Optica Applicata, Vol. XLI, No. 2, 2011

Injection, separation and fluorimetric detection of DNA in glass lab-on-a-chip for capillary gel electrophoresis

WOJCIECH KUBICKI*, RAFAŁ WALCZAK, JAN DZIUBAN

Department of Microengineering and Photovoltaics, Faculty of Microsystem Electronics and Photonics, Wrocław University of Technology, Janiszewskiego 11/17, 50-372 Wrocław, Poland

*Corresponding author: wojciech.kubicki@pwr.wroc.pl

Great attention is paid to develop lab-on-a-chip (LOC) for high-performance analysis of genetic material. Capillary gel electrophoresis is an efficient method of DNA analysis, including detection of potentially oncogenic genetic mutations. In this paper, glass LOC for capillary gel electrophoresis of DNA samples, as well as laboratory set-up for electrophoretic separation and fluorimetric detection of nucleic acids, are presented. Nanolitre genetic material specimen was on-chip injected and separated utilizing a high voltage automatic management system. Fluorochrome labeled DNA strands were sized by fragment length within a few minutes. Detection was performed by a laser/CCD-based miniature fluorimetric system equipped with specialized software for fluorescence signal recognition, analysis and storage.

Keywords: lab-on-a-chip, capillary gel electrophoresis, DNA.

1. Introduction

Capillary gel electrophoresis (CGE) is a high-performance method of genetic material analysis [1–3], including DNA sequencing [4] and separation of DNA restriction fragments [5]. The method is based on different mobility of electrically charged molecules migrating along a gel-filled capillary under external electric field. Negatively charged nucleic acids migrate towards anode. They are separated in a gel sieving matrix on the basis of fragment length or characteristic spatial structure (conformation). Individual components of the sample are detected at the end of the capillary by several detection methods (*e.g.*, optical, conductive, electrochemical). Among other methods, fluorimetric detection has extraordinary sensitivity and it is widely applied in commercially available gel electrophoresis instruments – slab or capillary. Despite high analytical resolution, unprecedented sensitivity and broad application, commercial CGE instrumentation is not wide-spread due to high cost of bulky apparatus and maintenance. The growing interest and market of miniaturized

devices for bioanalytical applications motivate development of labs-on-a-chips (LOCs) for rapid, high-resolution CGE, which are becoming relevant alternative to traditional instrumentation [6–8]. Compact LOC-based electrophoretic devices provide portability, automation and high efficiency of separation. Moreover, microengineering techniques enable integration of microfluidic components for nanovolume specimen holding, mixing, injection and separation without application of external and bulky mechanical devices. Among many materials used in LOCs microengineering, polymer-based CGE LOCs are the most common (*e.g.*, PDMS [9], PMMA [10], COC [11]). It is due to low cost of the material and relatively simple LOC technology. However, most polymeric LOCs suffer from drawbacks of hydrophobic and unstable surface properties. While fluorimetric detection is considered, high autofluorescence of some polymers must be taken into account [12, 13]. Therefore, great attention is paid to develop entirely glass CGE LOC [14]. Glass is chemically stable, biologically compatible and has high electrical resistivity, as well as low autofluorescence. These advantages ensure high efficiency of electrophoretic separation and sensitive detection by the fluorescence method. However, glass as LOCs material is technologically difficult, especially in comparison to polymers. On the other hand, due to high chemical resistivity, glass LOCs can be cleaned after single CGE. Thus, glass chips are reusable, while polymer chips are mostly disposable.

The aim of this work is to develop a LOC-based device for rapid CGE separation of genetic material in an entirely glass chip. The device would be applicable in a novel point-of-care molecular diagnostic instrumentation.

2. Experiment

Developed miniature system for CGE of genetic material consisted of: glass CGE LOC, chip holder, high voltage management system and fluorescence detection instrumentation.

