The Effect of Hydrophobic Patterning on Micromolding of Aqueous-Derived Silk Structures

Konstantinos Tsioris[1], Robert D White[1], David L Kaplan[2], and Peter Y Wong[1]
[1]Mechanical Engineering, Tufts University, Medford, MA, 02155
[2]Biomedical Engineering, Tufts University, Medford, MA, 02155

ABSTRACT

A novel micromolding approach was developed to process liquid biopolymers with high aqueous solvent contents (>90% water). Specifically silk fibroin was cast into a well-defined scaffold-like structures for potential tissue engineering applications. A method was developed to pattern the hydrophilicity and hydrophobicity of the polydimethylsiloxane (PDMS) mold surfaces. The water based biopolymer solution could then be directly applied to the desired regions on the cast surface. The variations in degree of hydrophilicity and hydrophobicity on the PDMS surfaces were quantified through contact angle measurements and compared to the outcome of the molded silk structures. Through this method free-standing structures (vs. relief surface-patterning) could be fabricated.

INTRODUCTION

Biopolymers, polymers synthesized by organisms, have many advantages including excellent biocompatibility and adjustable biodegradability [1]. Silk, for example, offers a wide spectrum of outstanding material properties such as good fracture toughness and excellent optical properties. Fabricating with biopolymers is also environmentally sound, since they can be processed in aqueous solutions. Due to these advantages biopolymers are often used as substrates in cell and tissue culture [1]. However they are rarely used in MEMS applications [2].

There is enormous potential for biopolymers in MEMS applications. In MEMS devices biopolymers could function as membranes or optical components. Devices which demand outstanding biocompatibility, such as implantable sensors, could be packed in or fully manufactured from biopolymers. The challenge today exists in understanding critical processing parameters in manufacturing structures with micron and submicron level features from biopolymers. In this research, the development of a micromolding technology, to produce microstructures from aqueous derived silk solutions was studied. In particular, well-defined cellular and tissue culture substrate (scaffold) fabrication was used as a model to study manufacturing methods.

The most important aspect of the proposed technology is the ability to produce freestanding structures vs. relief surface patterns through micromolding. In particular, the manufacturing challenges consist of processing materials with a high solvent content (> 90% water), producing well-defined structures and demolding the delicate structures. In this study we are addressing the molding process with the innovative solution of controlling the hydrophilicity and hydrophobicity of the cast surface to control the deposition of the biopolymer only in the cast cavities and not on cast surface.

EXPERIMENT

Soft lithography

Soft lithography is a well established method to create elastomer rubber stamps with micron size features [3, 4]. Briefly, standard UV lithography was performed with a transparency mask (Photoplotstore), SU-8 100 photoresist (Microchem®) of ~ 100 µm thickness on 4" silicon wafers to create a mold for the PDMS. The resists was exposed with an OAI 204 Aligner with a dose of 350 mJ/cm^2. We produced mold test pattern comprised a segmented band pattern of microchannels approximately 100 µm wide, 100 µm deep and with 200 µm interchannel spacing over an area of 10 mm x 10 mm. Furthermore, a variety of channels with wider dimensions was manufactured. PDMS was prepared with a ratio of 10:1 base to curing agent and degassed in a desiccator for approximately 1 hour. Subsequently, the liquid PDMS was poured over the microchannels. A pressurized nitrogen gun and a desiccator were used to remove air bubbles which where trapped during the molding process in the microchannels. The PDMS mold was cured at 60˚C for ~ 5 hours. The PDMS stamp was released after curing by cutting along the wafer edge with a razor blade and peeling it off. No SU-8/ PDMS demolding agents were used.

