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Miniature instrument for lab-on-a-chip capillary gel electrophoresis of DNA utilizing temperature control technique

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Abstract

A complete miniaturized instrument for chip-based capillary gel electrophoresis of DNA with fluorometric detection method and temperature control technique is presented. Separation of single-stranded and double-stranded DNA is obtained in a few minutes utilizing reusable glass chip. Real-time detection is performed using laser-induced-fluorescence and miniature CCD camera. Applied temperature control technique affects mobility of different DNA fragments in polymer sieving matrix. Electrokinetic sample plug injection and separation, temperature stabilization and fluorometric detection are automatically performed in the miniature docking station, controlled by dedicated software with real-time fluorescence signal recognition and digital data conditioning. Presented instrument is automatic, low-cost and portable solution, enabling future application of multitemperature single strand conformation polymorphism (MSSCP) genotyping technique for sensitive detection of minor genetic variants in DNA samples.

Keywords: electrophoresis; lab-on-a-chip; DNA; SSCP; temperature

1. Introduction

Currently, great attention is paid to develop low-cost and accurate chip-based instrumentation for rapid molecular diagnosis and detection of genetic variations, e.g. single-nucleotide polymorphisms and mutations. Among commonly applied molecular diagnostic techniques, single-stranded conformation polymorphism (SSCP) method of gene-scanning has been successfully adapted to chip-based solutions

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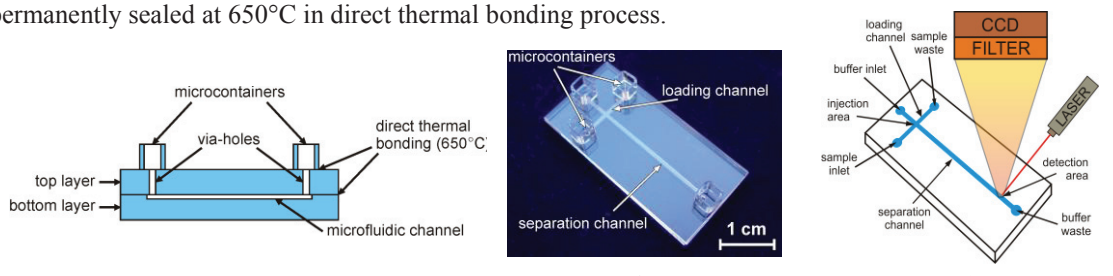
[1-4]. This technique utilizes differentiation of DNA conformations by electrophoretic separation in a gel sieving matrix. Sensitivity of genetic alterations detection by SSCP can be significantly increased by applying sequentially changed gel temperature during the separation. Multitemperature SSCP (MSSCP) technique [5] has proven its analytical and preparative capabilities in analysis of KRAS mutation samples [6], H1N1 seasonal and pandemic variants of influenza viruses [7,8] and has been applied e.g. in tumor biology, pharmacogenics and molecular diagnostic kits development [9-11].

Despite growing interest and market of miniaturized bioanalytical devices for genetic analysis, chip-based instrument for separation of DNA fragments utilizing MSSCP technique has, to the authors' knowledge, never been developed so far. The main goal of this work is to build miniature instrument for automatic MSSCP-based DNA separation utilizing reusable or disposable glass lab-on-a-chips (LOCs). In this paper, we present prototype instrument with stabilized LOC temperature, consisting of three components: the chip, the docking station and PC with dedicated software. This solution is a first step towards development of MSSCP LOC-based instrumentation.

2. The chip, samples and reagents

Glass lab-on-a-chip contains two 35 mm x 17 mm x 1 mm glass layers and glass microcontainers (Fig. 1a,b). A new series of borosilicate glass substrates (Borofloat®33 Optiplane, Schott), with laser-cut smooth side walls, were used to ensure high laser light coupling efficiency in utilized optical detection method (Fig. 1c). In the bottom layer, microfluidic channels (separation channel 25 mm long, 460 µm wide and 30 µm deep, crossed with loading/dosing channels 10 mm long) are fabricated utilizing HF:HCl wet-etching by photoresist masking layer (AZ®4562, MicroChemicals). Via-holes (0.8 mm diameter) for fluidic connection are mechanically machined in the top layer. Both layers and pre-machined glass microcontainers for fluid handling (approx. 30 µl volume each) are cleaned in Piranha solution and permanently sealed at 650°C in direct thermal bonding process.



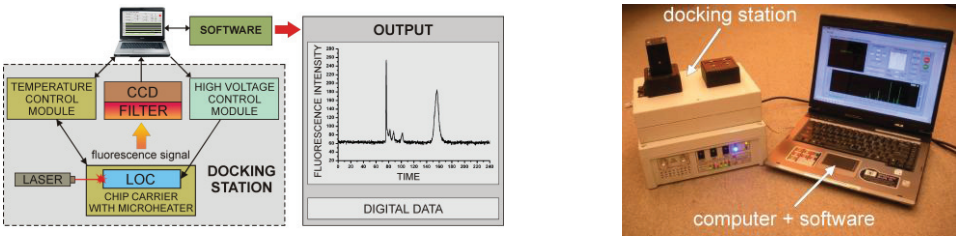
(a) (b) (c)

