SU-8 microchannels for live cell dielectrophoresis improvements

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Abstract

In this work a novel SU-8 fabrication technology is employed to construct microfluidic devices for sensitive dielectrophoretic (DEP) manipulation of budding yeast cells. Identical devices were produced with standard soft-lithography processes. In comparison to standard PDMS based soft-lithography, an SU-8 layer was used to construct the microchannel walls sealed by a flat sheet of PDMS to obtain the microfluidic channels. Direct bonding of PDMS to SU-8 surface was achieved by efficient wet chemical silanization combined with oxygen plasma treatment of the contact surface. The presented fabrication process significantly improved the alignment of the microstructures. In addition, PDMS delamination above electrode topologies was significantly decreased over standard soft-lithography devices. The fabrication time and costs of the proposed methodology were found to be roughly the same. Sensitivity of the devices was tested by discriminating Saccharomyces cerevisiae cells in the G1 phase from cells in the S/G2/M phase using dielectrophoresis. This level of sensitivity necessitated high precision electrode structure that was designed using an FEM

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model based approach. Attaining such high precision using standard soft-lithography can be difficult due to additional requirements of an alignment stage and its associated tight timing limits.

Keywords: Microfabrication, microfluidics, SU-8, silanization, dielectrophoresis, microchannels

1 Introduction

A charge neutral particle placed in an electric field is acted on by dielectrophoretic forces. These forces manifest themselves through polarization of the particle and its surrounding medium. Their magnitudes and directions are complex functions that depend on the dielectric properties of the entire molecular composition [1]. The sensitivity of the DEP sorting in microfluidics relies on precisely complied design parameters. The ability of sensitive DEP to sort the cells according to specific changes in their molecular composition will be beneficial to applications ranging between blood sample analysis and dynamic measurement of cell response [2, 3, 4].

Recently, soft-lithography processes have been viewed favorably for their low cost and fast prototyping of microfluidic devices [5]. However, the rough alignment of the PDMS microstructures with the underlying electrode structures makes it difficult to use in applications requiring higher precision.

2 Materials and methods

Microfluidic devices were designed and fabricated to explore the frequency dependence of the dielectrophoretic force acting on Saccharomyces cerevisiae cells.

Microelectrode structures were patterned on glass using standard photolithography processes. A 50 nm thick layer of Ti/W, and 500 nm thick layer of gold were deposited by sputter-coating. The layer of Ti/W was deposited first to improve the adhesion and stability of the deposited gold layer. After the metal deposition, a 1.1 μm thick layer of PFR7790 (JSR Micro) photoresist was spin-coated and patterned. The gold and Ti/W were etched by wet etching in the areas not covered by photoresist. The remaining photoresist was removed.

A 30 microns thick layer of SU-8 was spin-coated on top of the glass substrate to serve as a carrier of the microfluidic channel structures. The SU-8 photoresist was pre-baked immediately after spin-coating, exposed to

Figure 1: Fabrication of the SU-8 microfluidic channel structures.

UV light through a photomask, followed by post-exposure bake. The SU-8 development revealed the designed microstructures. Finally the wafer was hard-baked to increase thermal, chemical and physical stability of the developed resist structures. Fabrication process is schematically shown in Fig. 1.

Simple flat sheets of PDMS were fabricated to seal the microfluidic devices. Two-part silicone elastomer Sylgard 184 from Dow Corning was used to produce the PDMS sheets. The base part was mixed with sufficient amount of curing agent (10:1 ratio) and stirred well in a disposable plastic cup. The mixture was placed in a desiccator to remove the air bubbles introduced by mixing. PDMS mixture was poured into a plastic petri dish to form homogenous approximately 5 mm thick layer. The petri dish containing the mixture was then placed into an oven, for 2 hours at 80 °C. Perfect horizontal position was required to assure good planarity. The PDMS edges were cut off with a sharp tool and the PDMS sheet was gently peeled off the petri dish. The released PDMS sheet was cut into pieces equal in size to the individual microfluidic devices. Inlets and outlets were drilled carefully by sharp needle of the appropriate diameter at the desired locations. A paper template with the preprinted pattern may serve well for easier localisation of the inlets and outlets.

