Sensors and Actuators B 254 (2018) 153–158

Contents lists available at ScienceDirect

Sensors and Actuators B: Chemical

journal homepage: www.elsevier.com/locate/snb

Short communication

Miniaturized gel electrophoresis system for fast separation of nucleic acids

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ARTICLE INFO

Article history:
Received 17 April 2017
Received in revised form 6 July 2017
Accepted 12 July 2017
Available online 14 July 2017

Keywords:
Slab gel electrophoresis
Capillary electrophoresis
Polymerase chain reaction
Nucleic acids

ABSTRACT

Slab gel electrophoresis (SGE) is very common tool for DNA, RNA and protein analysis, but it is tedious, labor-intensive, skill-dependent, and relatively slow. Herein, we developed a biochip based SGE system, which can resolve the DNA fragments and record their separation process. By electrophoresis of 50 bp DNA ladder, we found that the 16 DNA fragments were effectively resolved within 14 min. In order to validate its feasibility and practicability, we take periodontal pathogens (e.g., *Porphyromonas gingivalis (P.g), Tannerela forsythia (T.f), and Treponema denticola (T.d)*) as an example by separating their polymerase chain reaction products. Experiments demonstrated that *P.g, T.d and T.f* were diagnosed within 12 min, and the electrophoresis of *P.g* showed that the detection limit of this system was about 6.4 ng/μl. Such a low cost system is easy to operate, and can effectively improve SGE in the biological experiment, especially for the labs in the third world countries.

1. Introduction

Nucleic acid separation is an important technology in molecular biology, following by polymerase chain reaction (PCR) analysis or before cloning, sequencing, northern or southern blotting. So far, high performance liquid chromatography (HPLC) [1], capillary electrophoresis (CE) [2], and slab gel electrophoresis (SGB) [3] are three common methods used for the separation of DNA, although research about the new methods are still under way [4–6]. In HPLC, the separation mechanism involves partitioning of analytes between a mobile and stationary phase. However, besides the separation performance, electrophoresis are deemed to have distinct advantages over HPLC, because instrumentation of HPLC is rather complex compared to the simplicity of CE or SGE system. Furthermore, the elution of large molecules can only be achieved by changing the selectivity during separation process (e.g. gradient elution), and expensive prepacked columns are required which have limited life time in HPLC [7].

Compared with HPLC, the separation channel in CE is an open or gel-filled tube, which was first developed by Mikkers et al. for separation of small anions [8]. It becomes an attractive analysis method in biochemistry field due to its numerous advantages [9,10], such as less reagent consumption, short running times, and high resolution. For example, Luraschi et al. described the use of CE coupled with immunosubtraction for the detection and characterization of low concentration of γ heavy chains in serum [11]. Godelieve Mariën attempted to detect and characterize the μ heavy chain disease by CE [12]. Our group has also applied CE for the detection of periodontal pathogens and *Escherichia coli* [13,14]. With the development of microfabrication technology, research on the CE chip became increasing popular in the past two decades. However, fabrication of the chip was based on expensive photolithography or tedious chemical etching techniques [15]. Walczak's group studied the separation and extraction of cell-free fetal DNA in a CE chip, which was etched in glass [16]. They also developed a miniaturized instrument based on this electrophoresis chip with laser induced fluorescence technology [17]. Although separation of single-stranded and double-stranded DNA was achieved in a

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http://dx.doi.org/10.1016/j.snb.2017.07.064
0925-4005/

Table 1
Primer sequence for periodontal pathogens.

Target Gene (16S rRNA gene)		Primers (5′-3′)	Product size (bp)
Porphyromonas gingivalis	FwRv	CTGATCTGCAGCAGGTAGCCTC GCACATAACCGGTAGCCTCTAC	132
Tannerella forsythia	FwRv	GCGTATGTAACCTGCCCGCA TGCTTCAGTGTCAGTTATACCT	641
Treponema denticola	FwRv	GAGTAGTCCGTCGGAGTCTATG AATCTCGGTATCCGGTTCGG	364

