DNA PURIFICATION IN CONTINUOUS NANOLITER FLOWS USING FLOW-INDUCED ELECTROKINETIC TRAPPING: INFLUENCE OF IONIC STRENGTH AND CHIP CONDITIONING

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ABSTRACT

Flow-induced electrokinetic trapping (FIET) is a continuous-flow phenomenon that involves the capture of particles in a recirculating flow pattern that arises from opposing pressure-driven and electro-osmotic flow in a non-uniform channel. Since particles are charged, they also exhibit electrophoresis in an applied electric field. Particle electrophoretic velocity, which is particle-specific, thus also plays a major role in the FIET process. DNA can be very simply trapped and preconcentrated using FIET, which makes FIET attractive for lab-chip microsystems for genetic analysis. With this in mind, the influence of ionic strength (IS) and channel conditioning on FIET of DNA were investigated in this study. At higher IS, the potential needed for trapping was observed to increase significantly, which may require the integration of an additional dilution step into an actual genetic analysis device employing FIET. A more extensive protocol for conditioning the channel yielded a stronger and more stable electro-osmotic flow.

KEYWORDS: Electro-osmosis; Recirculating flow; DNA preconcentration; Ionic strength; Chip conditioning

INTRODUCTION

This paper investigates the use of chip-based Flow-Induced Electrokinetic Trapping (FIET) [1] to preconcentrate DNA in a continuous nL/min flow, and how ionic strength (IS) and channel conditioning influence this. To date, DNA purification/enrichment has generally required solid-phase extraction of DNA. Our approach is unique in that no solid phase is required, with DNA captured instead in a stationary zone in a recirculating flow pattern. Possible applications include the purification of DNA from complex samples such as cell lysates for integrated microfluidic DNA analysis. To this end, the dependence of FIET on sample ionic strength (IS) and channel conditioning is investigated in this study.

THEORY

FIET depends on opposing electro-osmotic (EOF) and pressure-driven flows (PF) in a narrow microchannel to generate controlled bidirectional flow. Widening the channel at both ends yields a recirculating flow pattern, in which polymer microspheres [1] and biological particles (yeast, red blood cells, DNA) [2] can be trapped and concentrated (Fig. 1). As all particles are charged to some extent, particles also undergo electrophoresis (EP), affecting the flow conditions under which trapping occurs.

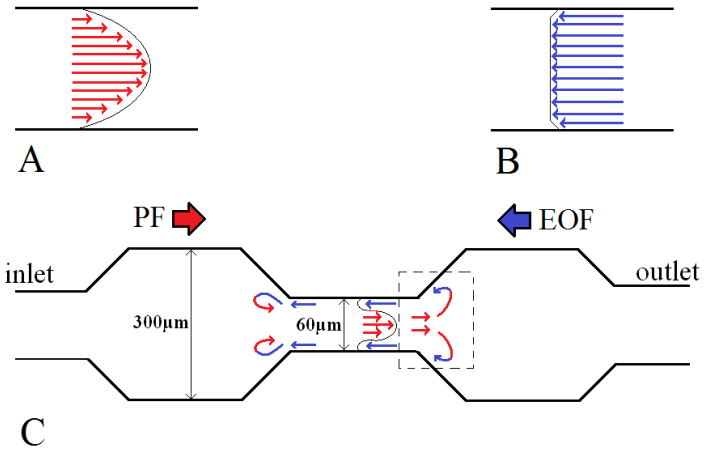

Figure 1: Viewed from above, flow profiles are shown of (A) pressure-driven flow, (B) electro-osmotic flow and (C) how they combine into a recirculating pattern in a diverging geometry. C also schematically shows the channel design. The region within the dashed box is where photographs were taken in FIET experiments.

Electro-osmotic flow and DNA electrophoresis are both dependent on the ionic strength of the buffer solution. EOF velocity is given in Equation 1.

$$V_{EOF} = E\frac{\varepsilon_0 \cdot \varepsilon \cdot \zeta_w}{4 \cdot \eta \cdot \pi} \qquad (1)$$

where ε_0 is vacuum permittivity, ε the relative permittivity of the solution, η the viscosity of the solution and ζ_w the zeta potential of the channel wall. When the ionic strength of a solution increases, the Debye length, which is the width of the electrical double layer at the channel walls, decreases, which in turn translates into a decrease of ζ_w. The permittivity of a solution also decreases with an increase in IS. Both of these decreases lead to a decrease in EOF velocity, as can be seen from Equation 1. DNA electrophoresis also decreases in a non-linear fashion with an increase of ionic strength [3]. DNA is not a spherical particle and can be present in multiple conformations. Therefore, DNA electrophoretic mobility and velocity are variable in our system and difficult to predict as a result. Since the EP and EOF components of DNA transport are also in opposite directions (EP towards the outlet, EOF towards the inlet), predictions of the overall effect of a change in IS are far from straightforward.