Surface treatment

After the PDMS molds were released, they were treated with oxygen plasma to change the surface properties from hydrophobic to hydrophilic [5]. The PDMS stamps were placed with the patterned surface side up in the plasma chamber (Tegal Plasmod). A vacuum of ~200 mTorr was achieved before the valve which controls the oxygen flow was opened and the chamber was flushed with oxygen for 1 min at atmospheric pressure. Subsequently the valve was closed and the chamber was evacuated until the pressure reached ~200 mTorr. The procedure was repeated three times. Finally the valve was fully closed and the chamber pressure was allowed to drop to ~100mTorr. The AC power was adjusted to 50 Watts and fine tuned to allow uniform, bright and stable plasma in the chamber. The exposure times were varied from 10s, 20s to 30s depending on the experiment. After oxygen plasma treatment the PDMS molds were immediately further processed. Three test groups were established: 1) The PDMS surface was not further modified after plasma treatment. 2) The hydrophilic top most PDMS surface was carefully swiped with a common stainless-steel razor blade to expose the underlying hydrophobic material (see figure 1) and 3) a thin layer of silicone-based vacuum grease (Dow Corning® High Vacuum Grease) was deposited at the very top surface of the mold to cover the hydrophilic surface with a hydrophobic film. To obtain the configuration of group 3, a thin grease film was deposited on a microscope slide and the PMDS stamp was placed on the slide and removed. Through this the hydrophobic grease was placed only on the very top surface of the PDMS master.

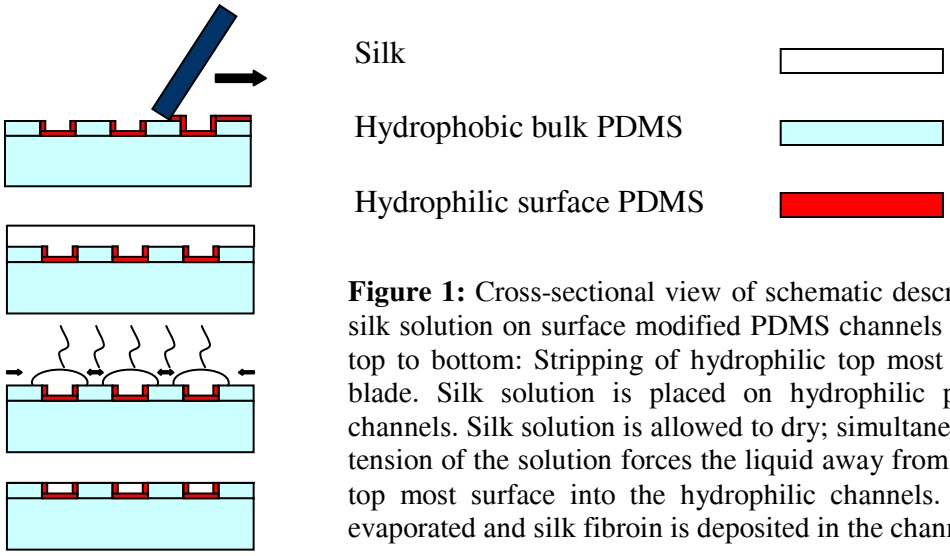

Figure 1: Cross-sectional view of schematic description of casting silk solution on surface modified PDMS channels (Group 2). From top to bottom: Stripping of hydrophilic top most layer with razor blade. Silk solution is placed on hydrophilic patterned PDMS channels. Silk solution is allowed to dry; simultaneously the surface tension of the solution forces the liquid away from the hydrophobic top most surface into the hydrophilic channels. Water has fully evaporated and silk fibroin is deposited in the channels.

Quantitative surface characterization

The degree of hydrophilicity or hydrophobicity was determined through contact angle measurement. Drops of water or silk solution (5µl each) were placed on the following surfaces: Glass, PDMS untreated, PDMS treated with oxygen plasma for 10s, 20s and 30s, PDMS treated with oxygen plasma for 20s and subsequently treated with a razor blade or vacuum grease. This led to 14 different surface/ liquid pairs (full data not shown). The images were recorded with a CCD camera and saved onto a PC. All images were recorded 10 seconds after the droplet was placed on the substrate. Further image analysis and automated contact angle measurement were performed with the ImageJ plug in LBADSA [6]. Three measurements, one droplet each, were conducted for each liquid/substrate pair. Statistical analysis (mean, standard deviation and t-test) was performed in Microsoft Excel®.

Silk fibroin extraction

A ~7.7% w/v silk fibroin solution was prepared from *B. mori* silk cocoons. The procedure of extracting silk fibroin from cocoons is described in detail by Sofia et al [7]. Briefly, fibroin is extracted in .02 M Na_2CO_3 solution. Subsequently the dried silk fibers are dissolved in a 9.3 M LiBr solution and dialyzed against DI water until the desired concentration is achieved.

