ON-CHIP CONTINUOUS FLOW INTERACTION STUDIES OF DNA AND PROTEIN COMPLEXED DNA

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ABSTRACT (100 words)
The investigation of DNA-protein interactions is a central issue in functional genomics. Here we present a hybrid micro-nanofluidic device, which allows the continuous separation of protein-complexed DNA from native DNA strands via electrodeless dielectrophoresis (DEP) . The central element of the device is a 3D-structured microfluidic channel with a constriction that reduces the channel height to about 670 nm. At this barrier, selective dielectrophoretic forces allow the immediate, effective and continuous separation of DNA from DNA-protein complexes. As a proof-of-principle, we demonstrate the separation of DNA/protein as well as DNA/antibiotic complexes from uncomplexed DNA.

KEYWORDS: Continuous Separation, DNA, Protein, Dielectrophoresis

INTRODUCTION

Quantitative interaction studies of DNA and proteins are essential in functional proteomics research. Commonly, these studies are performed by batch processing for example by electrophoretic mobility shift assays (EMSA) or micro arrays. Here, we present a novel micro-nano-fluidic chip design (Figure 1), which allows the continuous processing and separation of DNA/protein complexes from uncomplexed DNA strands by dielectrophoresis (DEP). The continuous operation offers several advantages like the convenient harvesting of molecules and the implementations of further downstream processing steps [1].

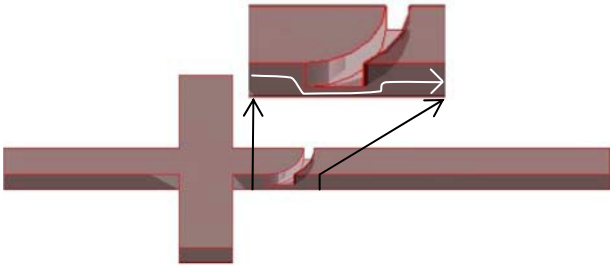

Figure 1: Sketch of 3D structured microfluidic channel with a constriction that reduces the channel height to 670 nm over a width of 200 µm with an resulting aspect ratio of 420:1. The inset is a detail of the bend constriction with indicated molecule trajectory.

In a first paper Regtmeier et al. successfully introduced dielectrophoretic separation of long DNA-fragments via electrodeless dielectrophoresis [2]. Using DEP at a bended constriction Hawkins et al. could continuously separate beads with diameter of more than 2 µm [3]. Kawabata et al. demonstrate manipulation of DNA-complexes by DEP using microelectrodes, field strengths as high as 1 MV/m were necessary for separation [4]. Based thereon, we designed a 3D microstructure fluidic chip, that consists of a cross injector and a bended constriction reducing the flow through height to about 670 nm over a width of 200 µm (Figure 1), yielding an aspect ratio of 420:1 that was realized with soft lithography.

In the following the fabrication procedure and first results of continuous flow separation of DNA and DNA/-protein complexes based on electrodeless dielectrophoresis will be presented.

THEORY

Dielectrophoresis is a molecular migration phenomenon occurring for a polarizable particle in an inhomogeneous electric field. In this work the necessary field is generated by applying a sinusoidal alternating voltage (AC) to a microfluidic channel with an insulating constriction. The resulting dielectrophoretic potential can be written as

$$W_{DEP} = \frac{1}{2}\alpha \vec{E}^2 \qquad (1)$$

α and $\vec{E}$ denote polarizability and electric field respectively. So for molecules with different polarizabilities the variable dielectrophoretic potential can be used for separation.

EXPERIMENTAL

We used dedicated soft lithography to build the microfluidic device with an extreme aspect ratio. First, two layers of SU8-photoresist were exposed and developed to create the required masterwafer. Pure poly(dimethylsiloxane) (Sylgard 184) failed to successfully replicate the masterwafer with the high aspect ratio, i.e. the nanoslit collapsed. To stably mold the constriction we used a double-layer of hard poly(dimethylsiloxane) (h-PDMS) and poly(dimethylsiloxane) [5].

For fluorescence video microscopy the DNA was labeled with YOYO-1 (~1 intercalator per 10 base pairs). To generate the dielectrophoretic potential well at the constriction we applied alternating voltages (AC). Floating injection was used to inject a narrow band of molecules towards the constriction by applying direct voltages (DC) at electrodes 1,2 and 3, whereas electrode 4 was grounded (Figure 2).