2.1. Lab-on-a-chip

The chip was fabricated utilizing microengineering techniques: chemical wet etching, mechanical drilling and direct bonding. Borosilicate glass substrates (Borofloat 33®,

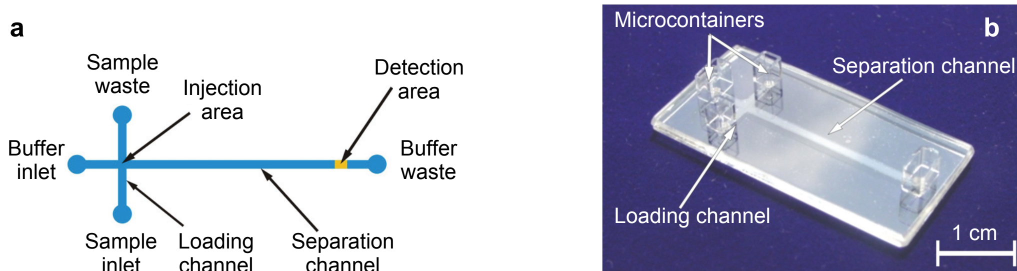

Fig. 1. Glass LOC for CGE: layout of microfluidic channels (**a**), ready-to-use structure (**b**).

Schott) were cut to 35 mm×17 mm×1.1 mm. Pattern of cross-shaped microfluidic channels was prepared in CAD software (Fig. 1**a**). Separation and loading channels (30 mm and 10 mm long, respectively) were 500 µm wide. The chip layout was transferred to chemically resistant foil by laser cutting. Then, the substrate was etched to the depth of 30 µm utilizing HF-based solution.

Via holes were mechanically drilled in the top substrate. Both structures and glass microcontainers for sample and buffer holding were cleaned in piranha bath, aligned and permanently sealed in direct thermal bonding process at the temperature above 600 °C (Fig. 1**b**). Finally, microfluidic channels were filled with linear polyacrylamide gel sieving matrix, prepared with procedure described by von Heeren *et al.* [8].

2.2. High voltage management system

Repeatable electrophoretic injections and separations of DNA samples were performed with the use of a high voltage (HV) automatic management system, consisting of regulated HV power supply, HV control unit, PC with digital to analog converter card and platinum wire electrodes inserted into inlets and outlets of LOC (Fig. 2**a**).

Control of the high voltage system was provided by developed Capillary Electrophoresis Lab-On-a-Chip Software (CELOCS), based on LabView platform. In

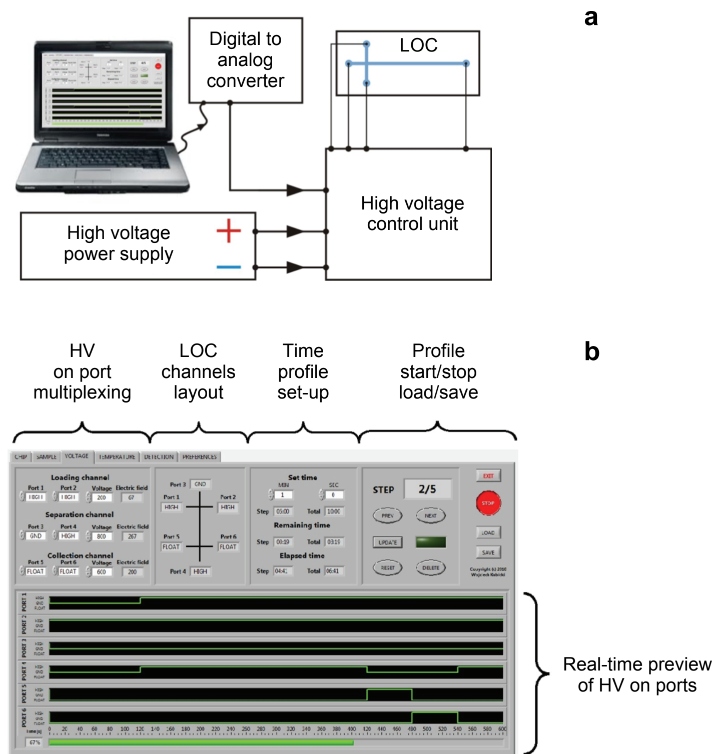

Fig. 2. High voltage management system: schematic view (**a**), HV control tab of CELOCS software (**b**).

order to precisely manage the specimen's flow in microfluidic channels network, the software allowed to generate, load and save voltage-time profiles for 6 electrodes independently (Fig. 2**b**). HV control unit was able to toggle voltages up to 1 kV. Full galvanic isolation of the computer was provided.

2.3. Fluorimetric detection system

Separated samples were detected at the distance of 20 mm from the injection area. Laser beam of a miniature semiconductor laser diode (635 nm wavelength) was laterally illuminating the detection area. Fluorescence signal from the detection area was collected by a miniature CCD sensor coupled with an optical filter and zoom lens (Fig. 3). Analog signal from the CCD was digitalized, processed and stored by CELOCS. Signal processing algorithm was used to transform an optical signal from the detection area into a digital plot of the fluorescence intensity in function of time (electropherogram).

