ON-DEMAND ELECTROPHORETIC SEPARATION OF DNA IN WRITTEN GEL LINES ON PLANAR SUBSTRATES

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ABSTRACT

We report a new approach to perform on-demand electrophoretic separation of DNA. In contrast to standard chip-based capillary electrophoresis in micromachined glass chips, we apply a planar polyimide substrate, write 200 µm wide gel lines bridging two Pt-electrodes and inject 500 pl sample volumes in non-contact manner. The gel is covered with mineral oil to inhibit evaporation. Subsequently, an electrical field is applied for 80 s and the separation of the DNA molecules (56 bp-Cy5 and 112 bp-Cy5, 10 µM) is successfully demonstrated.

KEYWORDS

Electrophoresis; open microfluidics; DNA separation; light induced fluorescence detection.

INTRODUCTION

Conventional Capillary Electrophoresis

Electrophoretic separation of DNA by size in gel is one of the most important techniques used in molecular biology up-to-date. Microfabricated glass chips are widely used for this purpose. Typical chips contain 50*50 µm² capillaries etched in glass and a standard T-structure to extract a small amount of the reagent to be separated *via* electrophoresis. Due to mass fabrication, there is no flexibility in design and operation time, price per run and reagent dead volume can be rather high. Several works have been focused on the rapid-prototyping of electrophoresis chips in PDMS or plastics [1, 2] in order to overcome these difficulties or even propose new alternatives like performing the separation of ions on a suspended thread [3].

Open Microfluidics

Open microfluidics is a rather novel field with aims at creating chips and fluidic networks without any hardcover by printing or writing the fluid directly onto a substrate. Main characteristics is therefore the existence of a free liquid/liquid or liquid/gas interface [4]. This way, extensive work in cleanroom facility can be avoided. During the last years several new applications like 3D prototyping of hydrogels or inks [5], rapid fabrication of PDMS chips [6] or western blotting [7] have been published. The flexibility of such a technology in association with a dramatic reduction of time-to-chip makes this approach a highly promising method to improve microfluidic-based operations, and in particular separation techniques like capillary electrophoresis.

EXPERIMENTAL SECTION

Approach

A line of polydimethylacrylamide gel is written on a substrate to function as a separation matrix between two electrodes sputtered on the substrate. The DNA sample is injected with a non-contact dispenser inside the gel at a known position avoiding the integration of a T-structure to inject a small amount of reagent. Figure 1 illustrates this approach.

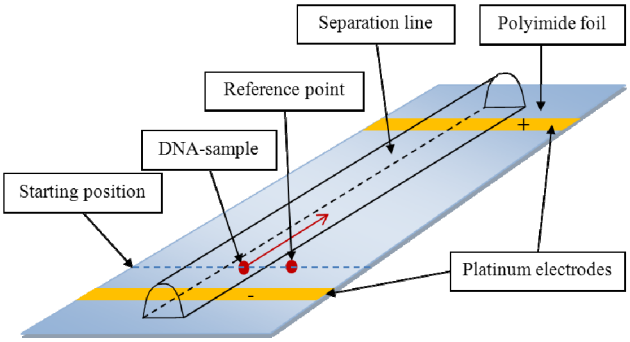

Figure 1: 3D-model of a written gel line. The second DNA spot is used as a reference to evaluate migration distances. DNA is injected near the negative electrode. The arrow points in the direction of DNA strand movement.

An electrical field is then applied between the two electrodes and the separation of the DNA strands occurs inside the printed matrix line. In order to avoid any effect from evaporation and to dissipate heat induced by the Joule effect an oil cover is dispensed onto the gel.

As the creation of liquid lines by droplet printing is prone to the emergence of a spherical shape boundary [8] (fig 2-a), we utilize a semi-contact method [9] (fig 2-b). Contrary to other printing methods using syringe pumps to drive the fluid, we only rely on capillary and hydrostatic force for liquid transport onto the surface.

Experimental setup

The substrate used is a polyimide foil with sputtered platinum electrodes (figure 3) exhibiting a pitch of 15 mm.