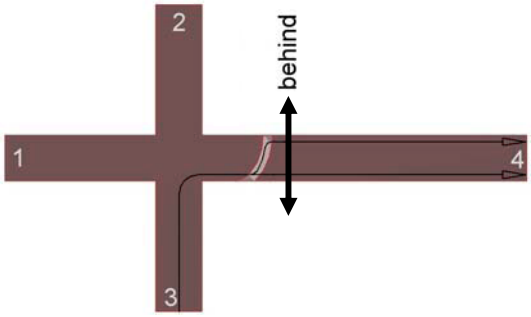

Figure 2: Top view representation of the device with different analyte trajectories (thin black arrows). A narrow band of molecules is led to the constriction by U_{DC} applied at reservoirs 1,2 and 3. Reservoir 4 is grounded. U_{AC} is applied between reservoir 1 and 4 to generate the dielectrophoretic potential well at the constriction. Behind the constriction, two separated bands of molecules can be detected. The thick black arrow indicates the pathway of fluorescent scanning during experiments leading to the fluorescence intensity plots (Figure 4).

For detection, the channel was scanned during the separation experiments (Figure 2 and Figure 3). DNA strands with 3000 and 6000 base pairs (bp) were used and *Escherichia coli* core RNA polymerase was complexed to 6 kbp DNA (1 polymerase per 125 bp).

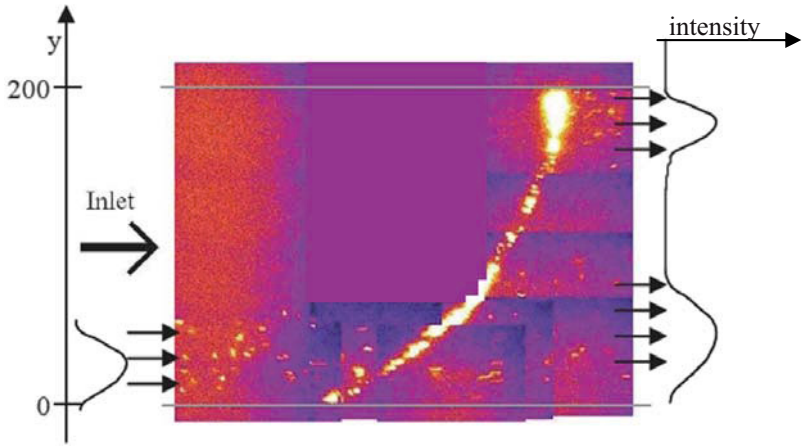

Figure 3 : Photocollage of images of the constriction during separation experiment with YOYO-1 labeled 3 kbp and 6 kbp DNA (~1 intercalator per 10 bp). The analyte is led as a narrow band to the constriction from the left. Behind the constriction, two bands of separated analyte molecules can be identified.

RESULTS AND DISCUSSION

As a proof of concept, we first separated 3 kbp from 6 kbp DNA fragments (Figure 3) and obtained a separation resolution of 1.19. In the next step, a mixture of complexed and uncomplexed DNA was tested. With a baseline separated resolution of 2.4, deflection of the DNA/protein complexes could be demonstrated, whereas the uncomplexed DNA passed the constriction unhindered (Figure 4).

The fluorescence intensity was plotted versus the position and fitted with a gaussian function. The peaks were identified by precedent measurements with single sorts of molecules under the conditions of the successful separation.

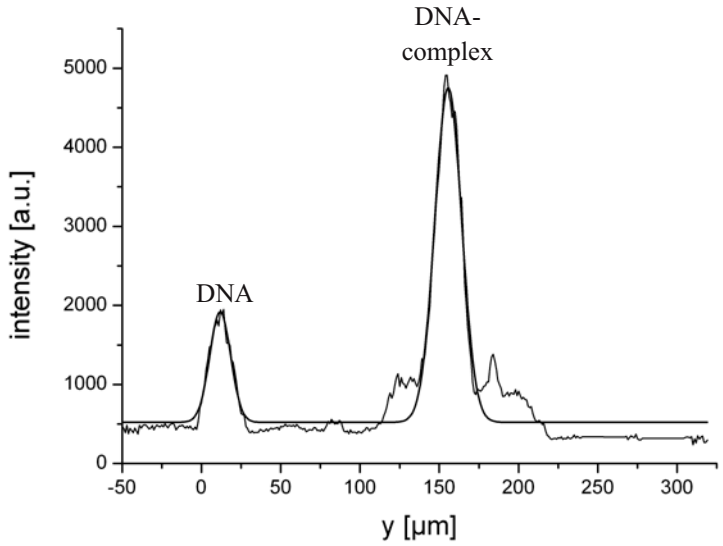

Figure 4 : Separation of uncomplexed 6 kbp DNA from DNA/protein complex. Plot of fluorescence intensity versus y-position in the microfluidic channel behind the constriction. The exact x-position is depicted in fig. 2. The two peaks can be assigned to the 6 kbp DNA and the DNA complex.

CONCLUSION

We have demonstrated for the first time a continuous flow separation of DNA and DNA/protein complexes with electrodeless dielectrophoresis in microfluidic chip format. This proves the high sensitivity of DEP for interaction studies with DNA. With this technique, continuous flow interaction studies become realizable which allow the immediate control and a further processing of the separated fractions downstream in Lab-on-a-chip devices.

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