2.4. Sample preparation

Genetic material samples, containing DNA strands in a range of 100–500 base pairs (bp) and concentration of 500 ng/µl, were labeled with Cy5 fluorochrome and mixed with

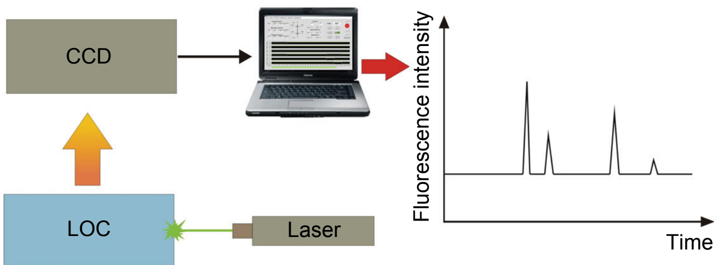

Fig. 3. Schematic view of the fluorimetric detection system.

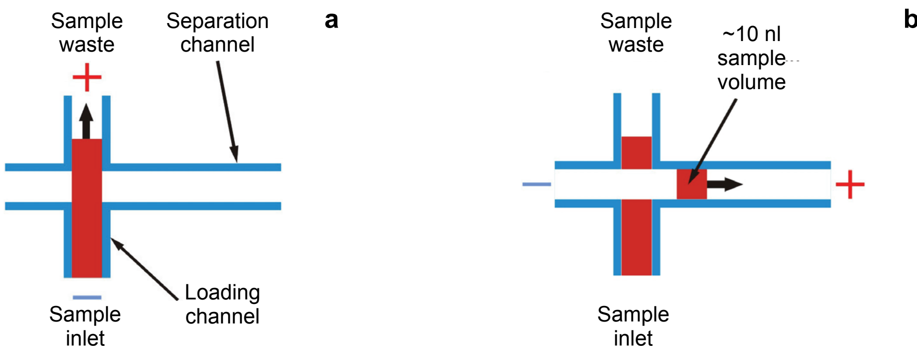

Fig. 4. Principle of on-chip sample injection at the intersection of microfluidic channels: filling of the loading channel with the sample (**a**), injection of the sample plug (**b**).

a loading buffer (BioVectis). Electrophoretic DNA fragment sizing was performed in the linear polyacrylamide gel utilizing TBE (Tris-boric acid-EDTA) electrophoretic buffer (BioVectis). In this case, the shorter DNA strand, the faster it was migrating through the gel sieving matrix and the corresponding peak occurred earlier in the electropherogram.

2.5. Experiment procedure

Glass microcontainers were filled with TBE buffer. Nanolitre sample injection was performed at the intersection of loading/separation channels in three steps: 1) specimen pipetting into the sample inlet, 2) electrokinetic specimen transport towards the sample waste (Fig. 4**a**), 3) voltage toggle and injection of approximately 10 nanolitres of the sample plug into the separation channel (Fig. 4**b**).

3. Results

DNA strands of various fragment size were separated during voltage-enforced migration towards the buffer waste. Laser beam was intersecting the terminal of the separation channel and exciting the fluorochrome dye binded to DNA strands (Fig. 5). Fluorescence signal was collected by the CCD-based detector and recorded in the computer. Collected data was processed by CELOCS module with fluorimetric signal recognition and plotted in the electropherogram. Positions of peaks in the fluorescence signal plot were corresponding to migration times of separated DNA strands passing the detection area. Electropherogram provided information about qualitative and quantitative sample parameters, including identification of the sample composition.

Electrophoretic separation was performed for mixture of 100, 200, 300 and 500 base pairs long DNA strands. Detection signal of separated fragments was sharp. Peaks