Micromolding process and characterization of structure

A volume of 0.2 ml of the silk solution was placed on the PDMS negative with a 1ml syringe. The tip of the syringe was used to spread the solution evenly across the patterned surface. The silk was allowed to dry; simultaneously the surface tension of the solution forced the liquid away from the hydrophobic top most surface into the hydrophilic channels. The silk solution was allowed to dry for 2-5 days at room temperature in order for the silk fibroin to deposit into the channels (figure 1). Subsequently the silk structures were released by manually peeling them from the PDMS mold. The scanning electron microscopy (SEM) images where taken on a JEOL

JSM 840A SEM. All manufacturing and testing procedures were kept as clean as possible by being performed in a class 1000 clean room.

DISCUSSION

Surface characterization

Lawton et al. reported a contact angle of water on PDMS of 110˚ [8]. The measurements in our study showed a mean contact angle and standard deviation (n=3) of 113˚ and 1˚ respectively. The variation from the value in the literature could be explained by a variation of the PDMS processing method, the drop size, air temperature or humidity. Therefore, one could argue that this result is in good agreement with the literature and the method to determine the contact angle is satisfactory.

Figure 2 shows the results of the contact angles of silk solution on a variety of PDMS surfaces. The most significant difference ($p = 0.00$) exists between the untreated PDMS surface and the surface treated for 20 s with oxygen plasma. Next we compared the surfaces of untreated PDMS and PDMS treated with oxygen plasma for 20 seconds and subsequently stripped or coated. No significant difference ($p = 0.94$) between untreated and stripped surface was found. The coated surface shows a significant difference ($p = 0.06$) in the contact angle. Table 1 shows the numerical values for mean and standard deviation associated with the bar graph of figure 2. The high standard deviation of the group *PDMS 20s stripped* and *–PDMS 20s coated* indicates the difficulty associated with the method of manually stripping and coating the surface. Though, one could argue that even if those methods are inconsistent it can still produce useful results with the stripping method for the micromolding technique (see figure 3). This could be explained by the stripping method returning the surface back close to the original surface configuration. PDMS consists of repeating $–O\text{-}Si(CH_3)_2$ units. Upon plasma treatment, polar groups (silanol groups (Si-OH)) are introduced on the surface and these groups replace the methyl groups ($Si\text{-}CH_3$)[5]. The silanol groups are subsequently removed or modified by the razor blade so that the surface becomes hydrophobic again. Figure 3 shows an association of the different surface treatments with the final outcome of the molded silk structures, details of the images will be discussed in the next section.

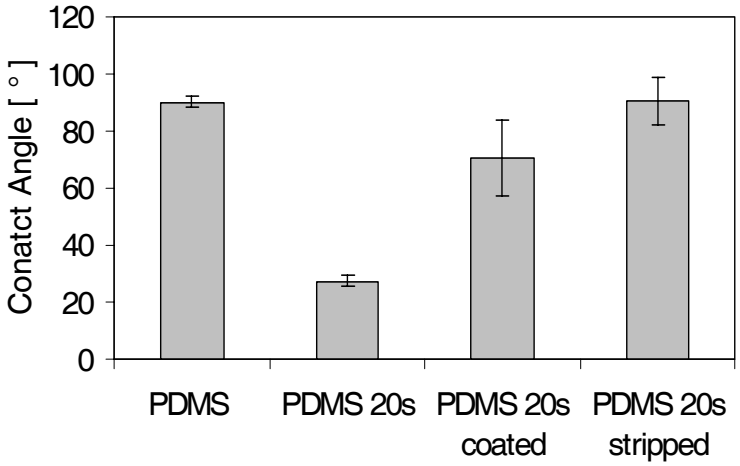

Figure 2: The bar graphs indicate contact angle measurements of silk solution droplets on treated and untreated PDMS surfaces (mean value n=3). Black bars indicate standard deviation. *20s* refers to oxygen plasma treatment for duration of 20 seconds. *Coated* refers to a deposited a thin silicone grease film on the surface. *Stripped* refers to removal of the top most layer of PDMS surface with a razor blade.

Table 1: Numerical values of mean and standard deviation (n=3) of contact angles from figure 2.

