Limitations of DNA High-frequency Anchoring and Stretching

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ABSTRACT

This paper reports measurements that characterize the immobilization of 48 kilobase-pair lambda DNA onto lifted-off microelectrodes by high-voltage and high-frequency dielectrophoresis. Measurements of voltage- and frequency-dependent immobilization of DNA onto microelectrodes by dielectrophoresis show significant reduction in the response as the frequency increases from 200 kHz to 1 MHz or decreases from 200 kHz to 100 kHz and also as the electric field is lower or higher than 0.4 Vp-p/m. We found that the immobilization and elongation of the DNA molecules is restricted by the geometry of the gap, and that by decreasing the electrode gap size, the DNA molecules have less chance for both immobilization and stretching. The produced electrodes with both random microscopic peaks and modified smooth edges are utilized to show the effect of electrode edge roughness. The results imply that more DNA molecules can be immobilized by microelectrodes having rough edges.

Keywords: DNA manipulation, DNA stretching, high frequency measurements, gap geometry, edge roughness

1 INTRODUCTION

Manipulation of single biomolecules such as DNA, RNA, and proteins facilitated significant advances in biology [1-5]. Technological advances in nanotechnology and high resolution visualizing systems facilitate single-molecule manipulation. Through high-resolution fluorescence microscopy, real-time restriction [6] and replication [7] of a single DNA molecule has been observed, and moreover, the transport properties of a single DNA molecule were obtained [8].

DNA molecules can be immobilized by various means including electrostatic, hydrodynamic, or magnetic forces. Washizu used dielectrophoresis and electroosmotic flow to immobilize DNA molecules [9]. Stretching by hydrodynamic force was performed either with free molecular ends or with one end immobilized to a solid surface [10,11].

Besides Atomic Force Microscopy and Scanning Tunneling Microscopy, a variety of techniques has been used including electric, magnetic and optical traps allowing to move and position nano-scale objects and molecules [12-14]. We present here an electric field based technique for the orientation and positioning of DNA molecules on micro-electrode silicon devices. Moreover, we have studied the parameters affecting the dielectrophoretic immobilization of DNA and it's dependencies as a function of the surface, micro-electrode, and bulk solution properties. We have optimized those parameters and been able to achieve more constant DNA immobilization.

The double-stranded DNA molecule is a long macromolecule consisting of two strands of deoxyribonucleotides held together by hydrogen bonding. Under slightly basic conditions, the phosphate groups within the backbone deprotonize and the DNA molecule is negatively charged. This charge leads to the formation of a counterion cloud surrounding the molecule to an extension given by the Debye length. A DNA molecule has essentially no net permanent dipole moment since the two helices forming the double-strand DNA point in opposite directions. However, the counterion cloud can be displaced in the presence of an electric field and it is expected to strongly increase the polarizability of the molecule (ionic polarizability). The suspended molecule can then be treated as a dielectric medium of given volume and shape placed in a continuum solution of different dielectric properties [15]. Electric manipulations of DNA molecules in microfabricated structures based on the induced dipole moment of the molecules have been carried out since the last decade [13]. Such devices allow to work with relatively high applied electric potentials (in the MV/m range) using low voltage sources. Furthermore, due to the small size of the structures, sample cells with small volumes (down to a few pico liters) can be used. So far, a relatively high number of molecules was manipulated within such devices [13, 16 and 17].

2 MATERIALS AND METHODS

2.1 Device Fabrication

Electrodes were fabricated on a thin Silicon wafer (100 μm Thickness). Photoresist (OFPR-800) was spun (see Fig. (1)), patterned by channel mask layer, and developed

