NEW NANOFLUIDIC DEVICE TO ACHIEVE A LENGTH DEPENDENT 
MOBILITY OF LONG DNA MOLECULES AND A SEPARATION 
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ABSTRACT
To achieve the size-dependent behavior of the long DNA molecules, a novel concept, the electrophoresis under pressure gradient in nanoslit device, is proposed in this report. We investigate length-dependent mobility of chromosome-sized DNAs (YOYO-1 stained λ-DNA (48.5 kbp) and T4-DNA (166kbp) molecules) in this nanofluidic device. A fluidic device of nanoslit style is simply fabricated on silicon wafer with microfabrication technique. Then, the electrode for electrophoresis was patterned on the fluid access holes and the PEEK® tubes for hydrodynamic pressure was installed and connected with a high performance liquid chromatography (HPLC) pump. The electric potential and the hydrodynamic pressure were applied simultaneously, but with opposite direction. As a result, the different two kinds of DNA show the length-dependent behavior.

KEYWORDS
DNA separation, electrophoresis, pressure gradient

INTRODUCTION
In the last decades, separation fundamentals of DNA molecules have been investigated extensively in the gel electrophoresis way. The uniformly charged polymer such as DNA molecule moves with length-independent mobility in the electric field because the friction force is proportional to DNA contour length as well as the electrostatic force [1]. This size-independent migration prevents separation in free buffer solution, and thus the sieving matrix such as agarose gels should be used. However, the DNAs above a critical length (typically ~20,000 basepairs) show the length-independent electrophoretical mobility even in sieving matrix, because the long DNA molecule becomes highly oriented along the direction of electric field in the gels [2]. As a result, the pulse field gel electrophoresis is generally used for the long DNA separation, which is typically one-day process [3]. Considering the importance of rapid mapping and fingerprinting in large-scale DNA sequencing projects, it is highly required to develop a new technique to separate long double-stranded DNA molecules. Therefore, many enhanced nanotechniques have been developed for investigations of dynamics of biomolecules in nanofluidic environments, such as nanoslits [4-5], entropic traps [6], pillars [7], and pressure driven in nanofluidics [8]. But, the separation was possible still under a strong electric field and the suggested nanodevices are very challenging to fabricate. Therefore, for practical applications, such as chromosome sizing, a novel mechanism of separating much longer DNA is demanded.

In this report, we present a simple, low-cost and high-throughput fabrication method for the nanoscale channel and slit devices and show the length-dependent mobility of long DNA molecules.

NANOCHANNEL DEVICE
The shadow evaporation technique is used to achieve the relevant dimensions for single-molecule DNA. Moreover, novel structures with alternating shapes of micro-scale chambers linked to nano-scale trenches on a (100)-type silicon substrate were fabricated using anisotropic wet etching with KOH depending on the crystallographic surface. After device fabrication, DNA electrophoresis is carried out with a λ-DNA and T4-DNA mixture. We found that the shorter λ-DNA migrated with a larger velocity due to the conformation change from the constriction of the device. By using the concepts of “DNA deformation induced by the device structures” and “DNA electrophoresis under pressure gradient”, the long DNA shows the length-dependent mobility. The fabrication process is easier and the condition of device running is more moderate than several established methods, such as nanopillar or slit-well devices.

Fig.1 (a) A fluidic device with chambers connected by nanochannels. (b) A cartoon of a DNA molecules migrating in the device. (c) Fluorescent sequential images in which a DNA molecule is escaping the micro-chamber and stretched in the nanochannel. (d) Typical fluorescent frame images of the intermittent movement of T4 DNA in the nanochannels (10 μm scale bar in (c) and (d)).
As shown in Fig.1, we proposed a new fabrication method for nano channels with zig-zag shape using thermal shadow evaporation and wet anisotropic KOH etching. A single DNA molecule could be driven in the nanofluidic device by electrophoresis, in which the linear stretching and electrophoretic migration of a DNA polymer could be observed at a single molecule level. Based on the phenomena of repetitive DNA deformation and recoil in the fabricated fluidic device, the shorter λ-DNA moved faster than the longer T4-DNA. (Fig.2)

![Fig.2](image)

*Fig.2 The length-dependent mobility of long DNAs in zig-zag shape nanochannel. (a) T4-DNA, (b) λ-DNA. The electric field is ~1 V/cm*

