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Short communication

Bias-free pneumatic sample injection in microchip electrophoresis

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Abstract

We have developed a new microfluidic chip capable of accurate metering, pneumatic sample injection, and subsequent electrophoretic separation. The pneumatic injection scheme, enabling us to introduce a solution without sampling bias unlike electrokinetic injection, is based upon the hydrophobicity and wettability of channel surfaces. An accurately metered solution of 10 nL could be injected by pneumatic pressure into a hydrophilic separation channel through Y-shaped hydrophobic valves, which consist of polydimethylsiloxane (PDMS) and fluorocarbon (FC) film layers. We demonstrated the successful pneumatic injection of a red ink solution into the separation channel as a proof of the concept. A mixture of fluorescein and dichlorofluorescein (DCF) could be baseline-separated using a single power source in microchip electrophoresis.

Keywords: Microchip electrophoresis; Pneumatic sample injection; Hydrophobic valve; Sampling bias

1. Introduction

Microchip electrophoresis, one of the examples of a successful lab-on-a-chip, is a promising analytical technique due to its high performance and ability to incorporate various channels. A variety of designs for microchip electrophoresis have been fabricated to mix, react, concentrate, and separate analytes [1,2]. These have facilitated chemical and biological analyses including enzyme assay, chiral separation, DNA sequencing, immunoassay, etc. [1–8].

Usually, sample solutions are introduced by electrokinetic methods, such as pinched injection [9,10] or gated injection [11,12] in microchip electrophoresis. Even though these methods are easy to use, there are problems such as sampling bias and difficulty in measuring the injection volume precisely [13]. Thus, many attempts have been made to overcome these problems in electrokinetic injection [14–17]. For bias-free sample injection, Slentz et al. introduced samples into the separation channel using the diffusion of molecules after turning off the applied potential [14]. Bai et al. investigated pressure pinched injection of nanoliter volumes by a multiport injection valve and syringe pumps [15]. Solignac and Gijs injected a sample hydrodynamically by applying a pressure pulse to a membrane on a reservoir using a mechanical actuator [16]. Tabuchi et al. also developed a pressurization technique to separate protein mixtures in a 12-microchannel array [17]. Such injection methods can solve the problem of sampling bias. However, the absolute amount injected is still ambiguous. Yamada and Seki developed a microdispenser system, which can measure and inject a fixed nanoliter-sized droplet pneumatically [18].

Recently, we have developed a novel nanoliter-fluidic chip, capable of nanoliter metering, reaction, and mixing, by controlling the capillary pressure and wettability of channel surfaces [19]. Precise quantitative sample handling and analysis for an enzymatic reaction were demonstrated with the chip. Applying this sample handling system to microchip electrophoresis is a promising technique since a desired vol-

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ume can be accurately measured and the complicated manipulation of high voltages avoided since only a single power source is required. The biggest problem to overcome, however, is to design an interface between the sample handling system and the electrophoretic separation channel. In this study, we introduce a new microchip electrophoresis system enabling precise sample metering, hydrodynamic injection, and electrophoretic separation. The sample manipulation in the microfluidic chip was performed by controlling the capillary pressure and the wettability of channel surface as before [19]. The injection and electrophoretic separation were demonstrated using a mixture of fluorescein and dichlorofluorescein (DCF).

2. Experimental

2.1. Materials

Sodium fluorescein was from Junsei (Tokyo, Japan). DCF and sodium borate were from Sigma (St. Louis, MO, USA). A run buffer for electrophoretic separation was prepared by adjusting the pH of 20 mM sodium borate with 0.1 M NaOH to pH 9.2. All reagents were of analytical grade and were used without further purification. De-ionized water was obtained from a NANOpure purification system (Barnstead, Dubuque, IA, USA).

2.2. Microchip electrophoresis

The schematic of microfluidic chip is shown in Fig. 1a. The microfluidic chip was fabricated using isotropic wet etching, lift-off patterning of the fluorocarbon (FC) film, and PDMS replica molding techniques, as described elsewhere [19]. The main structure of the microfluidic chip was composed of two layers; one fabricated on a glass wafer for electrophoretic separation and the other for microfluidic sample handling fabricated on polydimethylsiloxane (PDMS). On a glass surface, a separation microchannel (13 cm long, 150 μm wide, and 50 μm deep) was fabricated and then a hydrophobic FC film was patterned in order to adjust the surface interfacial energy of the hydrophilic glass wafer. The PDMS layer contains sample loading reservoirs, two metering microchannel networks, a serpentine mixing channel, a sample-injection hydrophobic valve, and air venting channels. The dimension of the assembled microfluidic chip was 46 mm × 25 mm.

