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Imaging Cross-section of DNA electrophoresis in a microfabricated glass device with CLSM

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Abstract—**In this paper, confocal laser scanning microscopy (CLSM) is used to detect and analyze DNA electrophoretic migration in a microchannel filled with in-situ polymerized cross-linked polyacrylamide. DNA distribution at the cross-section of the microchannel is observed to vary only slightly with the tested gel concentrations and the DNA sizes. However, in comparing with DNA electrophoresis using linear polymer solution (LPA), significant differences are observed in the distribution of DNA molecules on the observation plane using a cross-linked matrix (UV-polymerized polyacrylamide). Instead of a uniform signal boost upon the pass of one DNA band in linear polymer matrix, the electrophoretic process with cross-linked polyacrylamide presents a cross-section image with most DNA molecules aggregate near the vertical center of the microchannel during migration. This observation suggests an inhomogeneous nanostructure of in-situ polymerized cross-linked polyacrylamide in a microfabricated glass channel, consistent with SEM results. However, the phenomenon of DNA bands tending to focus in the vertical center along z-axis has not been previously reported. We also conclude from these observations that geometries containing fewer dead corners are desirable to minimize residual DNA in the microchannel.**

I. Introduction

DNA electrophoresis separation is an essential method for DNA analysis, which has been well investigated for decades, in both macro-scale (i.e. slab-gel) and micro-scale systems (i.e. capillary electrophoresis) to learn DNA 2-D electrophoretic behavior. Previous researchers have observed a wide variety of factors affecting 2-D DNA migration behavior, including electric field strength, ionic strength (e.g. buffer condition), temperature gradient, and so on. The motivation to further investigate DNA electrophoretic behavior, especially in a microchannel, comes from the high throughput/efficiency needs of bioanalysis process, which can be easily achieved by using microfabricated platforms. Numerous measures have been taken to improve the performance (i.e. sensitivity, resolution and reproducibility) of DNA electrophoretic separation in a microfabricated system mainly based on 2-D investigation from a macro-scale system [1], [2]. So far, DNA electrophoretic behavior in a 3-D network is not well investigated. A decent understanding of this area is important for both designing and optimizing components of micro systems for separation and detection, as the aspect ratio of microchannel cross-section approaches 1.

The mechanism of DNA electrophoresis was generally studied with a 2-D method (e.g. imaging the plane parallel to the migration direction), and three principle regimes have been proposed theoretically and confirmed by experiments in the past decade [3], [4]. According to reports of DNA electrophoresis in either a slab-gel or capillary system, the most important parameters are DNA size and gel pore size (for cross-linked gel) or tube size (for linear polymer solution), when the separation proceeds under the electric field strength lower than the threshold for DNA stretching. In a cross-linked gel, the electric force drives DNA molecules winding through a non-uniform 3-D network, which then induces a band distribution along the migration direction (e.g. dispersion) as a function of time. This distribution may also appear in the 3rd direction (e.g. distribution on the plane vertical to the migration direction) if the pore sizes change from the center to the inner wall-side. Also, the surface property of inner walls can cause the change in DNA distribution on the x-z plane (e.g. the plane perpendicular to the migration direction). For instance, when the electrophoresis occurs in a bare microchannel (glass) without inner wall coating, DNA migration behavior gets disturbed once the electroosmotic flow happens. This may also induce changes in band distribution at the cross-section. Besides the inner-wall surface condition, an inhomogeneous nanostructure of the sieving matrix, which can cause differentiation in DNA mobility, may eventually cause DNA band distributed in the direction with non-uniformity.

II. Experimental

A. Microfabricated DNA separation devices

Glass-to-glass DNA separation devices (Figure 1) were fabricated first by etching channels on a 500um thick 4-inch glass wafer with HF solution, using Cr/Au as mask. Then one diced piece with etched channel was UV-glued to a cover glass to assemble devices for electrophoresis using cross-linked polyacrylamide. Micro devices used for separation with linear gel solution were assembled after channel etched by wafer-to-wafer thermo-bonding at 640°C, in order to sustain the high loading pressure (~500psi for 6% LPA). Nanoport™ assemblies (Upchurch Scienctific) were bonded on the device over the buffer reservoir for monomer/polymer loading and buffer supply, as shown in

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Figure 1 (A). All the microchannels used in DNA electrophoresis with LPA get coated before loading the polymer solution following Hjertén's recipe [5].