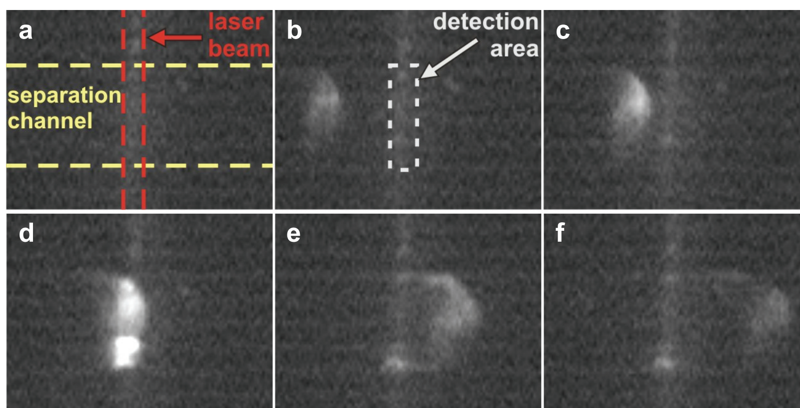

Fig. 5. Detection of the separated DNA fragment: view of the detection area with marked separation channel and laser beam (**a**), migration of DNA strands through the detection area (**b**–**f**) – images (**b**) and (**c**) show the sample approaching to the detection area, image (**d**) corresponds to the sample flowing through the detection area, whereas images (**e**) and (**f**) show receding sample.

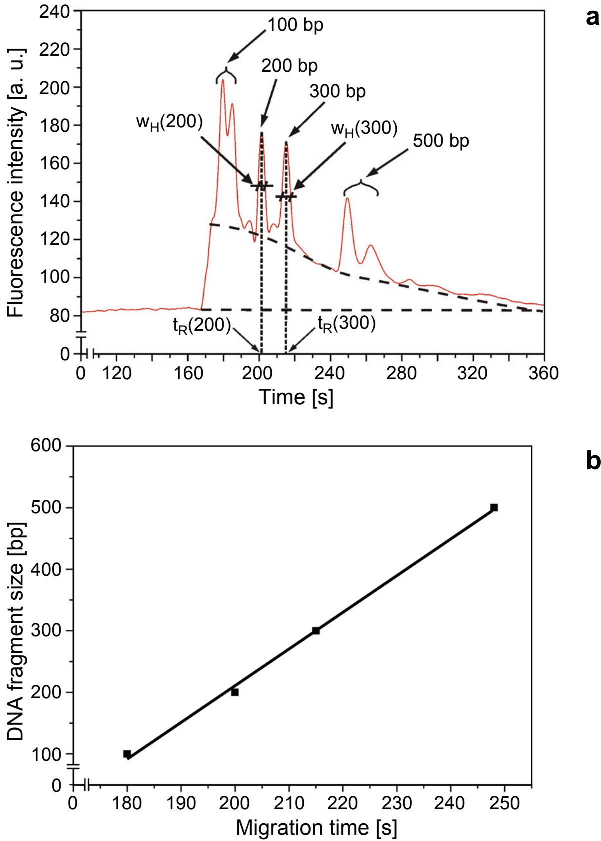

Fig. 6. LOC CGE fragment sizing of DNA: electropherogram (**a**), calibration curve (**b**).

corresponding to the strands were distinct (Fig. 6**a**). It has been found that peaks of 100 bp and 500 bp samples were divided, probably due to the thermal denaturation of DNA. On the basis of the recorded electropherogram, a calibration curve of migration time was plotted (Fig. 6**b**).

Separation efficiency was calculated on the basis of the following equation:

$$N = 5.55\left(\frac{t_R}{w_H}\right)^2$$

where t_R corresponds to component migration time and w_H is peak width at half-height. Obtained efficiency was embraced above 30000 theoretical plates (*e.g.*, $N = 33\,100$ for 200 bp) which gives about 1.5 million of theoretical plates per meter.

In comparison with standard CGE, theoretical plates as high as 10^7/m are possible when ethidium bromide intercalating additives are used for high resolution capillary electrophoresis [2]. However, typical theoretical plate values for classic CGE are in a range of hundred thousand per meter. Separation efficiency obtained in the reported

here CGE LOC is much higher (~1.5×10^6/m). It seems that further optimization of LOC layout, gel composition and separation conditions are possible towards improved separation efficiency.

Characteristic feature of presented CGE LOC is a very short separation time, which is important considering rapid DNA analysis. Fragment sizing of DNA sample containing 500 bp long nucleic acids took less than 5 minutes. In comparison with classic CGE instrumentation, LOC-based separation was at least 10 times faster. It must be also pointed out that investigated range of DNA fragments is typically used for analysis towards detection of oncogenic mutations in genetic material.