EXPERIMENTAL

The fabrication of the microchannels (20 μm deep) is described elsewhere [1]. Before use, the glass microchannels were conditioned with 0.1 M NaOH and TE buffer solution (10 mM Tris/ 1 mM EDTA / pH = 8.0) for 10 minutes each [1]. A solution of 48 kbp λ-DNA (0.3ng/μl) labelled with YOYO-1 in TE buffer was used; IS was increased as needed through addition of NaCl. DNA trapping was imaged with an inverted fluorescence microscope.

A second protocol for conditioning the channels was also used. In this case, channels were conditioned for 60 minutes with 0.1 M NaOH, followed by 10 minutes with native TE buffer solution.

A constant PF was generated by a difference in fluid height of 36.5 mm between the inlet and outlet reservoirs. EOF was generated by connecting platinum wires in the inlet and outlet to a power supply. Photographs of FIET processes were taken with an acquisition time of 61s each, with approximately five minutes between photographs. Between experiments, no potential was applied for 10 minutes.

RESULTS AND DISCUSSION

Figure 2 shows trapping for applied potentials of 200 V to 900 V for λ-DNA in TE buffer with (B) and without (A) 50 mM NaCl. These images show that higher potentials are required to obtain trapping at higher values of IS.

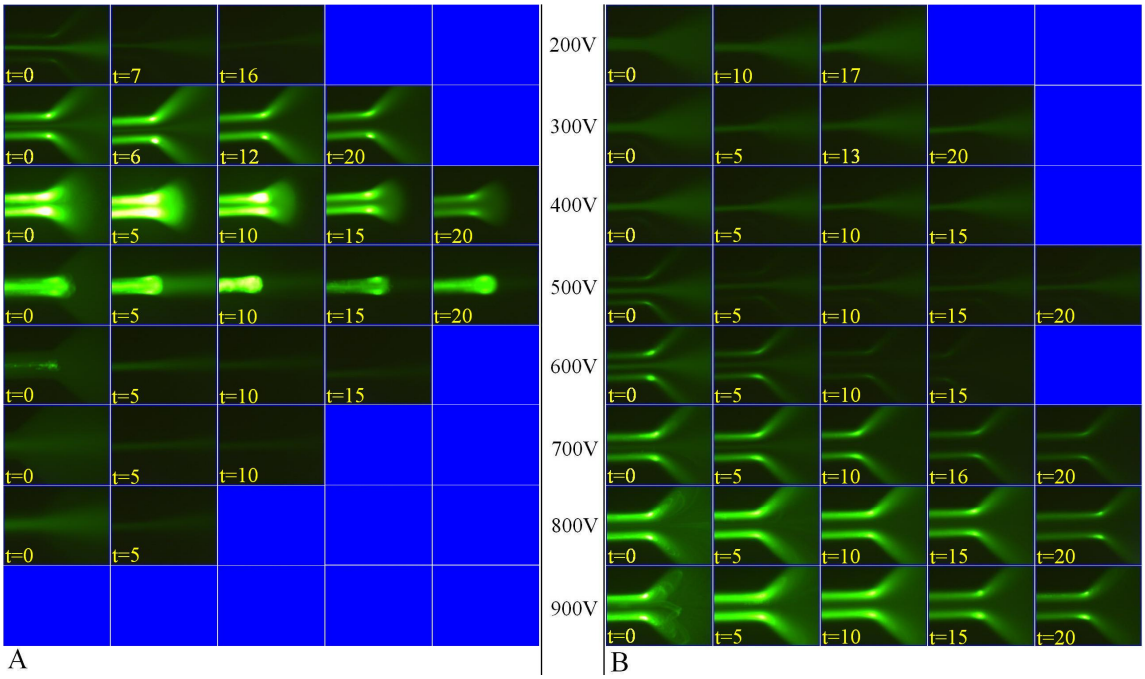

Figure 2: Trapping of 48 kbp lambda DNA (0.3 ng/μL) labelled with YOYO-1 dye. The time (minutes) at which image acquisition was started is given in each photograph. In A, TE buffer was used. In B, TE buffer with 50mM NaCl was used. When buffer with higher ionic strength is used, higher potentials are needed to obtain trapping.

