SURFACE MODIFICATION OF SiO_2 MICRO-NOZZLES FOR PATCH-CLAMP MEASUREMENTS ON-CHIP

T. Lehnert, A. Laine, and M.A.M. Gijs

Swiss Federal Institute of Technology Lausanne (EPFL), Institute of Microelectronics and Microsystems, CH-1015 Lausanne, Switzerland (Thomas.Lehnert@epfl.ch)

Abstract

A new surface modification has been applied to micro-nozzles that are integrated in a patch-clamp microsystem. In order to promote cell/surface interaction (Giga-seal formation), the SiO_2 micro-nozzles were coated with a sub-micron thin layer of polydimethylsiloxane (PDMS) and were subsequently exposed to an oxygen plasma. First measurements with living cells showed improved seal resistances.

Keywords: Patch-clamp on-chip, micro-nozzle, PDMS coating, cell handling

1. Introduction

An automated high-throughput patch-clamp system would be very beneficial for industrial drug screening. Several chip-based patch-clamp microsystems are under development to respond to this increasing demand [1]. However, the requirement of a very tight electrical seal (Giga-seal) between the cell membrane and a suitable aperture, seems to be more difficult to meet with micro-chips than with conventional glass pipettes. Specific surface treatments are often necessary. We present a new approach based on a plasma treatment of SiO_2 micro-nozzles that have been coated with a thin PDMS layer.

2. Nozzle-based microsystem and PDMS coating

Our microsystem is based on an integrated 3-dimensional Si/SiO_2 micro-nozzle structure [2]. A cross-section of the final hollow nozzle tube is shown in figure 1a. Similar nozzles have been developed previously for other applications [3]. In our system, a living cell can be immobilized on top of the nozzle by applying a negative pressure gradient from the backside. Although the present fabrication process has been optimized for smooth surfaces, only low seal resistances ($R \leq 0.15$ GΩ) have been obtained [2].
Previous work by K.G. Klemic et al. with mm-sized Xenopus oocytes has demonstrated that GΩ-seal formation between a large planar aperture (Ø 8 µm) in a plasma-oxidized PDMS substrate and a cell membrane is possible [4]. However, this approach has not been adapted for small cells so far. Slight mechanical stress/strain may alter the surface properties of the plasma-treated elastomer [5]. Therefore an important question is, whether deformation of a small elastomer aperture (Ø 1-2 µm) during cell immobilization by suction, will not be harmful for Giga-seal formation.

7th International Conference on Miniaturized Chemical and Biochemical Analysis Systems
October 5–9, 2003, Squaw Valley, California USA

0-9743611-0-0/µTAS2003/$15.00©2003TRF

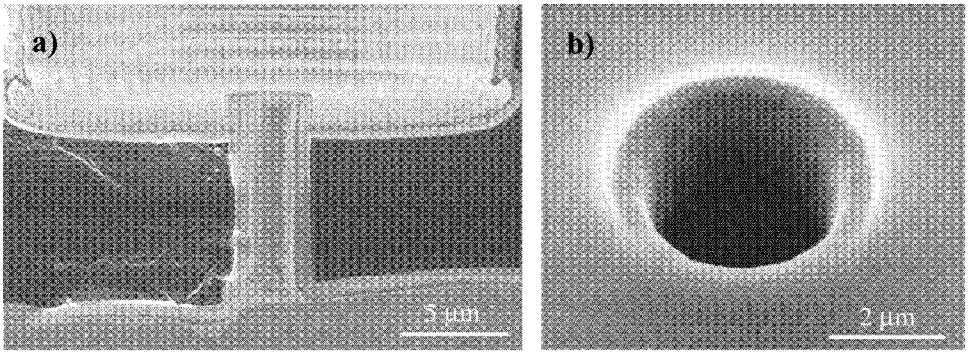

Figure 1: a) Micromachined SiO_2 nozzle (Ø 2.5 µm, cleaved Si substrate). The hollow oxide tube extends from the nozzle in the frontside pit to a large backside hole; b) enlarged top view of a SiO_2 nozzle coated with a thin PDMS layer.