Fig. 1. Glass lab-on-a-chip: (a) cross-sectional view; (b) the chip at a glance; (c) chip overview and detection principle

Microfluidic channels are flushed with methanol and DI water, dried, and subsequently filled with approx. 1 µl of polymer sieving matrix (POP-4™, Applied Biosystems) utilizing pressure of standard laboratory pipette. No special treatment of channel walls, e.g. chemical modification or coating, is applied. Electrophoretic buffer (Tris-borate-EDTA, BioVectis) is pipetted into microcontainers. DNA samples of 50 bp ladder (A&A Biotechnology) and 20-40 bp oligonucleotides mixed with 100, 200, 300 and 500 bp DNA fragments (BioVectis) are labeled with fluorescent dye (Cy5). Single-stranded (ss) DNA samples are prepared as follows: melting in 95ºC, subsequently chilling in -20ºC, mixing with loading buffer (BioVectis) and immediate pipetting into the sample inlet microcontainer. In preparation protocol of double-stranded (ds) DNA samples temperature steps were skipped.

3. The instrument

The chip is placed inside the docking station of the instrument (Fig. 2) and after that, all the analytical procedures are done automatically by setting adequate parameters in steering software. High voltage is supplied by needle-like platinum electrodes placed inside microcontainers. Precisely regulated voltage supply (up to 1 kV) is automatically managed in order to provide adequate dosing of nanoliter-range of sample plug into buffer stream and to ensure electrophoretic separation of eluents. Labeled DNA fragments are detected by fluorometric system in which solid-state laser beam (635 nm) is focused on transparent side wall of the chip and illuminates laterally ending of the separation channel. “Flashes” of the fluorescence light (~ 670 nm), emitted by eluents, are detected by CCD camera, equipped with optical long-pass filter (> 650 nm, Thorlabs). Collected data (images) are real-time processed by dedicated software and presented in electropherogram, as well as stored in digital database. During separation, temperature of the chip is changed by hotplate microheater with ±0.2ºC accuracy in a range from 5ºC to 95°C. The temperature may be precisely adjusted or ramped with an approximate gradient 1ºC/sec, providing thermal profile for MSSCP-based separation of DNA conformations.



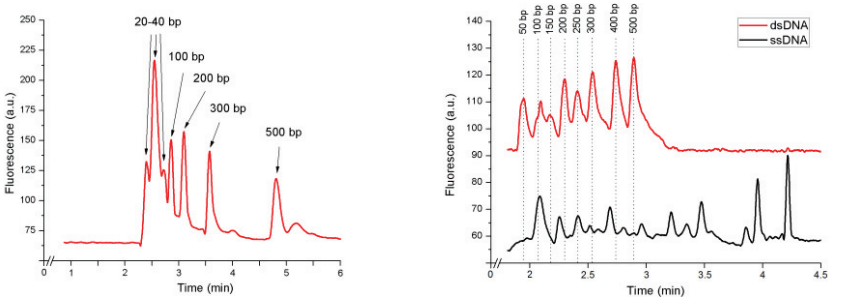
(a) (b)

Fig. 2. Miniaturized instrument for lab-on-a-chip capillary gel electrophoresis: (a) scheme; (b) view

The docking station has approximate volume of one cubic foot, power consumption below 100 W, it weights 3 kg and it is fully portable.

4. Experiment and results

The sample loading is performed by applying +200 V to the sample waste and ground to other microcontainers. Pinched injection mechanism is utilized for dosing about 5 nanolitres of the sample plug into the separation channel. DNA fragments are separated and detected 20 mm from the injection area.



(a) (b)

Fig. 3. Separation results of: (a) dsDNA sample containing 10-40 bp oligonucleotides and 100, 200, 300, 500 bp DNA fragments (22°C, 200 V/cm); (b) 50 bp ladder of ssDNA and dsDNA (15°C, 240 V/cm)

Calibration of the device is performed at 22°C by separating dsDNA fragments mixed with oligonucleotides (Fig. 1a). Average separation efficiency for 20-500 bp range is about 300 000 theoretical plates per meter (tp/m).

Separation results of 50 bp ssDNA and dsDNA ladder are presented in Fig. 3b. Estimated average separation efficiency for dsDNA at 15°C is 300 000 tp/m and for ssDNA it ranges from 0.2 mln tp/m (first fraction), up to 4.4 mln tp/m for the last two conformations. In comparison to slab-gel method, 20-fold reduced time of analysis has been noted.

Glass chip is reusable and it is cleaned utilizing methanol and DI water only, without special treatment.

5. Summary

Miniature instrument for LOC capillary gel electrophoretic separation of ssDNA and dsDNA utilizing temperature control technique is presented. The most advantageous features of the solution are: MSSCP-ready gel electrophoresis, reusable glass lab-on-a-chip, sensitive and simple real-time detection system, short time analysis, low power consumption, as well as, miniature size and portability. DNA fragments are separated within few minutes with temperature stabilization. Separation of ssDNA is performed with efficiency above 4 million of theoretical plates per meter and 20-fold reduction of time, in comparison to standard slab-gel separation method. These results will open the way for further development of MSSCP-based LOC instrumentation for rapid and sensitive genetic diagnosis.

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