4. Conclusions

Results of electrophoretic injection, separation and detection of the genetic material in the developed glass CGE LOC were presented. On-chip injections of 10 nanolitres of DNA samples at the intersection of cross-shaped microfluidic channels were obtained utilizing the high voltage automatic management system. Electric voltages up to 1 kV were toggled for every inlet/outlet channel of LOC independently. DNA samples (100–500 base pairs long) were separated and fragment sized within few minutes in the polyacrylamide gel-filled separation channel. Detection of Cy5 fluorochrome-labeled DNA strands was performed utilizing the laser/CCD fluorimetric detection system. High voltage control and fluorimetric signal recognition was provided by the developed Capillary Electrophoresis LOC Software (CELOCS). Specimen was identified on the basis of peaks position in the electropherogram. In comparison with classic macroscale, the CGE instrumentation separation efficiency is higher and the analysis time is much shorter. It has been concluded that further optimization is possible. Positive results of on-chip genetic material separation, obtained by the use of the advanced system for voltage-based sample manipulation in the microfluidic channels network and the sensitive fluorimetric detection system, open the way to develop a miniature, portable instrument for innovative, noninvasive prenatal diagnosis methods and rapid detection of oncogenic mutations in genetic material.

Acknowledgements – Authors would like to express their thanks to BioVectis (Poland) for supplying samples and reagents and for priceless discussion and support. This work was financially supported by the Ministry of Science and Higher Education from supervisor's research grant funds for years 2010–2011 (Grant No. N N515 537338).

References

[1] Cohen A.S., Paulus A., Karger B.L., *High-performance capillary electrophoresis using open tubes and gels*, Chromatographia **24**(1), 1987, pp. 15–24.

[2] Yan Xu, *Capillary electrophoresis*, Analytical Chemistry **65**(12), 1993, pp. 425–433.

[3] Ettre L.S., Guttman A., *The evolution of capillary gel electrophoresis: From proteins to DNA sequencing*, LCGC North America **22**(9), 2004, pp. 896–904.

[4] SWERDLOW H., GESTELAND R., *Capillary gel electrophoresis for rapid, high resolution DNA sequencing*, Nucleic Acids Research **18**(6), 1990, pp. 1415–1419.

[5] GUTTMAN A., SZÖKŐ E., *Capillary gel electrophoretic separation of DNA restriction fragments in a discontinuous buffer system*, Journal of Chromatography A **744**(1–2), 1996, pp. 321–324.

[6] XU Z.Q., HIROKAWA T., NISHINE T., ARAI A., *High-sensitivity capillary gel electrophoretic analysis of DNA fragments on an electrophoresis microchip using electrokinetic injection with transient isotachophoretic preconcentration*, Journal of Chromatography A **990**(1–2), 2003, pp. 53–61.

[7] SINVILLE R., SOPER S.A., *High-resolution DNA separations using microchip electrophoresis*, Journal of Separation Science **30**(11), 2007, pp. 1714–1728.

[8] VON HEEREN F., VERPOORTE E., MANZ A., THORMANN W., *Characterization of electrophoretic sample injection and separation in gel-filled cyclic planar microstructure*, Journal of Microcolumn Separations **8**(6), 1996, pp. 373–381.

[9] FUJII T., *PDMS-based microfluidic devices for biomedical applications*, Microelectronic Engineering **61–62**, 2002, pp. 907–914.

[10] FU-CHUN HUANG, CHIA-SHENG LIAO, GWO-BIN LEE, *An integrated microfluidic chip for DNA/RNA amplification, electrophoresis separation and on-line optical detection*, Electrophoresis **27**(16), 2006, pp. 3297–3305.

[11] KOH CH.G., TAN W., ZHAO M., RICCO A.J., FAN Z.H., *Integrating polymerase chain reaction, valving, and electrophoresis in a plastic device for bacterial detection*, Analytical Chemistry **75**(17), 2003, pp. 4591–4598.

[12] PIRUSKA A., NIKCEVIC I., SE HWAN LEE, CHONG AHN, HEINEMAN W.R., LIMBACH P.A., SELISKAR C.J., *The autofluorescence of plastic materials and chips measured under laser irradiation*, Lab on a Chip **5**(12), 2005, pp. 1348–1354.

[13] SZCZEPAŃSKA P., WALCZAK R., WOLFF A., DZIUBAN J., *SU-8 planar waveguides for real-time PCR chips*, Proceedings of XXII Eurosensors Conference, September 7–10, 2008, Dresden, Germany, pp. 383–386.

[14] EFFENHAUSER C.S., MANZ A., WIDMER H.M., *Glass chips for high-speed capillary electrophoresis separations with submicrometer plate heights*, Analytical Chemistry **65**(19), 1993, pp. 2637–2642.

Received September 25, 2010