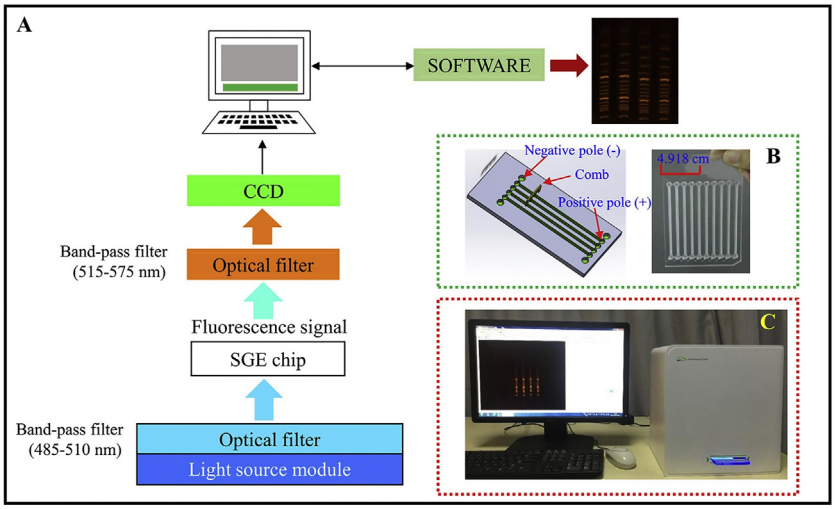

Fig. 1. The miniaturized SGE system: (A) Schematic diagram. (B) The real SGE instrument. (C) The SGE chip.

few minutes, the throughput is not high since only one sample can be separated in one experiment. In order to make it more ease of operation, fully automated and higher throughput commercial instruments for CE or microchip CE (e.g., Bioanalyzer 2100, 5100 ALP, SV110, MCE-2010) are available for routine clinical testing [18], but the high cost of such instrument hindered its prevalence, especially for the labs in the third world countries.

Slab gel electrophoresis (SGE) is the most common approach for separation and analysis of macromolecules (DNA [19], RNA [20] and proteins [21,22]) because of its low cost and high reproducibility. However, typical SGE experiment procedures are quite tedious, which involves preparing the agarose gel (30 ~ 50 min), sample loading (1 ~ 5 min), running electrophoresis (30 ~ 60 min), gel staining (15 ~ 30 min), and imaging DNA bands (1 ~ 5 min) [19]. Consequently, it requires quite a lot of instruments such as microwave to dissolve the agarose, 2-D electrophoresis cell and electrophoresis power supply for electrophoresis, gel-imaging system to check the DNA bands in the gel, and hence traditional SGE is labor-intensive, time-consuming, skill-dependent, and poor sensitivity.

Furthermore, the DNA molecules are usually visualized under ultraviolet light by staining with appropriated dyes such as ethidium bromide (EtBr) in traditional SGE experiment, but both ultraviolet and EtBr are suspected to be carcinogen. In order to overcome those drawbacks and increase the versatility of SGE, we developed an integrated miniaturized system which can separate the DNA fragments and record the DNA separation process during electrophoresis. Moreover, compared with the conventional SGE device, the system we developed can greatly save the experiment time (about 10 min) and take only 1/20 chemical reagents. Meanwhile, such a chip is reusable and the system is easy to operate. Finally, we take three harmful periodontal pathogens (e.g., *Porphyromonas gingivalis (P.g), Tannerela forsythia (T.f), and Treponema denticola (T.d)*) [23] as an example to validate our system.

2. Materials and methods

2.1. Materials and reagents

SpeedSTAR HS DNA Polymerase, 50 bp, 100 bp DNA ladder and 10 000 × SYBR Green I were from Takara (Shanghai, China). 10 × TBE (1 × TBE = 89 mM Tris/89 mM boric acid/2 mM EDTA, pH = 8.4) buffer and agarose powder were bought from Solarbio (Beijing, China). 1.0% agarose was obtained by diluting 0.1 g agarose into 10 ml 0.5 × TBE. The primers for the periodontal pathogens were synthesized by Sangon Biotech (Shanghai, China). Hydroxyethyl cellulose (HEC, 1300k) was from Polysciences (Warrington, PA, USA).

2.2. Capillary electrophoresis

The detailed information of the self-built CE was described in Ref [24]. The electric field is supplied by a high-voltage power supply (MODEL 610E, TREK, Medina, NY, USA). Total capillary length (ID/OD = 75/365 μm) is 6 cm (4 cm effective length), and the capillary is covalently coated with polyacrylamide [25,26]. The excitation wavelength from a mercury lamp is filtered to be 460–495 nm by the optical filter (U-MWIB-3, Olympus, Japan). The fluorescence signal from the conjugate of SYBR Green I and the nucleic acid is detected by a R928 photomultiplier tube (Hamamatsu Photonics, Japan). Power supply and data collection are controlled by LabVIEW (National Instrument, USA). The CE system is placed in a dark box. DNA sample is electrokinetically introduced into the capillary (100 V/cm, 1.0 s). The capillary is flushed by ster-