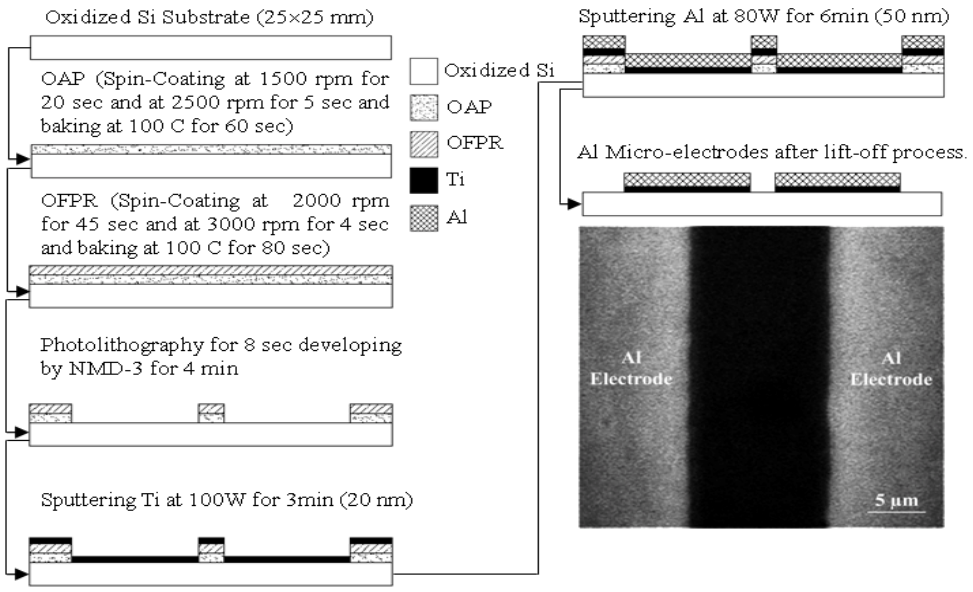

Fig.1 Schematic process of micro-fabricated electro-immobilization device.

in NMD-3 solution. Then, the 20 nm-thick titanium and 50 nm aluminum layers were deposited on silicon surface and were lifted-off by Acetone. The wafer was rinsed in DI water, dried by air, and diced into each pieces. Some wafers were annealed at 210o C for 45 min to improve the edge of electrode.

2.2 DNA Sample Preparation

Plain µ-phage DNA was prepared in this work. The DNA was labeled with fluorescent marker YOYO-1 at a dye-to-base-pair ratio of 1:5. DI water (pH 8, conductivity 2 µS/cm) was used as sample buffer. Labeled DNA molecules were mixed with DI buffer solution to make final concentration of DNA sample at 1 ng/µL.

2.3 Electric Field Application

Alternating current (ac) voltage was generated from a function generator (see Fig. (2)). The frequency was regulated from 1 Hz to 2.3 MHz, and the amplitude ranged from 2 to 25 Vp-p. Waveforms were monitored by an oscilloscope. The gradient of electric field around the electrode was estimated by electric field simulation (See Fig. (3)).

2.4 Fluorescence Microscope

A fluorescence microscope was used to observe the motion of single DNA molecules. A high-magnification X100 was used to get high resolution. The molecular behavior was visualized using a digital CCD camera. A 100-W mercury lamp with FITC filter was used as an excitation source.

3 DNA DIELECTROPHORETIC PRINCIPLES

In the presence of an electric field $\tilde{E}$, a DNA molecule in solution will bear an induced dipole moment given by $\tilde{p} = \alpha V \tilde{E}$ where α is the polarizability of the molecule per unit volume and V is the volume. The polarizability depends on the permittivities of the molecule and of the solution. The counterions cloud is expected to strongly enhance the polarizability of the molecule and the induced dipole moment will thus depend on the solution used and in particular on its ionic strength and pH. However, working with solutions containing a high concentration of ions would facilitate electrochemical reactions at the electrode-solution interface. Such a situation is detrimental to the experiment and can cause irreversible damages to the electrodes due to the relatively high fields applied. An advantage of the technique used here is that it allows working with ac electric fields in the frequency range of a few kHz up to a few MHz. Combining this with relatively low-conductivity buffers (typically below 100 µS/cm) allows to limit the voltage drop at the electrode-solution interface and helps reducing electrochemical reactions.