A handler for liquid sample injection and pneumatic solution control was constructed as before [19]. This was composed of plastic syringes, springs, a micrometer, and acrylic plastic housing (Fig. 1b). The rotational motion of the micrometer was converted into the linear motion of the piston of a syringe, letting a sample solution to be either pushed into or withdrawn from the microchannels. The motions of the sample solution were monitored using a CCD-camera-equipped optical microscope (Fig. 1c). After solution manipulations, the microchip was released from the handler and immediately

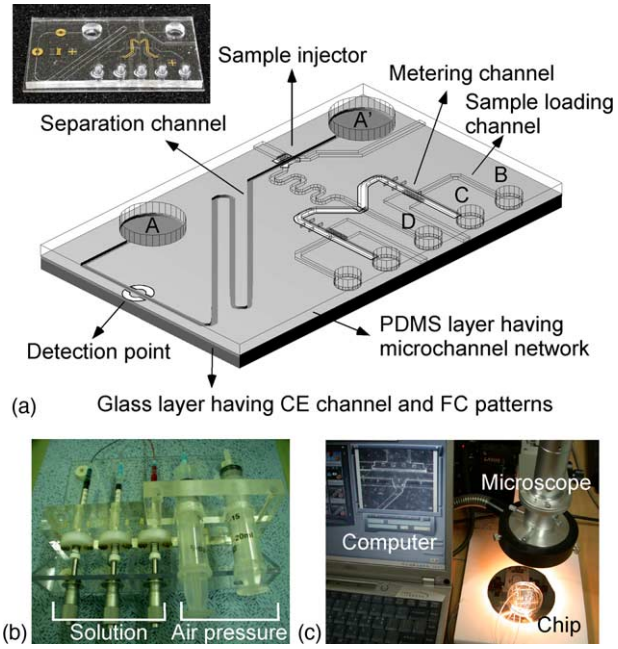

Fig. 1. Schematic of a microfluidic chip and a sample handling system. (a) Reservoirs are labeled as (A, A′) run buffer, (B) sample loading port, (C) metering port, and (D) pneumatic control port. The separation channel depth was 50 μm and width was 150 μm. The inset is an image of the fabricated microfluidic chip (size: 46 mm × 25 mm). Half of the sample loading and metering channel was designed for later use. (b) A handler for liquid sample injection and pneumatic solution control. (c) Setup to monitor sample manipulations using a CCD-camera-equipped optical microscope.

mounted on a stage for microchip electrophoresis. The electrophoretic separation was performed using a single power source, as described previously [5,6]. A laser-induced fluorescence detection system was used to detect the analytes with an excitation/detection at 488 nm/520 nm, respectively.

3. Results and discussion

For a quantitative analysis, it is important to meter precisely the amount of solution injected into a microchannel. The scheme developed by Lee et al. [19] was used. Due to the coating of a hydrophobic FC film on the glass surface (white area in Fig. 2a), an aqueous solution from reservoir B stopped in front of the metering channel. The solution could

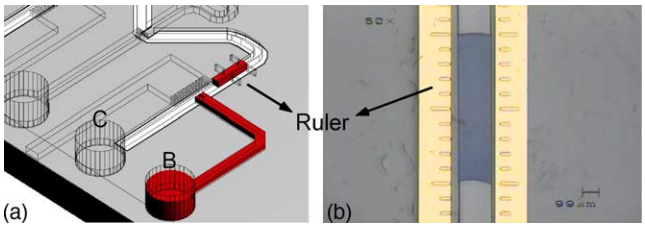

Fig. 2. Metering of a sample solution. (a) Simplified schematic of a metering channel. The injected amount was measured using a ruler. The illustration was not drawn to scale. (b) Microscope image of a 10-nL metered solution. The volume between two scale bars corresponds to 1 nL.