Fabrication of Biodegradable Zein Films by Using Soft Lithography

B. Altunakar*, J. Luecha** and J.L. Kokini***

* University of Illinois at Urbana-Champaign,Urbana, IL, USA, bilge.uiuc@gmail.com
** University of Illinois at Urbana-Champaign,Urbana, IL, USA, jluecha2@illinois.edu
*** University of Illinois at Urbana-Champaign,Urbana, IL, USA, kokini@illinois.edu

ABSTRACT

Zein films were precisely nanofabricated with various features such as channels, wells, poles and grids. Master templates were created by using photolithography and soft lithography techniques were applied to develop stamps from master templates. Polydimethysiloxane (PDMS) stamps with precisely controlled nano-scale patterns were used to transfer the patterns on to zein films. Scanning electron microscopy images revealed that pattern formation and transfer on to zein films were successful. The soft lithography techniques were also used to fabricate microfluidic patterns on the zein membrane. The zein matrix could well-capture the features even the complicated one that contained small channels with several posts.The developed biocompatible patterned surfaces can further be functionalized to specifically bind to certain substrates such as proteins or cells. The application areas of this technology can be used as a tool in biomedical technologies to track specific molecules, to capture different types of biological substrate in design of lab-on-a-chip devices as well as building biodegradable and biocompatible scaffolds in tissue engineering.

Keywords: zein, nanofabrication, soft lithography, biodegradable films, microfluidic device.

1 INTRODUCTION

The applications of nanotechnology in food and agriculture are relatively new as compared to its use in medicine and pharmaceuticals. The two approaches of nanotechnology; bottom-up and top-down build up the basics of this century's frontier technology [1].

Lithography, one of the tools of nanotechnology, is a technique of stenciling to transfer a pattern of choice onto another surface. Photolithography is a commonly used technique for surface patterning. It requires passing light through a mask for patterning a surface with desired topography where a photosensitive resist is deposited on the substrate. The irradiation of the surface by a high-energy beam, typically UV light, through a photomask causes alterations on the surface in the form of ablation of the photoresist layer, breaking of a chemical bond that releases an attached molecule, initiation of polymerization or formation of a chemical bond which at the end results in multi-dimentional surface patterns [2]. The process involves the use of a photoresist (in liquid form) to make a selected material photosensitive by coating the surface of a silicon wafer with a curable photoresist solution. Next, the cured silicon wafer is exposed to UV light for certain with desired patterns is fabricated, it is then used as a template to produce PDMS stamps with soft lithography.

Soft lithography is a technique based on self-assembly and replica molding for nanofabrication that is developed for the need to pattern surfaces without the experimental difficulties and high cost associated with photolithography. The technique is used for surface patterning has been used for microcontact printing, microfluid patterning, and micromolding. The technique involves the production of a master using photolithography that is patterned as the negative of the design of interest. This master is then utilized to form a patterned substrate, typically composed of a cross-linked elastomeric polymer like PDMS. The soft lithography process involves: fabrication of the elastomeric elements and use of these elements to pattern features in geometries defined by the element's relief structure. The structure from which the stamp is derived is called the 'master' and can be fabricated by producing well-defined structures of relief on a surface [3]. Polymers such as PDMS are used due to their chemical flexibility, ease of processing and inexpensiveness. In this process the PDMS solution is poured onto the patterned template (silicon wafer) and after drying is peeled off from the surface with the same patterns. PDMS stamps are used in a variety of applications in nanotechnology research to pattern surfaces and one of the most common practices occurs in the field of microfluidics and nanosensors.