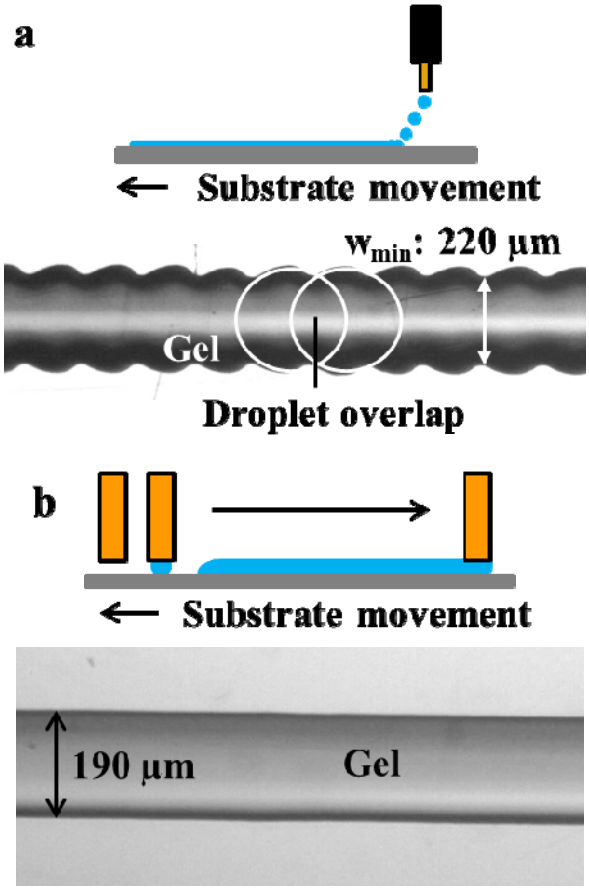

Figure 2: Two different methods of fluidic line generation on a substrate a) ejection of droplets in a non-contact manner (single droplet volume ~ 7 nL) b) direct-writing via *capillary bridge between nozzle and substrate.*

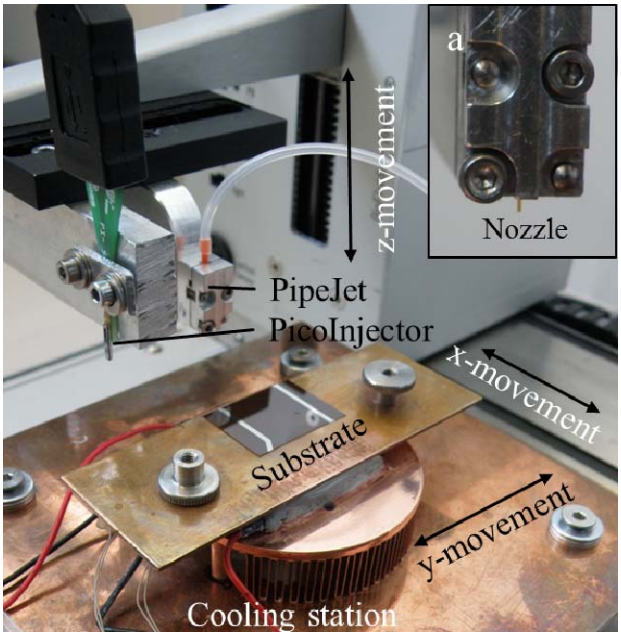

Figure 3: 3-axis robot with cooling station, mounted PI-substrate with sputtered Pt-electrode and the two needed dispensers. a) Detailed view on the PipeJet P9 (BioFluidix GmbH) nozzle and tube used for semi-contact writing.

To write the line and inject DNA samples, we use the 3-axis robot with mounted dispensers depicted in figure 3. The gel is dispensed and written by a PipeJet P9 dispenser (BioFluidix GmbH) [10] equipped with a 200 µm inner diameter tube. The DNA sample to be analyzed is loaded into a pL dispenser (Picoinjector, IMTEK [11]) which delivers sample aliquots in 10 pl droplets by non-contact dispensing.

The substrate is placed onto a Peltier cooler which regulates the temperature to 10°C (2°C above the dew point in laboratory environment) during the printing process thus reducing evaporation of the gel solvent.

Writing of gel lines

The PipeJet dispenser (P9, BioFluidix) is primed with a solution of polydimethylacrylamide dissolved in a tris-tricine buffer which is dedicated to the separation of DNA molecules between 25 and 500 base pairs (bp). By approaching the surface of the polyimide foil close to proximity, a capillary bridge is established between the nozzle of the PipeJet dispenser and the substrate (figure 4-a). By moving the substrate at a controlled speed, a fluid line is then written on the substrate (figure 4-b). The line dimensions are dependent on the speed of the displacement - in our case the width was slightly smaller than the tube inner diameter (193.2 µm +/- 8.2 µm compared to 200 µm).