PDMS	PDMS 20s	PDMS 20s coated	PDMS 20s stripped
90	27	70	91
2	2	13	8

Micromolding

The structure seen in figure 4B was obtained by using the surface stripping approach to generate the differences in hydrophillicity and hydrophobicity. Figure 4A shows a micrograph of an approximately 100 µm x 100 µm PDMS microchannel. The channel sidewalls are vertical and good replication of the corners was achieved from the PDMS on SU-8 molding process. The bottom is perfectly smooth and shows a replica of the top SU-8 surface. The sidewalls show some roughness which can be contributed to the low quality of the low cost lithography mask. Figure 4B shows the casted silk structure with similar dimensions to the PDMS microchannel. The corners where replicated with high accuracy with a radius of approximately a few microns.

Figure 1 shows a schematic description of the casting process and mechanisms that lead to the separation of the silk solution and the deposition into the individual channels. Figure 3 shows an association of the different surface treatments with the final outcome of the casted

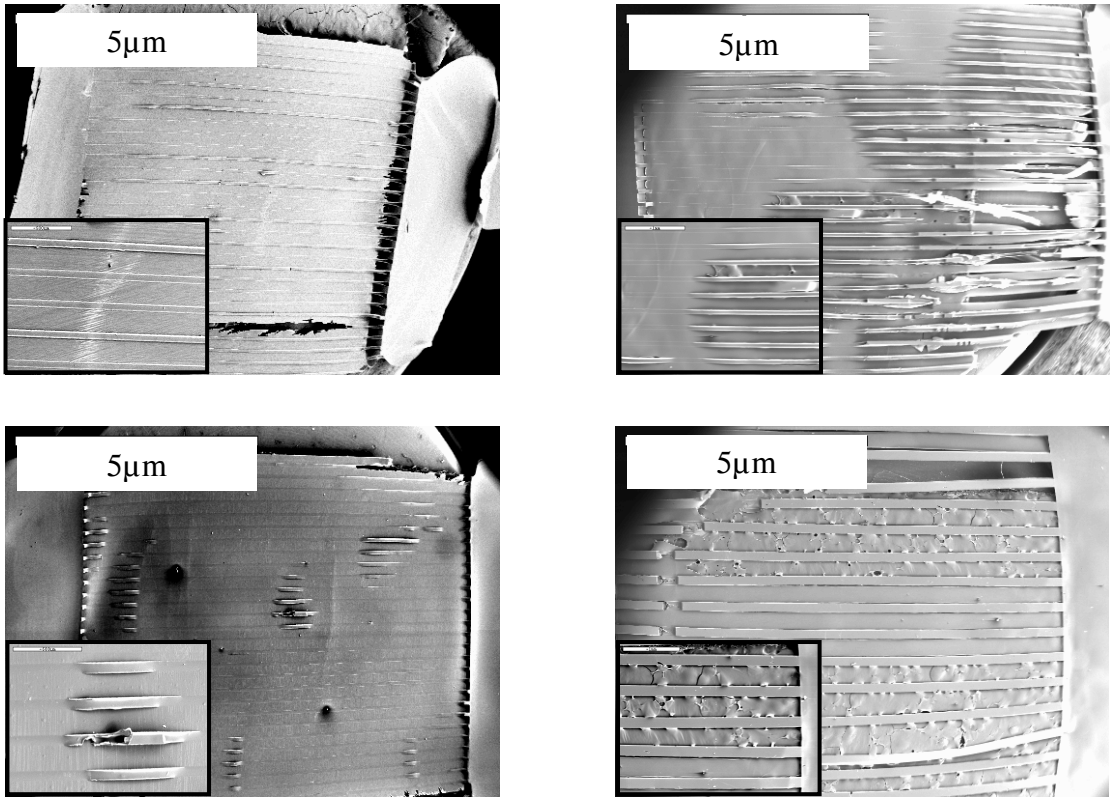

Figure 3: Decasted silk films, clockwise from top left, PDMS untreated, PDMS treated for 20 seconds with oxygen plasma, PDMS treated for 20 seconds with oxygen plasma and surface subsequently stripped with a razor blade, and PDMS treated for 20 seconds with oxygen plasma and surface subsequently coated with vacuum grease. Bottom left corner in all images shows a magnified region of a section of the image.

structures. The method using a razor blade led to the desired free standing segmented band patterns as seen in the micrograph in the bottom right corner of figure 3. The other three cast preparation methods led to only surface patterning with insufficient imprints of the features.