One of the potential applications of soft lithography is the fabrication of zein microfluidic devices. Microfluidic components are widely used in the design of both bioanalytical and diagnostic microdevices. A reduced sample volume together with modifications of the surface chemistry and localization of molecules within microfluid channels has opened many potential application areas in engineering and biomedical research fields [1]. The miniaturized devices are superior to conventional techniques in term of rapid analysis, small sample size requirement, user-friendly and cheap [4]. The applications of microfluidic devices are in micro-analysis of substances, micro-mixing as a bench-top scale mixing, and cell culture just to mention a few [5]. Recently, the demands of environmentally friendly polymers such as natural derived polymers for replacing petroleum-based polymers are remarkably raised including the polymers used in microfluidic devices [6 and 7]. Moreover, the biodegradability and biocompatibility of biopolymers can potentially create the more *in vivo* like environment which is desirable for microfluidic devices in biological applications [8]. Zein is a corn prolamine which can be processed to obtain water-insoluble, clear, edible film coatings for a wide range of food materials as well as encapsulation of nutraceuticals and drug components. Commercially it is produced from corn gluten meal which is a side product of bio-ethanol industry [9]. Zein has excellent film forming ability due to formation of hydrophobic, hydrogen and disulfide bonds within zein chains.

Therefore, the objective of this study is to present the feasibility of using biodegradable zein polymers as a substrate for fabrication of microfluidic membranes.Zein films were precisely nanofabricated with various features such as channels, wells, poles and grids. Master templates were created by using photolithography and soft lithography techniques were applied to create stamps from master templates. PDMS stamps with precisely controlled nano-scale patterns were used to transfer the patterns on to zein films. Scanning electron microscopy images revealed that pattern formation and transfer on to zein films were successful. The developed biocompatible patterned surfaces can further be functionalized to specifically bind to certain substrates such as proteins or cells. The application areas of this technology can be used as a tool in biomedical technologies to track specific molecules, to capture different types of biological substrate in design of lab-on-a-chip devices as well as building biodegradable and biocompatible scaffolds in tissue engineering.

2 MATERIALS AND METHODS

3-inch silicon wafers were used to fabricate masters and were subjected to oxygen plasma prior to coating to release impurities that exists on the surface of the wafer. Silicon wafers were spin coated with photoresist (SU-8, Microchem, Newton, MA) material based on the instructions provided by the manufacturer. The coated wafers were subjected to baking step called soft bake to get rid of the excess solvent within the photoresist as well as to stick the polymer layer onto the wafer. During soft bake process photoresist coated silicon wafers were heated gradually upto 95°C. A UV light photolithograph was used to transfer selected patterns onto the coated wafers. Exposure time was calculated by:

Exposure time = exposure energy (Dose)/ Measured intensity of UV source

Following the UV exposure, a post bake step is completed by gradually heating the UV-exposed wafers to fix the transferred pattern onto the wafer. Following the post-bake step, photoresist coated silicon wafers were developed with a photoresist developer material (Microchem, Newton, MA). Excess photoresist that is not polarized with UV was washed away in this process leaving the visible pattern on the surface. When the pattern becomes visible, wafers were rinsed with 2-propanol and blow-dried with nitrogen followed by a hard bake step to create tougher surface. Fabricated wafers were inspected with a microscope (AxioScope A1 with AxioCam ICc3 color camera, Carl Zeiss, Germany) for imperfections. In order to establish hydrophobicity for easy peeling off the PDMS layer, the surfaces were later washed by a surfactant.

PDMS (Sylgard ® 184 Silicone elastomer base, Dow Corning Corp., MI) solution was prepared by mixing 20 grams of PDMS with 2 grams of silicon curing agent (Sylgard ® 184 Silicone elastomer curing agent, Dow Corning Corp., MI) and the mixture was kept under vacuum for 30 minutes to remove bubbles. When the bubbles are released, the PDMS solution is poured onto the wafer. After 24 hours, the PDMS will be ready to be peeled from the wafer and the pattern will be visible. The whole process was completed in a clean room to avoid dust contamination.

