Integrated Microfluidic System for DNA Analysis
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ABSTRACT — In this paper, we report the fabrication of a Si based microfluidic system, which is integrated with electrodes and polymer structures. Low temperature Si to glass bonding using PMMA as an intermediate layer has been developed to integrate electrodes and PMMA structures with Si channels. The electrodes in the microchannel are used for electrokinetic-based DNA stretching. DNA molecules are stretched using high frequency ac electric fields inside Si channels that are 100 µm wide and 20 µm deep.

Index Terms — Microfluidic, DNA, PMMA, bonding, electrokinetic

I. INTRODUCTION

Electrokinetic techniques such as dielectrophoresis and electro-rotation have been utilized for many years for manipulation, separation and analysis of cellular-scale particles [1]. Proteins as small as 5 nm have been manipulated using both attractive [2] and repulsive forces [3]. Dielectrophoresis, concerned with manipulation of molecules in solution, where wet environments are necessary for bio-molecules, requires microfluidic system with integrated electrodes [4].

Recently DNA manipulation has been demonstrated using ac electrokinetics [5]. The integration of electrodes inside channels involved UV glue bonding of glass channels. This bonding technique cannot be used in case of submicrometer channels since the UV glue will seep into and block the channels. We have developed a low temperature PMMA bonding technique to integrate electrodes inside Si channels. These electrodes can be used to manipulate biological molecules such as DNA and proteins inside very small channels.

Stretching of DNA molecules inside microchannels by combing has been demonstrated [6]. The disadvantage of this technique is that the DNA molecules are dried from the solution in the channel, which would result in DNA not being biologically active. We have used electrokinetic-based DNA stretching technique to demonstrate that DNA immobilization and stretching can be achieved inside sealed microchannels.

II. FABRICATION TECHNOLOGY

Integrating electrodes inside Si channels requires bonding of Si and glass using PMMA as an adhesive layer. The procedure is illustrated in Fig 1. To ensure good bonding, the wafer surface has to be free of particles and impurities. The 100 µm thick glass is immersed in 1:1 H2O2:H2SO4 solution for 20 min, followed by dehydration at 200 °C for 1 h in an oven. 20/50 nm thick Cr/Au layer is deposited on glass using electron beam evaporation. Photoresist is spun and patterned on the glass using optical lithography. The pattern is then transferred to metal layers by wet etchants with photoresist as the mask. Photoresist is then removed using PRS 2000 heated at 100 °C, leaving Cr/Au electrodes on the glass, as shown in Fig. 1(a). It is very important to ensure that there are no PR residues left on the glass since this will result in poor bonding. The glass wafer is dehydrated again at 200 °C for 1 h in an oven. 6% 950K PMMA is spin-coated on the glass wafer containing the electrodes at 2.5 krpm, resulting in 600 nm thick layer, as shown in Fig. 1(b). 10 nm thick Ti layer is evaporated on the PMMA. To define areas over which the PMMA is etched, photoresist is spun and patterned on the Ti layer, as shown in Fig. 1(c). The thin Ti layer prevents the solvents in the resist and the PMMA layers from interacting with each other. Following photoresist patterning, Ti layer is etched in 20:1:1 H2O:H2O2:HF solution [7]. The exposed PMMA is then etched in O2 plasma with 100 W rf power, 250 mTorr, 100 sccm of O2 for 12 min as shown in Fig. 1(d). Photoresist is then removed in PRS 2000 at room temperature, since removal in higher temperature solvent would result in PMMA peeling off from the glass surface. The last step involves Ti layer etched in 20:1:1 H2O:H2O2:HF solution for 10 s, leaving Cr/Au electrodes and patterned PMMA on glass as shown in Fig. 1(e).

The channels are etched in Si using plasma etching. The etch process is switched between the SF6 etch step and the C4F8 passivation step to obtain fast Si etch rate and vertical etch profile. A source power of 75 W and stage power of 12 W are used at 26 mTorr with 105 sccm SF6 in the 14-s etch cycles. A 75 W source power is applied with no stage power at 16 mTorr and 40 sccm C4F8 in the 4-s passivation...
cycles. Fluidic ports of 300 µm diameter to access the channels are defined by etching through the 550 µm thick wafer using plasma etching. A source power of 75 W and stage power of 20 W are used at 26 mTorr with 160 sccm SF₆ in the 13-s etch cycle. A 75 W source power was applied with no stage power at 16 mTorr and 85 sccm C₄F₈ in the 7-s passivation cycles. The total etching time is 3 h. The Si wafer with etched channels and fluidic ports is cleaned in 1:1 H₂O₂:H₂SO₄ before bonding.

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The wafers are first aligned and then transferred to the bonding tool for bonding. Bonding of the wafers is carried out in vacuum at 75 Torr pressure. This prevents the formation of air gaps and helps to improve the contact uniformity across the wafer. The bonding is performed above the glass transition temperature of PMMA (109 °C) for 2 h at a pressure of 0.4 MPa, resulting in electrodes integrated inside fluidic channels as illustrated in Fig. 1(f) [8].

