DIELECTROPHORESIS AT A NANOSLIT: CONTINUOUS-FLOW SEPARATION AND ANALYSIS OF GENE VACCINES

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ABSTRACT

Minicircle DNA is very promising in the field of gene vaccination [1]. Along production, three DNA species appear that must be fully separated without damaging the DNA. Dielectrophoresis is well known for its non-invasive use and application in bio-separation techniques. Here, we present a versatile nano-microfluidic device that is capable to continuous-flow separation and analysis of the fabrication products of minicircle DNA exploiting dielectrophoresis. Several enhancement iterations were performed for best separation efficiency, fast response time, and baseline-separated resolution. Finally, the DNA products could be investigated according to the efficiency and purity of the production process within two minutes.

KEYWORDS: Dielectrophoresis, DNA, continuous separation, gene vaccines

INTRODUCTION

Dielectrophoresis (DEP) is known as a non-invasive method for investigation, manipulation and separation of biological probes [2]. In previous works, we have already demonstrated successful continuous-flow separation of two linear DNA species at an insulating ridge [3].

Here, we present an enhanced hybrid nano-microfluidic device that is capable to continuous-flow separation and analysis of the complete fabrication products of gene vaccines -circular DNA- by insulator dielectrophoresis. Several enhancement iterations were performed, including the general device layout for separation of more than two species and the enhancement of separation resolution and efficiency.

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 consisting insulating ridges (Figure 1). The resulting dielectrophoretic trapping potential can be written as [2]

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

with α and E polarizability and electric field, respectively. Thus, for molecules with different polarizabilities the dielectrophoretic potential can be used for label-free separation (Figure 1b).

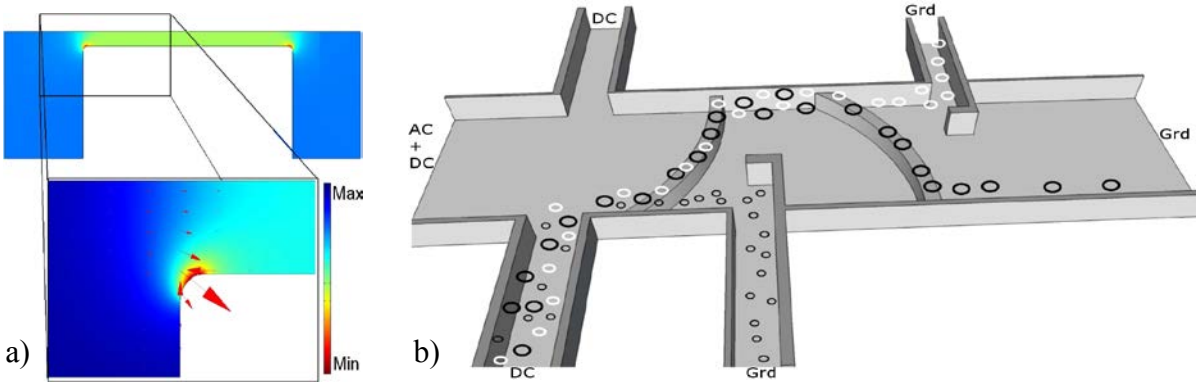

Figure 1: a) Simulation of DEP potential at insulating ridge. b) Sketch of device layout and separation principle. A DNA mixture (circles) was led towards the ridges. Due to different DEP potentials of the DNA species size dependent trajectories appeared at the ridges and resulted iterative separation of the three species.

EXPERIMENTAL

The device consisted of a cross-injector, a main channel that split into three channels, and two arc-shaped insulating ridges that spanned the microchannels laterally (Figure 1b). The channel heights were reduced to 500 nm at the ridges. Thus, a strong gradient appeared in the vicinity of the nanoslit when an electric field was applied. Floating injection was used to induce a narrow band of DNA molecules towards the constriction by superimposed direct voltages.

The device was custom made by PDMS soft lithography [4], which allowed fast and easy adaption of the channel design. Though, the DNA molecules were fluorescent labeled that was only to enable detection via fluorescence video microscopy and not necessary for the separating mechanism itself.

RESULTS AND DISCUSSION

The ratio of the dielectrophoretic potentials at the two ridges depended on the channel geometries and was calculated beforehand. The ratio of first to second potential was 4:1. Therefore, the three DNA species -parental, miniplasmid, and minicircle DNA – could be separated iteratively (Figure 2).

At the first ridge the larger ones, miniplasmid and parental plasmid, were trapped dielectrophoretically in the nanoslit and migrated along the slit towards the opposite channel wall where it escaped. In contrast, the smaller minicircle DNA passed the slit unaffected. The DEP potential was lower at the second ridge due to the channel geometries. Thus, only the larger parental DNA was deflected whereas the smaller miniplasmid DNA passed the slit unaffected and migrated towards a separate outlet.

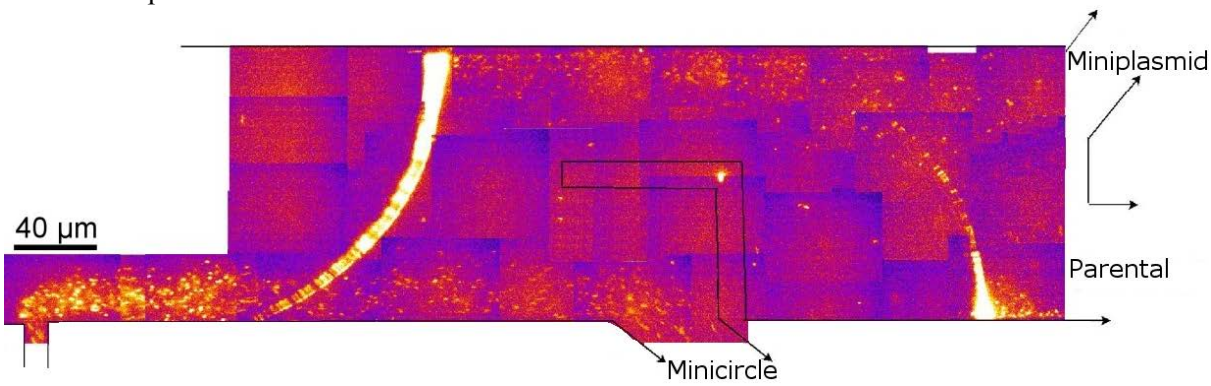

Figure 2: Collage of fluorescence video microscopy images (channel was scanned during experiment). Each yellow dot is one single YOYO-1 labeled DNA molecule.

CONCLUSION

In summary, we were able to design and produce a hybrid nano-microfluidic device with which dielectrophoresis based continuous-flow separation of three circular DNA species was performed with high separation resolution and efficiency. With this approach, continuous-flow production control of gene vaccines became realizable, which allows the immediate control and further processing of the separated fractions downstream in Lab-on-a-chip devices as well as harvesting of the samples.

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