We take advantage of these reported specific surface properties by coating a suitable rigid microstructure, i.e. a SiO_2 micro-nozzle, with a sub-micron thin PDMS layer (Sylgard^{TM} 184). This elastomer establishes conformal contact with patterned surfaces on sub-µm scale, as is widely demonstrated in µm- and nm-molding and replication. The technical challenge consists in coating nozzles with diameters down to 2.5 µm without plugging the hollow oxide tube. Reproducible results have been achieved by injecting PDMS with moderate positive pressure through the nozzle tube from the backside of the chip until it emerged in the frontside pit. This process was controlled visually under an optical microscope. To avoid plugging, suction was applied to the nozzle during PDMS curing. Figure 1b shows a coated SiO_2 nozzle. The rim and inner surface appear to be smoother than for as-fabricated nozzle surfaces. In addition to the modified surface chemistry, this feature is expected to be beneficial for a tight cell/material contact.

3. Surface modification of PDMS by oxygen plasma treatment

The exact nature of the interaction during Giga-seal formation between the lipid head groups of a cell membrane and the material surface is not well understood. However, it is known that in order to promote the attachment of the hydrophilic cell membrane, the material surface also needs to be hydrophilic. The naturally hydrophobic PDMS surface can be made hydrophilic by an oxygen plasma treatment. This effect is generally attributed to the formation of a nm-thin silica-like surface layer [5, and refs therein]. However, continuous hydrophobic recovery is observed. Possible explanations for the hydrophobic recovery are the diffusion of low molar mass PDMS molecules to the surface or the reorientation of polar hydroxyl groups from the surface into the bulk material [5,6].

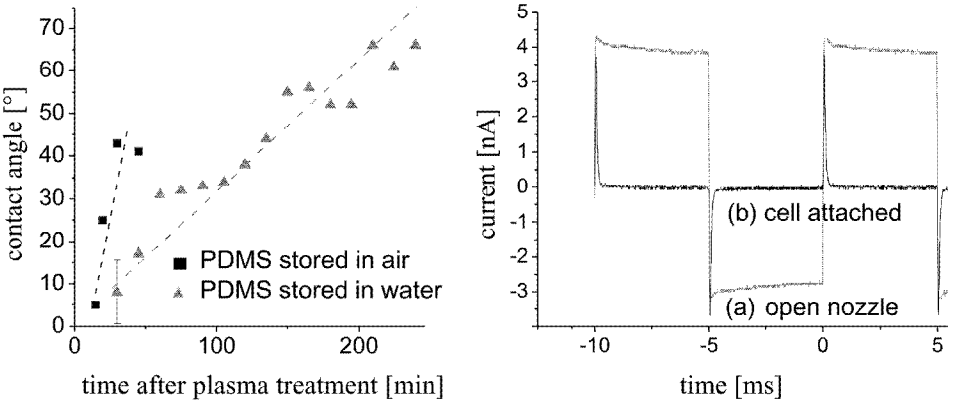

Figure 2 (left): Temporal increase of the water contact angle on PDMS surfaces, i.e. loss of hydrophilicity, after RF oxygen plasma treatment.
Figure 3 (right): Measurement with a PDMS coated nozzle (∅ 2.5 µm, current response to a ± 5 mV/5 ms test signal, PBS); a) open nozzle without cell ⇒ R = 1.5 MΩ; b) Jurkat cell attached to the nozzle ⇒ R ≈ 1 to 1.5 GΩ.