III. INTEGRATED ELECTRODES AND PMMA STRUCTURES IN SI CHANNELS

Figure 2 is an optical micrograph showing electrodes on a 100 µm thick glass aligned and bonded to Si wafer containing channels perpendicular to each other. Conditions used for bonding the wafers are 110 °C, 0.4 MPa for 2 h in 75 Torr vacuum. The Cr/Au electrodes on the glass are 20/50 nm thick and the channels in Si are 30 µm deep, 3 and 10 µm wide. To etch the channels, a total of 45 cycles of switching is carried out with a total etch time of 15 min. The area over which the PMMA is etched is illustrated in the figure.

Figure 3 shows a fluorescent micrograph of PMMA structure integrated inside a 100 µm wide and 1 µm deep Si channel. The fabrication process is similar to the one described above. PMMA is spun and patterned on the glass. Instead of Ti, Au is used as an intermediate layer between PMMA and photoresist. The bonding conditions used are similar to the one mentioned above.

IV. DNA MANIPULATION IN CHANNELS

Electrodes in the channel are used to stretch the DNA molecules using ac electric fields. Channel leak is tested before applying the electric field. It is important there are no leaks because the presence of leaks drives the fluid and DNA molecules inside the channel by capillary force, which results in disturbing the position of the DNA molecules inside the channel with respect to the applied electric field.

The forces acting on the DNA molecules and the fluid by ac electric fields have been described by Ramos et al [9]. The electrokinetic stretching of DNA molecule in a fluidic medium involves several forces acting on the...
DNA. One of them is the dielectrophoretic force caused by the induced dipole along the backbone of the DNA molecule. This force is directed towards the electrode edges. The second force is the torque exerted on the induced dipole by the electric field. For a DNA molecule with one end immobilized on the electrode, the torque tends to elongate the DNA parallel to the electric field. The third force is the electrothermal force acting on the fluid medium. This force arises from the thermal gradients in fluid created by the variations of the electric field. The electrothermal force induces a circulatory motion of the fluid near the electrodes. The fluid medium used in this case is 1:1 DI water:morpholinoethanesulfonic (MES) buffer (50 mM, 5.5 pH). When DI water alone is used as the fluid medium, the electrothermal force acting on the fluid is high due to its low conductivity. The flip side is, the probability that one end of the DNA molecule would get immobilized on the electrode in DI water is low. When MES buffer alone is used as the fluid medium, the electrothermal force acting on it is comparatively lower due to its higher conductivity, but the probability that one end of the DNA would be immobilized on the electrode is higher. Taking the above factors into account, the fluid medium chosen is a mixture of equal amounts of DI water and MES buffer.

3 ng/µl of λ-DNA in 1:1 DI water:MES buffer is introduced into the channels. 100 KHz sinusoidal ac voltage starting from 0.5 V is slowly ramped up. The DNA molecules start moving towards the electrodes at around 2.2 V due to the combination of dielectrophoretic force and the circulating fluid. During this process some of the DNA molecules that get attracted to the electrode, have one of their ends immobilized on the electrode. After ramping to about 6 V the tethered DNA molecules start stretching out as shown in Fig 4. The Cr/Au electrodes consist of straight edge electrode on the left that is separated by 30 µm from the tip of the pointed electrode on the right. These electrodes are integrated in a 100 µm wide and 20 µm deep Si channel. The thickness of the Cr/Au electrodes is 20/50 nm. More DNA molecules are immobilized on the pointed electrode due to higher electric field gradient at the pointed tip. After DNA immobilization, the voltage is ramped up, which results in torque and electrothermal force exerted on the attached DNA molecules. The torque acting on the molecule combined with the electrothermal fluid flow contribute to the elongation of DNA [10]. The DNA molecules attached to the electrodes are stretched out as seen in the Fig 4.

Figure 5 shows the variation of stretched lengths of DNA molecule with applied voltage. The DNA stretched length corresponds to a DNA molecule that is immobilized on the electrode and stretched from the tip of the electrode. With increase in voltage, the electric field increases, resulting in an increase in torque and electrothermal force. The increase in forces result in elongation of the DNA molecules. After DNA has stretched to around 11.5 µm, the length remains constant, indicating that the DNA might be stretched to the full contour length. The actual length of the fully stretched DNA would be longer than measured because the attached end of DNA could be located a distance away from the electrode edge.

Fig. 4. λ-DNA immobilized and stretched using electrokinetics in a 100 µm wide and 20 µm deep Si channel. The applied ac voltage is 100 KHz sinusoidal and 10.8 V peak-to-peak.

Fig. 5. Variation of stretched lengths of a λ-DNA molecule due to applied ac fields. The length is measured from the tip of the electrode to the edge of the stretched DNA molecule.

V. CONCLUSION

We have demonstrated the fabrication of Si based microfluidic system integrated with electrodes and polymer structures. A low temperature PMMA bonding of Si channels to glass has been developed which allows us
to integrate electrodes and PMMA structures inside Si channels. The electrodes in the microchannel have been used for electrokinetic-based DNA stretching. DNA immobilization to electrodes and stretching have been demonstrated using high frequency ac electric fields. Maximum DNA stretched length of 11.5 µm has been obtained in microchannels that are 100 µm wide and 20 µm deep.

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