Short oxygen plasma treatment of the SU-8 surface and subsequent chemical silanization process was performed to enable direct irreversible bonding of the SU-8 structures to the PDMS [6]. First, the oxygen plasma treatment allowed binding of silanes to the SU-8 surface through opening of the epoxy rings at the SU-8 surface. Subsequently, SU-8 structures were immersed into (3-Aminopropyl)trimethoxysilane (APTMS) solution diluted with methanol to 9% by mass. The samples were dried at 110 °C for 30 minutes. Silanization process is illustrated in Fig. 2.

The microfluidic devices were sealed with prepared flat sheets of PDMS. Individual flat sheets of PDMS were cleaned properly with adhesive tape. The PDMS and SU-8 surface was treated by oxygen plasma for 20 seconds. The oxygen plasma affects the PDMS backbone and forms reactive silanol functional groups (Si-OH) enabling formation of permanent irreversible bond of the PMDS to the silanized SU-8 surface [7]. The oxygen

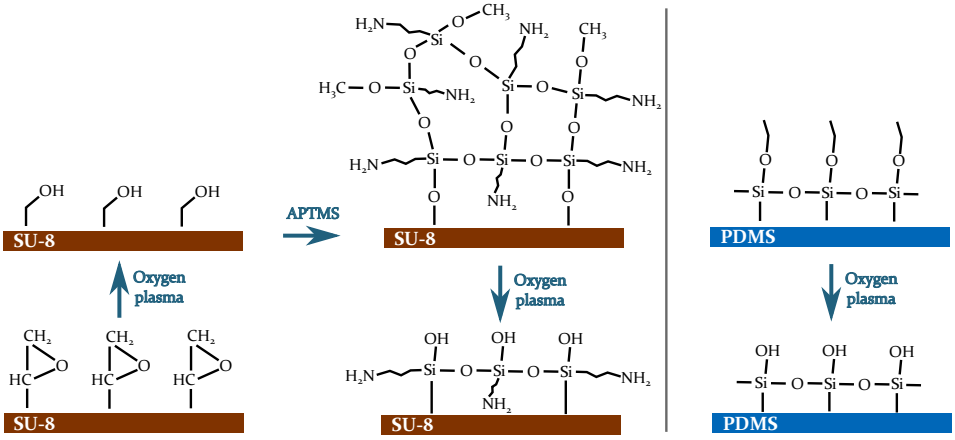

Figure 2: Illustration of the silanization process and the final oxygen plasma activation of the SU-8 (left). Oxygen plasma activation of the PDMS (right).

plasma activation is illustrated in Fig. 2.

In addition, the PDMS treatment with oxygen plasma is beneficial as it avoids nonspecific adsorption, decreases cell clogging and turns the PDMS to hydrophilic, that facilitates the future microchannel wetting [8]. Immediately after the oxygen plasma treatment, small droplet of methanol was poured over the SU-8 surface to avoid instantaneous bonding of the activated PDMS to the SU-8. The methanol between the SU-8 and the PDMS increased the time necessary for alignment of the PDMS microstructures above the SU-8 microstructures to approximately one minute. Alternatively, methanol may be replaced by less dangerous isopropyl alcohol. The alignment of the flat PDMS sheets was performed manually. The bonded devices were placed in the oven at 80 °C for 5 minutes. Finally, the devices were placed at room temperature covered with a petri dish to avoid contamination. All inlets and outlets were sealed by adhesive tape after 24 hours. The bonding of the flat PDMS sheet to the SU-8 layer is illustrated in Fig. 3.

Identical devices were fabricated using standard soft-lithography processes.