The manipulations of the molecules are based on the interaction of the induced dipole moment and the applied electric field. The translational motion of the molecules is caused by the application of a non-uniform electric field to the solution which is realized in our case by the smooth edged electrodes. In a non-uniform field, a neutral object of polarizability α and volume V will undergo a force proportional to the square of the applied field $\tilde{E}$ and given

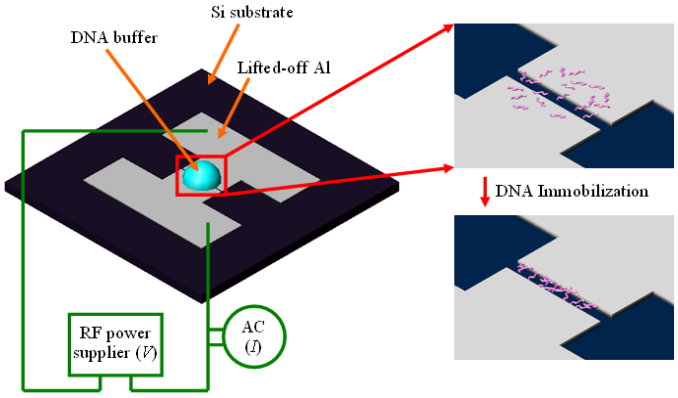

Fig.2 Electric field application. The frequency was regulated from 1 Hz to 2.3 MHz, and the amplitude ranged from 2 to 25 Vp-p

by $\widetilde{F}_d = 0.5\alpha V \widetilde{\nabla}|E|^2$. For a dielectric ellipsoid of volume $r^2 l$ (r: radius, l: length) and permittivity ε_2 immersed in a medium with permittivity ε_1, the force at equilibrium can be estimated by [8]

$$\widetilde{F}_d \approx r^2 l \varepsilon_2 \varepsilon_1 \widetilde{\nabla}|E|^2 \qquad (1)$$

as long as $\varepsilon_2 >> \varepsilon_1$ which is expected for DNA in an aqueous buffer. A rough estimate of the electric field value E_{th} necessary to overcome thermal fluctuations can be obtained by integrating Eq. (1) and comparing the result to $k_B T$. Since the DNA molecule is a long, flexible polymer, we will consider here the force exerted on one segment of the molecule. Taking the permittivity of water and using a radius of 1 nm for a λ-DNA molecule and a persistence length of 100 nm, we obtain $E_{th} < 10^7 V/m$ showing the necessity to use relatively high electric fields. In this particular case where the dielectric object is more polarizable than the medium, the force will be directed towards the highest intensity point of the electric field (positive dielectrophoresis).

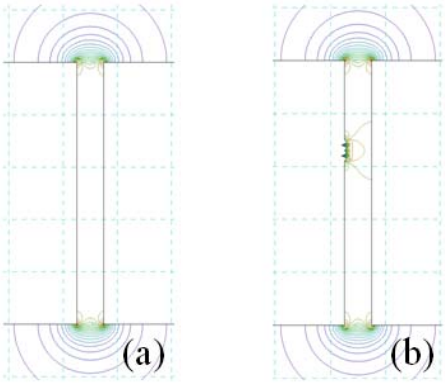

Fig.3 Simulation of the gradient of electric field around the electrodes (a) a smooth electrode edge and (b) a rough electrode edge.

The interaction of the induced dipole moment with the applied electric field will also cause electrically non-symmetrical molecules to sense a torque $\widetilde{T} = \widetilde{p} \times \widetilde{E}$. High aspect-ratio molecules will thus tend to align themselves with their longest axis parallel to the applied field. In the case of a long flexible molecule such as DNA, the orientation effect is expected to take place for every segment of the molecule, resulting in an uncoiling of the molecule to its full length.