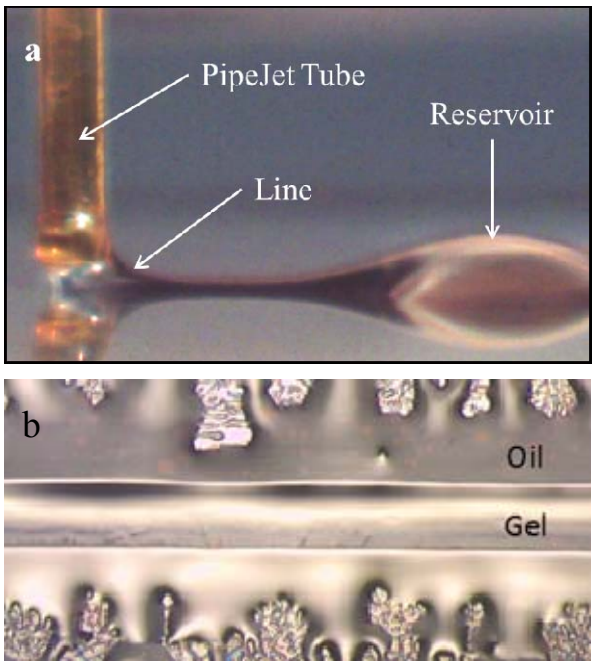

Figure 4: a) Reservoir and capillary bridge between dispenser tube and polyimide substrate. b) 190 µm wide directly written gel line with mineral oil cover.

The measurement of the matrix gel contact angle on the substrate as well as the electric resistance of the fluid line has allowed us to compute the theoretical height of the written line to be approximately 20 µm at the center.

As shown on figure 4-a, gel reservoirs (around 1.5 µL each) were printed at the respective ends of the line directly on the electrodes to avoid any charge carrier

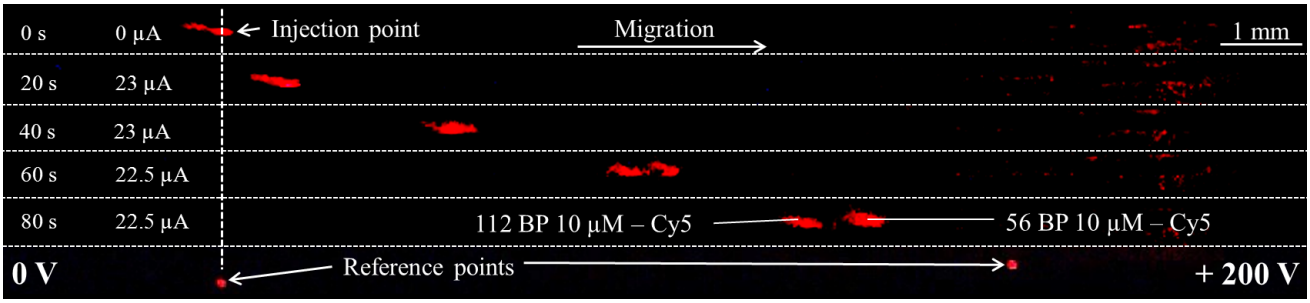

Figure 5: Live observation of the DNA strand separation (56 and 112 bp, labeled with Cy5). Injected volume was 500 pl at 10 µM. Electrical current measured in the line is indicated for each timestamp. The electrical field applied amounts to 133 V/cm. Total migration time equals 80 seconds.

depletion during electrophoretic separation. In the absence of these reservoirs, the electrical resistance of the line is not constant over more than a few seconds.

Electrophoretic separation

After line writing, a volume of 500 pl (50 droplets) of artificial DNA molecules fluorescently labeled with Cy5 (biomers.net) is injected near the negative electrode (figures 1 and 5). A mineral oil cover (Qiagen) is manually dispensed on top of the line to prevent evaporation and to increase heat transfer with ambient air (figure 4-b). The substrate is then positioned on a high-voltage supply and an electrical field of 133 V/cm is then applied between the two Pt-electrodes.

The separation of the DNA fragments is observed live with a setup comprising a microscope (Leica MZ12.5), a diode laser (635 nm, Lasiris, StockerYale Inc.) with laser line optics, a sensitive CCD camera (Zeiss Axiocam MRC) and a band-pass filter (Semrock, 670 +/-20 nm). The laser illumination spot covers the complete gel line allowing the observation of DNA strand migration from the injection point to the positive electrode. The electrical current is also monitored and gives valuable information on the integrity and stability of the gel line during the process.

The total process from prototyping the chip to observing the separation takes less than 5 minutes. Figure 5, 6 and 7 illustrate typical results showing the separated bands of two different DNA molecules (56 bp-Cy5 and 112 bp-Cy5, 10 µM).

Results

Figure 5 shows the separation of two differently sized DNA strands during the process at different timestamps whereas the two strands can be clearly distinguished after migration. The displacement observed in the first 20 seconds is slower than afterwards because the electrical field is not fully established. After that, the current stabilizes at 23 µA and the electrophoretic separation takes place. After 60 seconds, the two different fragments are visible and after 80 seconds (and 8 mm in the line) we obtain two clear peaks (figure 6).