3 Bio-compatibility

PDMS material is generally considered as bio-compatible. However, the use of PDMS with sensitive cell lines may not always be so straightforward. It has been reported that PDMS exerted toxicity while growing 3T3 mouse

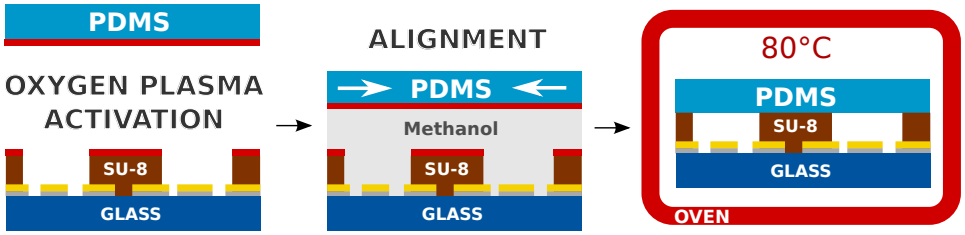

Figure 3: Bonding of PDMS to SU-8.

fibroblasts [9]. These issues were solved by rinsing the microchannels 24 hours prior to the experiment and a subsequent autoclaving. After curing of PDMS, there is up to 5% of the weight of the PDMS in the form of un-crosslinked oligomers. These oligomers may be released, contaminate the culture medium, and suppress the growth and proliferation of present cells, e.g. primary neurons [10]. The un-crosslinked PDMS oligomers can be decreased or avoided by extraction of the oligomers with highly soluble solvents [11]. Other experiments have proven the influence of PDMS on gene expression in PC12 cells [12].

Unlike the PDMS, SU-8 shows perfect biocompatibility without any additional processing. It has been widely used in applications ranging between cell encapsulation [13] and neuronal probes [14]. Several deep analysis were performed *in vitro* and even *in vivo* to prove the biocompatibility. The SU-8 showed no influence on cell growth and proliferation in *in vitro* experiments with rabbit muscle stem cells [15], SH-SY5Y human neuroblastoma cells [16], human skin fibroblast cells, Schwann cells and explanted neurons from dorsal root ganglia [14]. No significant reactions on the presence of SU-8 in *in vivo* experiments on mice have been observed [17].

4 Prerequisites and comparison to soft-lithography

Microfluidic devices exploiting interactions of live cells with electric field require electrodes to be patterned on a substrate. However, it is difficult to obtain perfect alignment with standard soft-lithography due to the manual alignment. Here we utilize mask aligning system to perform a high precision alignment prior to the UV exposure of the SU-8 layer.

The presented method requires patterning of the microfluidic channels to be performed for each set of microfluidic devices. On the other hand, the soft-lithography process requires single fabrication of the silicon (or SU-8) master necessary for PDMS mold replication. These additional replications

do not require a mask aligner, a spin processor or a UV exposure system.

In terms of time requirements the two methods are roughly the same with the exception of the electrode to channel alignment. While the standard PDMS requires each device to be aligned separately, the method presented herein involves single alignment stage per wafer (containing up to tens of devices).

5 Live cell dielectrophoresis

We developed a model of a Saccharomyces cerevisiae cell and simulated the dielectrophoretic response using analytical DEP solutions. These simulations combined with an FEM model-based approach were used to optimize the electrode structures. Matlab and Comsol Multiphysics with LiveLink^{TM} for Matlab were used to implement the cell and electrode models. This approach showed that a relatively small 10-micron gap is optimal for obtaining sensitive and efficient low voltage DEP. The electrode optimization enhanced the capability of the DEP to reveal significantly smaller dielectric differences between individual cells. The results suggest that accurate alignment of electrode structures with microfluidic channels is indeed important.

6 Results and discussion

The devices fabricated with the SU-8 formed microchannels showed perfect alignment of the microchannel structures with the electrodes, as well as perfect adjoining of the SU-8 layer to the glass substrate with patterned electrodes. No noticeable PDMS delamination at the electrode-PDMS contact surface or microchannel shrinkage during the PDMS hardening was observed. Delamination of the PDMS above electrode structures and the imprecise alignment common for soft-lithography fabricated devices is shown in Fig. 4. Sensitivity of the devices was tested by discriminating Saccharomyces cerevisiae cells in the G1 phase from cells in the S/G2/M phase using dielectrophoresis. Preliminary data comparing dielectrophoretic responses in different life cycle phases are shown in Fig. 5.

