end{tabular}
\caption{Luminescence measurement of cell culture on the chip.}
\end{table}
maintaining a normal morphology, proliferate and reach similar luciferase expression level upon drug stimulation as in commercial well plate without the need for collagen coating.

4. Conclusions

PS has become an increasingly attractive alternative in microfluidics due to its high mechanical strength and biocompatibility. The Si-SIIIL method that we have developed makes it possible for conveniently prototyping PS based microfluidics with micron-sized channels and high aspect ratios. Our results show that the molded PS surface exhibited almost the same surface properties as the original PS. Cell culture tests were also performed, suggesting that the Si-SIIIL does not have any adverse effect on cell growth. The results show that PS, with the aid of Si-SIIIL, can be an alternative material to PDMS in building microfluidic chips.

However, the Si-SIIIL technology at the current stage comes with some drawbacks. First, we note that the PS/mold release time is long. Even with a vacuum chamber, it takes about 1.5 h to separate the PS chip from the mold, which is a bottleneck in the rapid prototyping. Second, the extinction coefficient is changed to $2 \text{mm}^{-1}$ from $0.08 \text{mm}^{-1}$ after acetone immersion due to gel layer formation, which leads to the transmittance reduction of the PS chip. Although high transmittance may not be needed for many applications, improved optical transmission quality will certainly broaden the utility of the Si-SIIIL. Third, compared to SIIIL, three additional processing steps are added, spreading acetone on the PS surface, adding a PDMS cushion, and immersing another PS slab. Finally, the number of re-uses is relatively low for the Si mold with extremely high aspect ratios and long channels.

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References

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