In the zones where fluorescence is most intense (white), aggregation of DNA is observed under the microscope. This is unexpected, as DNA is negatively charged and molecules should repel each other. Our hypothesis is that aggregation is entropy-driven, due perhaps to a rearrangement of bound ions and/or water molecules as DNA molecules are forced to interact at the high concentrations [4]. The loss in intensity over time is believed to be caused by photocleavage, as the dye forms radicals upon longer UV exposure, which cleave the DNA strands.

As experiments showed variability, extended conditioning procedures were tried. When the NaOH step was increased to 60 minutes, lower potentials were required to obtain trapping, as shown in Figure 3. Figure 3 also demonstrates that a stable system was obtained in this channel, as trapping patterns at a fixed potential did not change notably during the entire experiment. When variation was observed in other experiments, the initial trapping pattern could be recovered by re-conditioning the channel with NaOH solution and TE buffer for 10 minutes each.

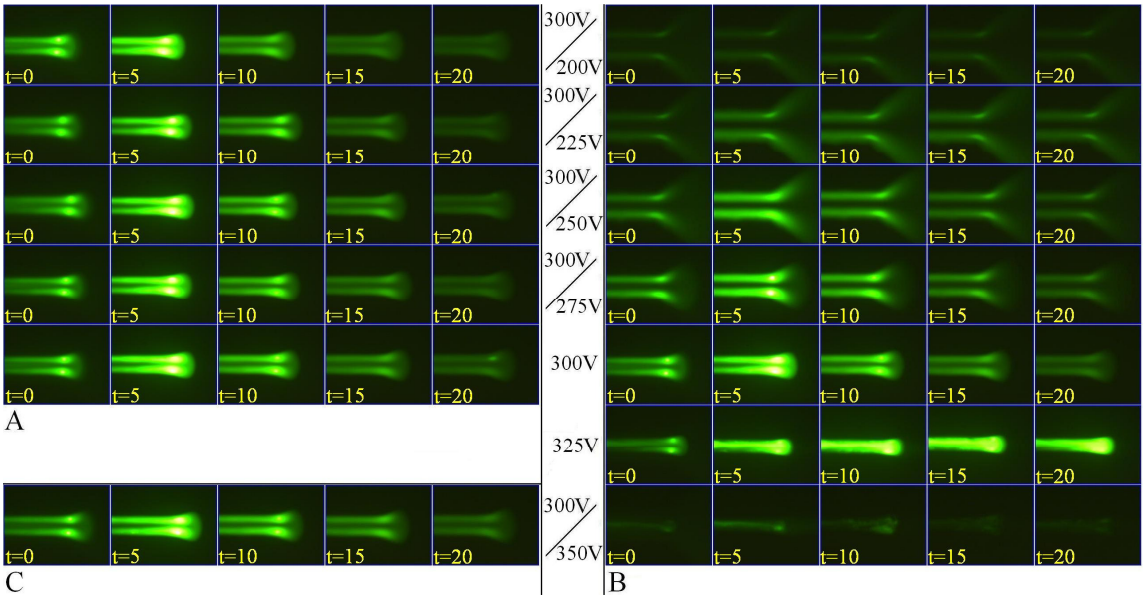

Figure 3: Trapping of 48 kbp lambda DNA (0.3 ng/µL) labelled with YOYO-1. Native TE buffer was used. The channel was conditioned with 0.1 M NaOH solution and TE buffer solution for 60 and 10 minutes, respectively. Trapping was performed consecutively in the same channel at (A) 300V, (B) 200V to 350V and (C) again 300V. The time (minutes) at which image acquisition was started is given in each photograph. After extended microchannel conditioning, lower potentials were required for trapping. The trapping pattern at fixed potential remained the same during the entire experiment.

CONCLUSION

An increase in ionic strength of the buffer requires an increase in potential to compensate for lower EOF and DNA EP. Lower values of ionic strength are thus favourable for microfluidic DNA purification, and integration of an on-chip dilution step may be required if FIET is to be used for genetic analysis. Extending the procedure for microchannel conditioning with NaOH solution provides better stability. Reproducibility is best ensured when the channel is re-conditioned between experiments. Future experiments will include investigation of DNA aggregation and purification from cell lysate.

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