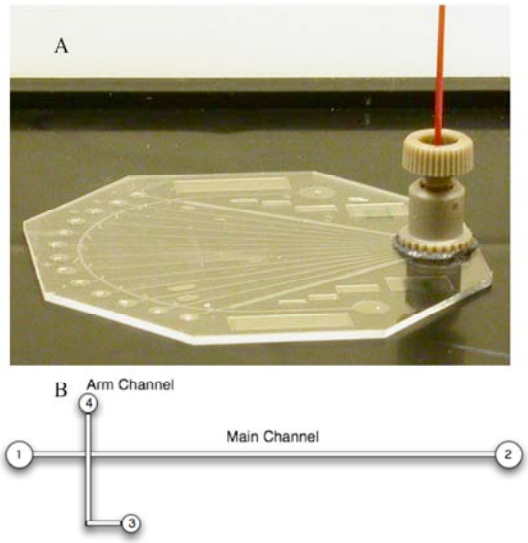

Figure 1: (A) one fully assembled glass-to-glass device with attached buffer tank and connections. (B) Sketch of the microchannel for electrophoresis, arm channel and main channel are denoted in the figure.Final Stage

B. Sieving Matrix Loading and DNA Electrophoresis

Cross-linked polyacrylamide gel was formed first by loading the mixture of acrylmaide monomer, initiator and buffer into the microchannel. The whole device was then exposed to UV light, which triggered polymerization with cross-linking. Gel with an interface at the T-section was formed by masking the injection region during UV exposure. Concentration of cross-linked gel was changed by varying the monomer volume ratio to the total mixture volume. Linear polymer solution (LPA 6%) was commercially purchased and loaded into the channel with a high-pressure loading system, using helium gas.

DNA electrophoresis was then performed in the microchannel with off-chip electrodes connected to a power supply. For sieving matrix without gel interface, including linear polymer and mask-formed cross-linked gel, DNA sample was injected into the T-section by applying 10V potential across the arm channel and then 150V potential over the separation channel for electrophoresis. Otherwise, DNA sample was injected into the gel interface by applying 150V over the separation channel for 10 seconds and the uninjected solution was quickly removed by aspirator followed with buffer rinse. Then 150V potential was applied for the separation across the main channel.

C. CLSM Detection

Cross-section images of microchannel during DNA electrophoresis are taken with CLSM by using xzt-scan mode.

III. Results and Discussion

Cross-section (x-z plane) images of DNA electrophoresis using LPA (6%) are obtained with CLSM both before and while the DNA band is passing through the observation plane (Figure 2). In comparison with the fluorescence intensity at the beginning (Figure 2A), there is an even signal boost all over the cross-section (Figure 2B) when DNA molecules reach the plane, and no significant gradient in distribution can be detected. This observation implies that there is no observable inhomogeneity of DNA distribution on the x-z plane, which is consistent with the characterization of a linear polymer sieving matrix. It also validates the assumption that electroosmotic flow is fully suppressed by coating the inner walls.

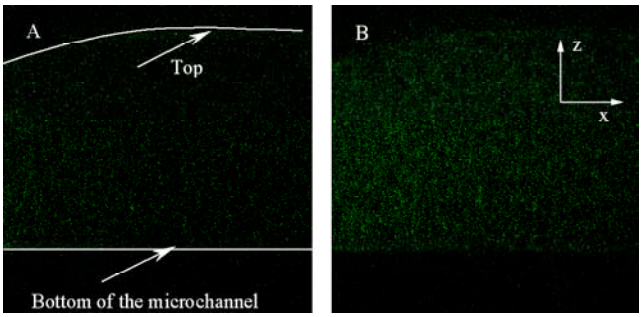

Figure 2: Image of DNA distribution at the cross-section (x-z plane) during electrophoresis within 6% LPA: (A) before one band passing b, (B) when a band passing.