The soft lithography procedure was used for fabricating microfluidic channels as mentioned previously. However, the geometries of the patterns were different and an improved formulation of zein solution was used. The simple cross microfluidic channel (50mm × 33mm × 0.05 mm) with reservoirs at the four ends (three with 3mm and one with 5mm diameters) was prepared. The second pattern which is a common geometry for cell culture application was the microfluidic channel (50mm width and 1mm diameter) with some posts. The zein with 70 wt % oleic acid and 5 wt% Tween were dissolved in warm (~ 60 ^{o}C) aqueous ethanol in the ratio of zein:ethanol as 20 g:100 ml. The chemicals were supplied from Sigma-aldrich (St. Louis, MO). Then the mixture was poured on top on the PDMS negative mold with designed microchannels and dried in desiccators for 24 hours. The patterned zein membranes were obtained by peeling off the PDMS negative molds and evaluated by both light and scanning electron microscopy.

3 RESULTS AND DISCUSSION

Nano wells with 35 micron depth and 30 microns length were successfully patterned on both master and PDMS wafers (Figure 1). Fabricated PDMS stamps were used to develop patterns on zein film surface (Figure 2). Patterned zein film was easily peeled off from PDMS surface and light microscopy indicated that pattern transfer was successful (Figure 3). The results were also confirmed by the SEM images (Figure 4).

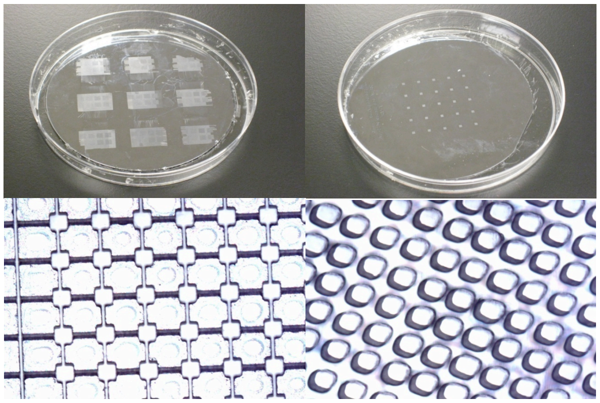

Figure 1: Microscopic images of master and PDMS stamp.

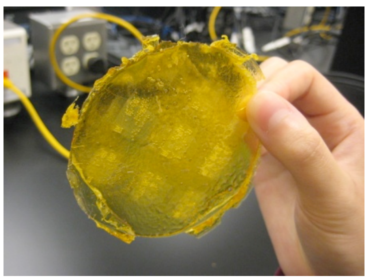

Figure 2: Nano-patterned zein film.

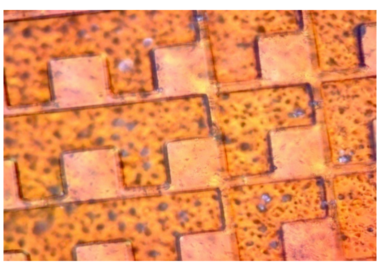

Figure 3: Microscopic image of nano-patterned zein film.

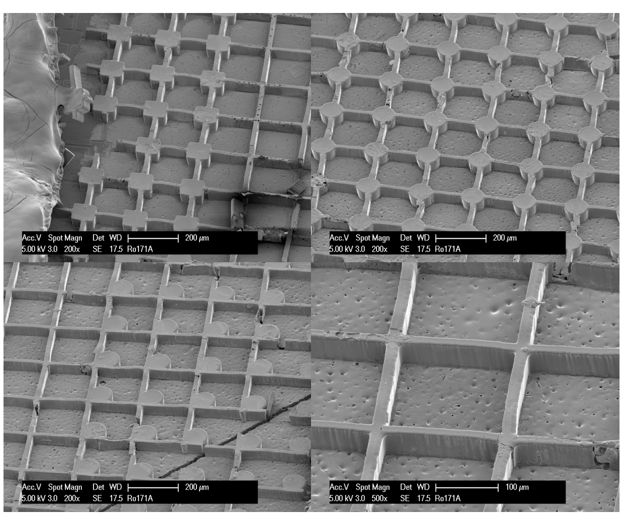

Figure 4: SEM images of patterned zein films.

The clear yellowish zein microfluidic membranes with simple cross channels were 500 microns in thickness (Figure 5). It can be seen that the replica-molded zein membranes cast on PDMS negative molds was a promising technique which transferred microchannels and reservoirs with high degree of feature fidelity (Figure 6). It is well-known that PDMS itself is deformable. Hence it is acceptable that the patterns transferred to zein membranes showed slightly deflection especially at the edges.