In our experiments, plasma treatments were performed either with a barrel-type reactor (Tepla 300, RF plasma, pure O_2) or with a parallel-plate system (Edwards Scancoat Six in etch mode, DC plasma, ambient air). The typical plasma exposure times were very short (10 to 60 s) with minimized power. For more intense plasma exposures, cracks developed on the PDMS surface during and after treatment. The increase of hydrophilicity was characterized by water contact angle measurements on flat PDMS test samples. With both plasma systems the PDMS surface could be made temporarily hydrophilic. Figure 2 shows results obtained with the Tepla system (RF, P = 200 W, p_{O2} = 1.1 mbar, t = 10 s). After treatment, the contact angle on PDMS decreased from initially 85° (hydrophobic) to 5±3° (hydrophilic). After 30 min, hydrophobicity is partially recovered (i.e. the angle increased to 40-45°). The time constant of hydrophobic recovery can be significantly prolonged by storage in water, as indicated in figure 2 (the contact angle increased only to about 15° after 30 min). This observation is qualitatively comparable to results mentioned in [4], however, a more quantitative comparison with published data is hardly possible, as plasma parameters are very system specific. The initial contact angle of the untreated material was generally not fully recovered, even after several days. For PDMS surfaces treated in the parallel-plate sputter system filled with air (DC, U = 0.6 kV, I = 45 mA, p_{air} = 0.35 mbar, t = 60 s) the lowest contact angle was similar as with the barrel-type reactor, but the hydrophobic recovery time was about two times faster.

4. Electrical measurements with PDMS coated nozzles

Electrical seal resistance measurements have been performed with suspended Jurkat cells (Ø 15 µm). Plasma treatment of the PDMS coated nozzles was carried out in the parallel-plate system in ambient air. Cells have been positioned by applying very gentle suction to the nozzle. It was observed that cell adhesion to plasma-treated surfaces is much stronger than to untreated ones. This holds for bare Si/SiO_2 as well as for PDMS-coated surfaces. However, oxygen plasma treatment of Si/SiO_2 surfaces did not significantly improve the seal. With the coated nozzles, seal resistances increased typically to about 300 MΩ, and the possible formation of Giga-seals was observed. One of such resistance measurements is shown in figure 3. After cell positioning, the resistance continued to increased above 1 GΩ, even when the negative pressure under the nozzle was released. The seal was stable for several minutes. Such a behavior, that was not observed in previous experiments with uncoated nozzles, is comparable to the seal formation in conventional patch-clamp experiments with glass pipettes. The presented values are preliminary results, the time being no systematic characterization has been carried out. As hydrophilicity of the PDMS surface diminishes rapidly with time, measurements were carried out within 10 min after plasma treatment.

5. Conclusion

PDMS coating of SiO_2 nozzles for patch-clamp measurements on-chip can be realized with satisfying yield. This novel technique is also expected to be very well-suited for small apertures in thin membranes. Suitable plasma oxidation parameters have been found in order to generate temporarily the required hydrophilic PDMS surface without introducing surface damage (micro-cracks). Our electrical measurements indicate that the technique is beneficial for seal formation with living cells and results in higher seal resistances than with bare SiO_2 surfaces.

Acknowledgements

The authors would like to thank Prof. J.-D. Horisberger (University of Lausanne, Switzerland) and Dr. R. Netzer (Evotec OAI, Hamburg, Germany) for helpful discussions, as well as Dr. C. Vandevyver (EPFL-SB-ISP) for cell culturing.

References

1. J. Xu et al., *Drug Discovery Today* **6**, 1278 (2001).
2. T. Lehnert, M. Gijs, R. Netzer and U. Bischoff, *Appl. Phys. Lett.* **81**, 5063 (2002).
3. Ph. Luginbuhl et al., *Sensors and Actuators* **B 63**, 167 (2000).
4. K. Klemic, J. Klemic, M. Reed and F. Sigworth, *Biosens. Bioelectr.* **17**, 597 (2002).
5. H. Hillborg and U. Gedde, *Polymer* **39**, 1991 (1998).
6. J. Kim et al., *IEEE Trans. Dielectrics and Electrical Insulation* **6**, 695 (1999).