Micropatterning Thin Polystyrene Films For Single Cell Culture

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Abstract

Isolating cells play a critical role in studying cell characteristics such as differentiation, proliferation and migration. The purpose of this research is to micropattern thin polystyrene films in order to study single cell cultures. Our goal is to collect data for different concentrations of polystyrene and generate graphs. Our goal was also to be able to pattern smaller diameter wells of approximately 10 microns to 20 microns for single cell culture. The main processes used in this research are photolithography, silanization, soft lithography and hot embossing. The depth of the wells was measured using a profilometer. We were able to generate accurate graphs for well depth vs. RPM and for well depth vs. number of layers of polystyrene for 500 um diameter wells. We were unable to pattern 20 um diameter wells. The smallest possible wells we were able to pattern was 68 um.

Keywords: *photolithography, polydimethylsiloxane, soft lithography, micropatterning*

1. Introduction

Cell micropatterning is a technique that is used for controlling the placement of living cells on a surface[1]. In most cases, cell behavior is studied using a pool of cells in a petri dish. This increases the cell-cell interactions and may lead to misinterpretation of data[2]. Hence, micropatterning of micro wells can be used in studies related to isolated cell behavior and cell shape in a three-dimensional environment under different conditions. This process can also be used in various applications such as cellular analyses, tissue engineering and drug testing. Cell micropatterning can be used to understand fundamental mechanisms in a cell[1]. Micropatterning can be used in tissue engineering procedures such as transplantation by replicating the microenvironment surrounding the cells[1]. It can also be used to detect effects of a drug on a single cell[1].

The main materials used in our cell micropatterning process were su-8 photoresist, polydimethylsiloxane (PDMS) and polystyrene. The photoresist used is an epoxy based negative resist that is sensitive to near UV radiation. It is widely used in permanent use applications. Polydimethylsiloxane (PDMS) is a silicone-based polymer that has elastic properties and can be molded into any shape with necessary surface features. PDMS is a non-toxic polymer and is hydrophobic in nature. However, it can be made hydrophilic by plasma treatment. Polystyrene is also a commonly used polymer for fabrication of micro and nano structures[3].

2. Methods

Cell micropatterning involves several procedures such as photolithography, silanization, soft lithography, embossing, well depth measurement and cell culture.

2.1 Photolithography

500 um diameter wells: Silicon masters were prepared using a technique called photolithography as shown in figure 1. The silicon wafers were first washed with acetone, methanol and iso-propanol. It was then cured at 125°C for 10 minutes. The wafers were then plasma treated for 1-2 minutes. A thin coat of su-8 2150 was then spin-coated on the wafer for 10 sec at 500 rpm at acceleration of 100 m/s^{-1} and 30 sec at 1600 rpm at acceleration of 300 m/s^{-1}. The wafers were then soft-baked at 65°C for 5 minutes and at 95°C for 16 minutes. The soft-baked wafers were then exposed to a mask with 500 um wells under the UV light for 43 seconds, based on the intensity of the UV light. The wafers were then cured at 65°C for 5 minutes and at 95°C for 10 minutes. After the post exposure bake, the wafers were developed in a used su-8 developer for approximately 10 minutes and then developed in new su-8 developer for approximately 5 minutes. The wafers were then placed on a profiler to measure the height of the wells.

68 um diameter wells: The silicon wafers were first washed with acetone, methanol and iso-propanol. It was then cured at 125°C for 10 minutes. The wafers

were then plasma treated for 1-2 minutes. A thin coat of su-8 2050 was then spin-coated on the wafer for 10 sec at 500 rpm at acceleration of 100 m/s^{-1} and 30 sec at 3250 rpm at acceleration of 300 m/s^{-1}. The wafers were then soft-baked at 65°C for 6 minutes and at 95°C for 20 minutes. The soft-baked wafers were then exposed to a mask with 500 um wells under the UV light for 32 seconds, based on the intensity of the UV light. The wafers were then cured at 65°C for 1 minutes and at 95°C for 6 minutes. After the post exposure bake, the wafers were developed in a used su-8 developer for approximately 5 minutes and then developed in new su-8 developer for approximately 2 minutes. The wafers were then placed on a profiler to measure the height of the wells.

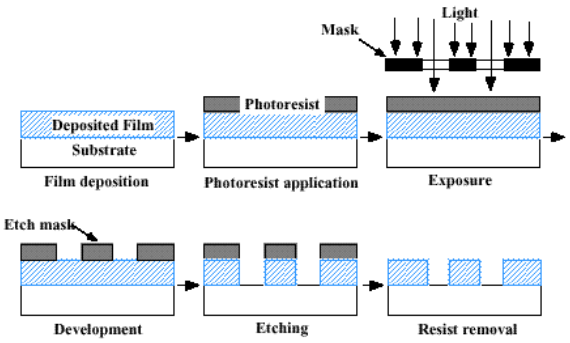

Figure 1. Schematic for photolithography[4]. Once the photoresist is applied on the silicon substrate, it is exposed to the mask under the UV light. After the post exposure bake, the wafer is developed in su-8 developer.

2.2 Silanization

The patterned silicon master was placed in a vacuum chamber along with 30 uL of Tridecafluoro-1,2,2-Tetrahydrooctyl-1-Trichlorosilane for 2 hours. A thin silane coating was formed on the master to facilitate easy separation of PDMS.

2.3 Soft Lithography

The PDMS mold was prepared using a technique called soft lithography. Sylgard 184 silicone elastomer consisting of a base and curing agent were mixed using a weight ratio of 10:1. The mixture was placed in a vacuum to remove bubbles. PDMS was then poured on the silicon master and cured at 80°C for 2 hours. The silicon master with the PDMS was then placed in a freezer at -80°C for 15 minutes. The PDMS was then peeled off from the master.