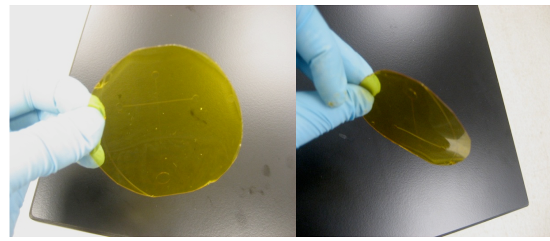

Figure 5: The zein membrane with a pattern of simple cross microfluidic channels.

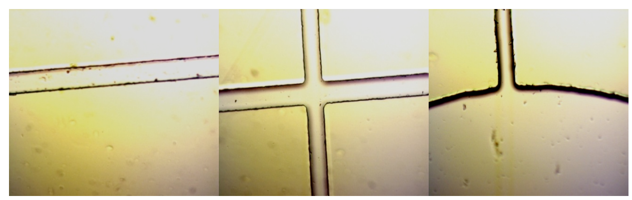

Figure 6: The microscopic images of zein microfluidics, the channel (left), the cross (middle), and the reservoir (right).

In terms of complex geometry, zein biopolymer could replicate the small features from the PDMS mold (Figure 7). This indicates the high quality of pattern transferring from PDMS molds to zein matrix using this current technique.

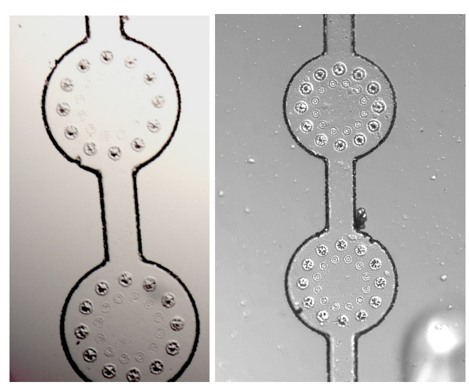

Figure 7: The microscopic images of PDMS mold (left) and replica zein microfluidic membrane (right).

4 CONCLUSIONS

Nanofabrication of zein films with soft lithography was a key step to pattern biodegredable surfaces for potential appliations. All used geometries including poles, wells and channels were able to be patterned on the zein surface with perfection. Precision and ease of patterning on a biodegrable surface was accepted as an intermediate step to fabricate microfluidic membranes for the upcoming applications. The application of soft lithography technique on zein substrate was able to fabricate zein microfluidic membranes with high quality geometries. This potential technique of patterning biodegredable surfaces can further be developed into the fabrication of zein biodegradable microfluidic chips that can facilitate agriculture, food and biotechnology applications.

5 ACKNOWLEDGEMENTS

The authors would like to thank Dr. Liu's research group of Micro and Nanotechnology Laboratory for technical discussions and assistance in microfabrication.

REFERENCES

[1] N. Sozer and J.L. Kokini, Trends in Biotechnology, 27, 82-89, 2009.

[2] Hook at al, Patterned and swithable surfaces for biomolecular manipulation, Acta Biomaterialia, 5,7,2350-2370, 2009.

[3] Rogers and Nuzzo, Recent progress in soft lithography, 8, 50-56, 2005.

[4] Y. Xiao and G.M. Whitesides, Annual Reveiw Material Science, 28:153–84, 1998.

[5] O. Skurtys and J. M. Aguilera, Food Biophysics, 3,1–15, 2008.

[6] A. L. Paguirigan and D. J.Beebe, Nature Protocols, 2, 1782- 1788, 2007.

[7] C. J. Bettinger et al, Advanced Materials, 19, 2847-2850, 2007.

[8] A. L. Paguirigan and D. J. Beebe, lab on a Chip, 6, 407-413, 2006.

[9] Shukla and Cheryan, Industrial Corps and Products, 13, 171-192, 2001.