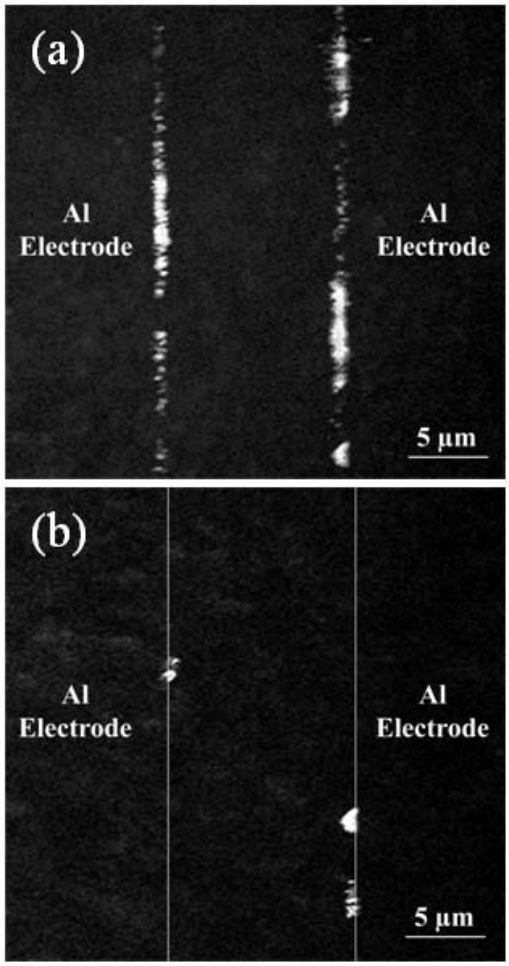

Fig.4 DNA immobilization using (a) a rough electrode edge and (b) a smooth electrode edge. Many molecules were attracted to the random peaks of the rough electrode edge while just a few number of DNAs were immobilized on electrode edges after annealing process.

4 RESULTS AND DISCUSSIONS

The device we constructed for DNA immobilization uses dielectrophoresis to capture the DNA molecules. By

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using an electric field generated by a straight electrode (high field gradient), we can control the movement as shown in Fig. (4). Generally, DNA molecules are attracted to the higher field gradient region at high frequency (~200 kHz). The directional movement of the molecules is frequency dependent in accordance with their dielectric properties. That is, the transition frequency at which the dielectrophoretic force reverses from positive to negative will depend on the molecule's dielectric properties. Molecules were repelled from high field gradient regions if frequencies higher than 700 kHz were used. DNA immobilization is a strong function of solution conditions such as polymer concentration, conductivity, and pH values. A low-conductivity, pH 8 buffer is optimal for DNA immobilization. Experiments were performed with solutions of pH 4, 8, and 10, but immobilization of DNA could only be obtained in the pH 8 solution, indicating that dielectrophoretic immobilization occurs best near physiological pH values. Low-conductivity solutions are also best for dielectrophoretic immobilization. High-conductivity solutions produce a large amount of Joule heating, and resulting molecular movement depends on the temperature increase in the fluid and not on the dielectric force. Moreover, a high level of bubble generation was observed that disrupted the immobilization process. Electrode edge smoothness plays an important role in attracting DNA molecules to the intended sharp area. Rough electrode edges provide many small points along the electrode's edge that generate high electric field gradients resulting in molecular movement. Fig. (4a) shows how DNA molecules are attracted to the rough edges of the electrode, while Fig. (4b) shows how the number of immobilized DNA molecules is reduced due to annealing process.

In conclusion, we have presented a silicon-based device allowing a satisfactory immobilization and capture of a relatively small number of DNA molecules in free-flow.

We expect that such devices will be useful for the handling of minute amounts of molecules in a variety of experiments involving DNA separation, amplification or detection. The further development of integrated systems such as biosensors or "lab-on-a-chip" devices for biochemical experiments requiring several steps will certainly make use of high intensity electric fields for an efficient handling of the molecules involved.

Moreover, the optimum conditions for robust and controllable DNA immobilization have been investigated. By optimizing the solutions conditions, electric field strength, and shape, and the surface properties, the success of DNA immobilization can approach 90%. Molecular manipulation by dielectrophoresis is a relatively common technique, but there are still unexplored topics in this area. Additional studies are needed to investigate other parameters such as temperature, surface property, and buffer ion dependence on stretching. With the design of more reliable micro-fabricated DNA immobilization and stretching devices, integrated systems may be possible that perform multiple analysis operations on a single DNA molecule extracted from a single cell or other minute samples.

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