From the live migration of the DNA strands inside our gel line, we can calculate the migration velocities for both the strands. Figure 7 shows the velocity dependence of each DNA strand size in comparison to the electrical field. As expected, the velocity is proportional to the electrical field strength as nevertheless, the ratio between the two velocities remains approximately the same (0.86 and 0.88). This is in a good agreement with the electrophoretic theory which stipulates that this ratio should be independent of the electrical field strength.

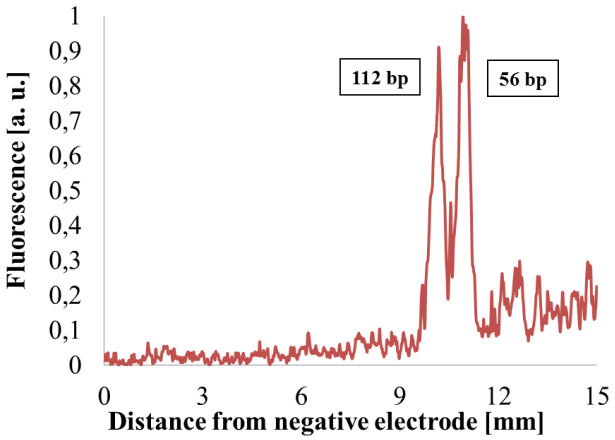

Figure 6: Migration distance after 80 s for the two DNA fragments. The left peak is the 112 bp-Cy5 fragment moving slower than the right peak representing 56 bp-Cy5.

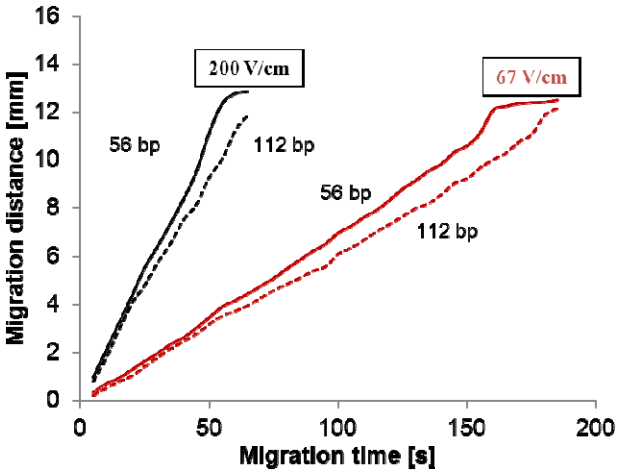

Figure 7: Migration distances of the two fragments in respect to migration time for two different electrical field strengths (67 and 200 V/cm). Triplication of the electrical field results in triplication of velocities.

The power consumption during the process is estimated to be 4.6 mW (current: 23 µA and electrical field: 200 V). No charge carrier depletion could be measured in the line for an excess of 300 seconds with 1.5 µL reservoirs and 200 V/cm.

DISCUSSION

Compared to standard chip-based capillary electrophoresis, our approach exhibits the following advantages: i) all materials can easily be fabricated at the point-of-use in a low-cost and fast manner, ii) all fabrication and detection steps can easily be done in automated fashion on one instrument, iii) the time from fabrication to analytical result is less than 5 minutes compared to 30 minutes for preparation of commercial chips where the gel has to be loaded into micro-channels before use, iv) design of separation line can easily be adapted to different applications, v) the non-contact sample injection turns complex sample injection and metering via T-structures known from standard capillary electrophoresis obsolete. Still, the following optimizations could harvest the full potential of our approach. A fluidic T-structure can be for example added to store the different solutions to be analyzed or the reagents can be dispensed inside the line consecutively to perform several analyses in the same unique line. The different substances, once separated, can be extracted from the line either by electrical field or aspiration-pipettes.

Moreover the actual detection scheme is composed of a red-line laser that allows a complete observation of the separation line. This is highly useful to assess the validity of our approach. Nevertheless, in order to observe a lower intensity fluorescence signal, an alternate detection scheme respectively system has to be applied. The use of a high-performance point-detection scheme will largely increase the sensitivity of our method and decrease the needed reagent volume and concentrations. Alternately, by simply printing more inter-digitated electrodes on the substrate, we can also perform an electrochemical detection scheme for the different species in solution similar to [3].

CONCLUSION AND OUTLOOK

We successfully demonstrated on-demand electrophoretic separation of two DNA strands in an open microfluidic system comprising a planar polyimide substrate equipped with two Pt-electrodes and a liquid separation channel covered with oil.

Future work will be dedicated to the optimization of the writing and especially the detection process. This way, the base pair resolution could be largely improved for smaller concentrations. By printing single cells inside the written lines, the presented method could open the way to on-demand fast in-line single cell protein electrophoresis.

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