Significant difference was then observed with DNA electrophoresis using cross-linked polyacrylamide. The images showing the distribution of DNA molecules at the channel cross-section only change slightly with respect to the gel concentration, but significantly with the gel formation condition (Figure 3A vs. 3B), e.g. the exposure time to UV-light, as well as the separation distance (Figure 4A vs. 4B). The most interesting finding is the center accumulation phenomenon that DNA molecules tend to migrate through the gel in the center of the channel instead of near the wall for most of the separation condition (Figure 3A). This phenomenon keeps consistent with different gel concentration when the UV exposure time is maintained at 600sec during gel formation.

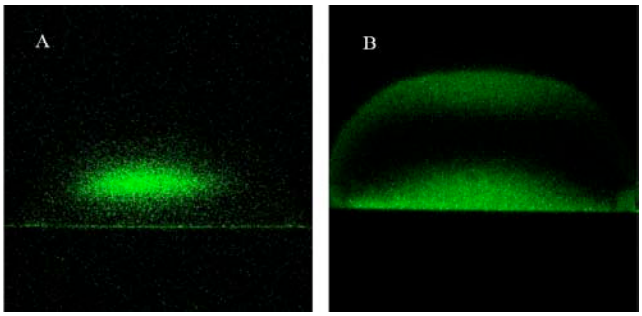

Figure 3: Cross-section mages of DNA electrophoresis process in a cross-linked polyacrylamide (10%T, 2.6%C) with changing UV exposure time. (A): Gel with UV exposure time of 600sec, (B) Gel with UV exposure time of 900sec.

The centering phenomenon also happens for those cases with an even initial distribution (at shorter distance). DNA molecules tend to aggregate back to the middle of the channel after they migrate further. This happens when we design one experiment to exclude the possibility of injection-induced distribution, which might cause the DNA molecules center at the beginning. In this test, direct electrophoresis without any injection steps is performed and DNA molecules are driven from the sample inlet straight into the gel. All the other processes and conditions are the same. In this case, we observed relatively even distribution in a shorter separation distance (~0.8cm) but a centered distribution after the DNA molecules migrating downstream (~2cm) as shown and compared in Figure 4.

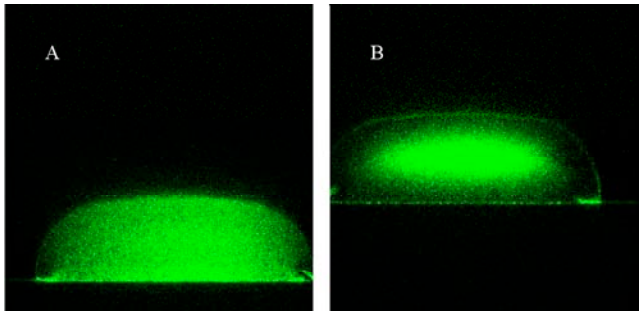

Figure 4: DNA electrophoresis in cross-linked gel without injection: (A) DNA distribution at the cross-section of the microchannel at a migration distance around 0.8cm. (B) Focused DNA distribution at the cross-section in the same run at a migration distance around 2cm.

Another observation with a cross-linked system is very useful in interpreting DNA electrophoretic behavior near an internal defect of the sieving matrix. In our previous research, we have noticed that the separation resolution is not significantly affected by sporadic air bubbles trapped/formed in the gel. By imaging the cross-section where the air bubble is located, we observe that DNA can migrate around the defect without being trapped. Furthermore, the band gets re-focused after it passes the defect (Figure 5). We have not yet understood the mechanism of how the DNA strands migrate around the air bubble but surely this can be considered one merit of a cross-linked separation system, in term of robustness.

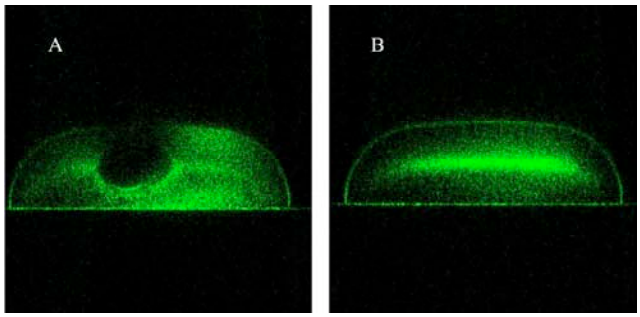

Figure 5: DNA molecules passing around a air bubble in the cross-linked polyacrylamide. (A) DNA distribution along the air bubble during electrophoresis. (B) Re-focused DNA band after passing the bubble.

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