7 Conclusion

In this paper, we present an innovative fabrication process of microfluidic devices suitable for applications requiring high precision but not limited to them. We showed that permanent bonding of SU-8 to PDMS is achieved

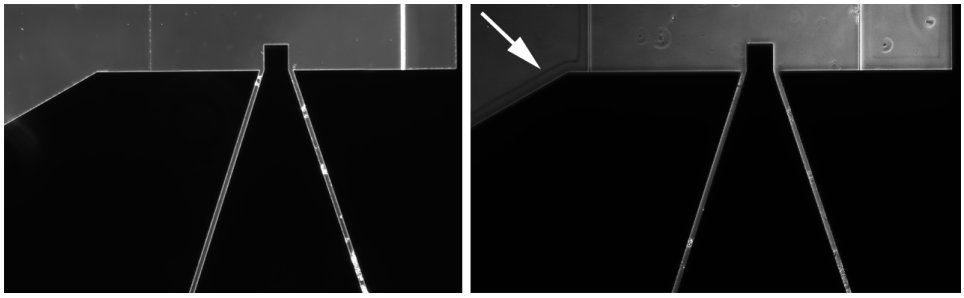

Figure 4: The device with SU-8 formed microchannels (left) and the device fabricated with standard soft-lithography showing misalignment and delamination above electrode structures (right).

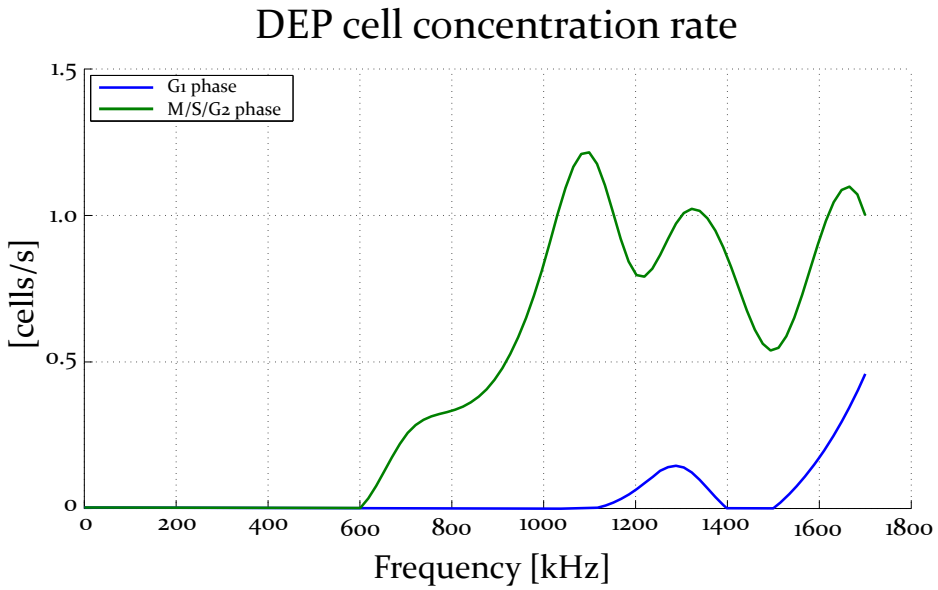

Figure 5: Preliminary results on the dielectrophoretic response of the Saccharomyces cerevisiae cells.

by simple and efficient wet chemical silanization step in combination with oxygen plasma treatment. Fabricated devices showed significant improvement in alignment of the SU-8 microstructures above electrode topologies, higher reliability and resistance to pressure. The presented method is easily reproducible and enables simple, low cost, fast prototyping of microfluidic

devices suitable for live cell handling.

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