2.4 Embossing

Glass cover slips were coated with polystyrene by spinning 300 uL of different concentrations of polystyrene on it for 30 seconds at acceleration of 900 m/s^{-1} and at 1000, 2000, 3000 and 4000 rpm respectively. The cover slips were also coated with 2, 3, 4 and 5 layers of polystyrene by spinning it for 30 seconds at an acceleration of 900 m/s^{-1} at 3000 rpm. The peeled-off PDMS was placed on the glass cover slips coated with polystyrene as seen in figure 2. The cover slips with PDMS on it were then placed on a glass slide in a petri dish. A weight of 0.5 lb was placed on top of the PDMS and was cured at 180°C for 10 minutes. The cover slips were then allowed to cool and then observed under the microscope.

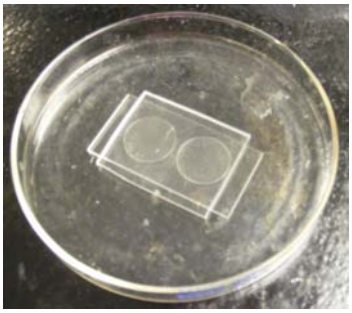

Figure 2. The PDMS mold is placed on the glass cover slips coated with polystyrene and cured on hot plate to transfer the pattern onto the cover slips.

2.5 Well depth measurement

After patterning wells in polystyrene, the well depths on the glass cover slips were measured using a profilometer. A stylus was moved across a know length and a minimum of 3 values for the height were collected.

2.6 Cell Culture

The glass cover slip with 68 um wells were sterilized and MDCK-Madin-Darby Canine Kidney Cells were seeded. The cells were allowed to adhere to the polystyrene and the excess cells were washed away.

3. Results

A minimum of three values was collected for well depth vs. RPM at which polystyrene was spun on the glass cover slip and the values were plotted as seen in figure 3. Also, a minimum of three values was collected for well depth vs. number of layers of

polystyrene and the values were plotted as seen in figure 4.

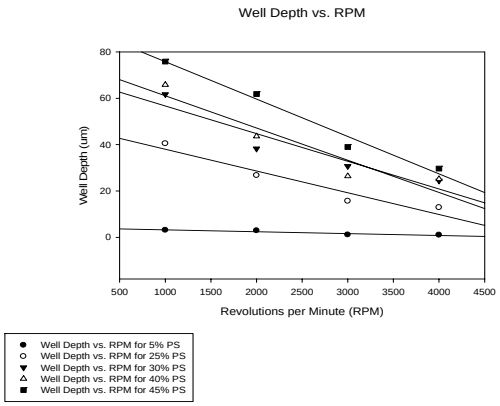

Figure 3. The graph shows the well depth at 1000, 2000, 3000 and 4000 rpm's for 5%, 25%, 30%, 40% and 45% polystyrene for 500 um diameter wells.

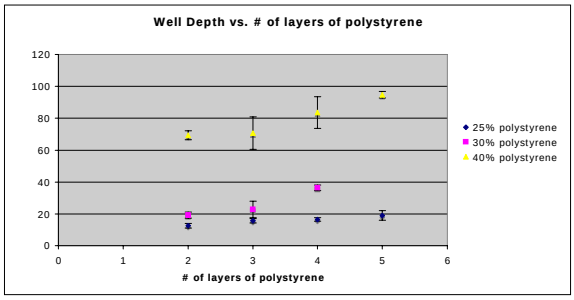

Figure 4. The graph shows the well depth at 2, 3, 4 and 5 layers of polystyrene for 25%, 30% and 40% polystyrene for 500 um diameter wells.

The smallest wells we were able to pattern were 68 um. MDCK cells were seeded in the 68 um diameter wells. We were able to 4 cells in a single well as shown in figure 5.

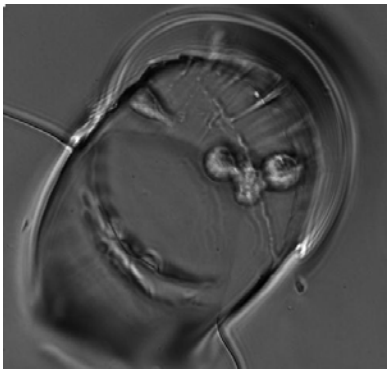

Figure 5. 4 MDCK cells are seen in a 68 um well patterned on a glass cover slip coated with polystyrene.

4. Discussion

Silanization technique was used while patterning wells less than 100 um. This was because smaller features are more prone to breaking. Silanization process helped reduce the adhesion strength between the PDMS and the silicon master, which in turn facilitated easy separation of PDMS[5].

The smallest wells we were able to pattern were 68 um. This was because wells smaller than the 68 um had a tendency to smear, which prevented them from having distinct vertical walls. This in turn would have prevented cells from settling in the wells.

The graphs seen in figures 3 and 4 can be used in the future to predict what concentration of polystyrene and what RPM to use or how many number of layers of polystyrene to use based on the desired well depth. As seen in figure 5, 4 MDCK cells were able to adhere to the polystyrene in a 68 um wells.

5. Conclusions and Future Work

Micropatterning is a new approach when it comes to three dimensional cell cultures. It allows us to control the microenvironment surrounding the cell as well as helps us to control cell-cell interactions and cell shape[6]. We were able to pattern 500 um diameter wells for 5%, 25%, 30%, 40% and 45% polystyrene and were able to collect data for well depth vs. RPM as well as for well depth vs. number of layers of polystyrene. The smallest well we were able to pattern was 68 um. We were not able to pattern wells smaller than 68 um.

In future, further experimentation will be performed in order to pattern smaller diameter wells ranging from 10 to 20 um. This will in turn help in culturing single cell in a single well.

6. Aknowledgements

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5. References

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