Limitations

The major difficulty with micromolding is the decasting process of the delicate structures [9, 10]. In this study the minimum feature size was mainly determined by the decasting process. The smallest features that could be replicated were rectangular fiber with a ~ 100 µm x 100 µm cross section (Figure 4). Consistently reproducible, the smallest structures were ~ 200 µm wide and 100 µm thick (bottom right corner Figure 3). The major reason for this limitation relate to the attachment of the silk structures to the PDMS side walls. The inconsistency in producing the smallest features is due to the limits of the process parameters of the plasma tool and the inconsistency caused by manually modifying the top surface of the cast substrate with a razor blade.

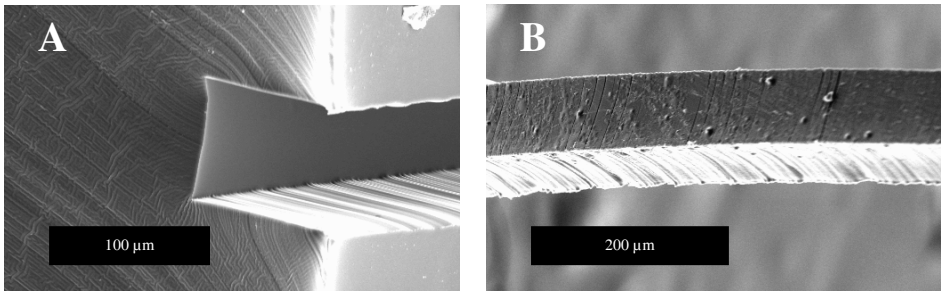

Figure 4: SEM image A on the left is showing a PDMS casting channel with width and height dimensions of approximately 100 µm x 100 µm. Image B on the right is showing a single casted silk fiber with the same dimensions.

CONCLUSION

The specific micromolding method of plasma treating and swiping the topmost surface allows fabricating free standing structures (vs. structures supported by a layer on one side, what could also be considered relief surface patterning). The two critical parts to success are (1) filling the channel features without having liquid on the top surface of the PDMS stamp and (2) decasting the structures without severe deformation. The method is able to fill the channels well; however, the feature sizes are limited by the decasting process in which the geometries and material properties of the silk are important and have to be consider.

REFERENCES

1. Meinel, L., et al., *Engineering cartilage-like tissue using human mesenchymal stem cells and silk protein scaffolds.* Biotechnol Bioeng, 2004. **88**(3): p. 379-91.
2. Bettinger, C.J., Cyr, K. M., Matsumoto, A., LangerR., Borenstein J. T., Kaplan D. L., *Silk Fibroin Microfluidic Devices.* Biomaterials, 2007 (in press).
3. Kim, E., Y.N. Xia, and G.M. Whitesides, *Micromolding in capillaries: Applications in materials science.* Journal of the American Chemical Society, 1996. **118**(24): p. 5722-5731.

4. Xia, Y.N. and G.M. Whitesides, *Soft lithography.* Annual Review of Materials Science, 1998. **28**: p. 153-184.
5. Rangel, E.C., G.Z. Gadioli, and N.C. Cruz, *Investigations on the stability of plasma modified silicone surfaces.* Plasmas and Polymers, 2004. **9**(1): p. 35-48.
6. Stalder, A., et al., *A snake-based approach to accurate determination of both contact points and contact angles.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2006. **286**(1): p. 92-103.
7. Sofia, S., et al., *Functionalized silk-based biomaterials for bone formation.* J Biomed Mater Res, 2001. **54**(1): p. 139-48.
8. Lawton, R., et al., *Air plasma treatment of submicron thick PDMS polymer films: effect of oxidation time and storage conditions.* Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2005. **253**(1): p. 213-215.
9. Guo, Y.H., et al., *Analysis of the demolding forces during hot embossing.* Microsystem Technologies-Micro-and Nanosystems-Information Storage and Processing Systems, 2007. **13**(5-6): p. 411-415.
10. Heckele, M. and W.K. Schomburg, *Review on micro molding of thermoplastic polymers.* Journal of Micromechanics and Microengineering, 2004. **14**(3): p. R1-R14.