**NANOCHANNEL / SLIT DEVICE WITH HYDRODYNAMIC DRIFT**

On the other hands, when the pressure gradient is applied with opposite direction to electrophoretic direction of DNAs, the long DNA shows the length-dependent mobility. In our experimental set-up, the drift of DNA molecules happens often, particularly at the beginning step of experiment. The device should be wet before electrophoresis, and thus the buffer solution is introduced prior to DNA injection. Although DNAs are typically injected after a few minutes from the device wetting, the natural drift is likely to happen due to the temporary height-level-difference between the fluidic reservoirs. Therefore, the drift of DNA solution tells us the existence of pressure gradient. Under the fluid drift caused by the imbalance of fluidic reservoirs, the length-dependent mobility is also observed. Fig.3 shows the length-dependent electrophoretic mobility under the hydrodynamic drift. As observed in Fig.4, the longer T4-DNA moves faster than the shorter λ-DNA, which the resolution can be enhanced much better than the entropic trapping dynamics shown in Fig.2.

![Fig.3](image)

*Fig. 3 The drift of DNA molecule in the nanochannel. The DNA goes back upwards as soon as the electric switch is off due to the hydrodynamic force caused by the fluid level difference between the reservoirs.*

![Fig.4](image)

*Fig. 4 (a) The sequential movie frames show the length dependent electrophoretic mobility under the fluid drift. The long T4-DNA moves faster than the small λ-DNA. The time gap between frames is ~ 90 ms, and the width of each frame is ~ 27 µm. The relative velocity between two DNAs is ~ 3.5. (b) The relationship between the velocity and the intensities.*
Based on this observation, we can manipulate the specific DNA in nanoslit device with the simultaneous effect of electric and non-electric forces. Takamura et al. [10] previously reported a similar case of size dependent mobility of DNA in electric and hydro drag force field at the conference of µTAS. They measured the migration velocities of DNA in taper-shape channels, where both the hydro pressure and the electric force were applied at the same time in mutually reverse directions. They found the large T4 DNA moves faster than the small λ-DNA. As shown in Fig.5, the electric potential and the hydrodynamic pressure were applied simultaneously, but with opposite direction in a nanoslit device, finally we can separate successfully different lengths of long DNAs.

![Fig. 5 DNA electrophoresis under pressure gradient in 200-nm high nanoslit.](image)

The bigger DNAs (T4 DNA ; the white circles) move along the direction of the electric field (move fast), while the smaller DNAs (λ-DNA ; the red rectangles) move oppositely, along the direction of the pressure gradient.

The scale bar is 20 µm and the time gap is ~ 90 ms. The electric field is 80 V/cm, and the pressure is 0.1Mpa.

CONCLUSION

This work represents new work in fabrication of a novel nanofluidic device for achieving a length-dependent mobility of long DNA molecules. Fluidic devices with alternating structures, wide chambers, and narrow trenches were fabricated using the shadow evaporation and anisotropic etching of a silicon substrate with KOH aqueous solution. The novel shape of the resulting nano-channels with micro-chambers resulted in a length-dependent electrophoretical mobility between long λ- and T4-DNA. This device represents a new experimental concept for separating single DNA molecules with a length-dependent dynamic mobility.

In order to investigate the length-dependent electrophoretical movement of DNA molecules, we fabricated a nano-fluidic device that led to DNA conformational change. In order to reach the relevant dimension for DNA size with typical lab-scale equipment, including conventional UV-lithography, shadow evaporation over a 500 nm high photoresist structure was performed, which resulted in 50–100 nm wide gap with rough edges. Using a zigzag pattern of lines oriented 90° to each other, a device with alternating structures of chambers and channels was fabricated by anisotropic etching in KOH solution. By adjusting some variables, the dimension of the device could be controlled. In the corner of the zigzag pattern, chambers with microw size were formed and lines were turned into narrow channels with a nanometer size. Based on the phenomena of repetitive DNA deformation and recoil in the fabricated fluidic device, the length-dependent electrophoretical mobility was investigated. We found that shorter λ-DNA moved faster than longer T4 DNA by ~ 5 µm/sec under an electric field of 5 V/cm. This length-dependence was quite different than the results for nano slit-well devices, where longer DNA moved faster than shorter DNA. Our result cannot be explained by the contact area concept used in the interpretation of slit-well devices. Otherwise, when the pressure gradient is applied with opposite direction to electrophoretic direction of DNAs, the longer DNA moved faster than the shorter one.

These nano fluidic devices may become an important tool for further biophysical and biochemical investigations of long DNA molecules as well as interactions with proteins. In particular, we hope to study the further effect of different ionic strength, temperature, other sieving effects [9] from polyvinylpyrrolidone (PVP) to prevent electro-osmotic flow, intrinsic curvature and flexibility, leading to the phenomena of DNA condensation with various smaller nano-confining devices.

REFERENCES


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