SLIDABLE AND VALVELESS POLYMERASE CHAIN REACTION–CAPILLARY ELECTROPHORESIS MICRODEVICE FOR PATHOGEN DETECTION

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ABSTRACT

In this experiment, we developed a novel integrated microdevice which could perform the sample loading, polymerase chain reaction (PCR), capillary electrophoresis (CE) without any microvalves or an external pump system. Microfabricated movable slide, called a slidable chamber, was switched from the sample loading to PCR amplification to CE separation. The movement of the slidable chamber was simply operated manually, and the leakage of the PCR cocktail could be minimized by the surface treatment on a chip. With a miniaturized portable genetic analyzing system, we successfully identified *Staphylococcus aureus* (*S. aureus*) on the slidable PCR-CE microdevice.

KEYWORDS: valveless microdevice, polymerase chain reaction, capillary electrophoresis, pathogen detection

INTRODUCTION

The integrated polymerase chain reaction-capillary electrophoresis (PCR-CE) microsystem has brought a revolution to the pathogen/infectious disease detection due to its portability, rapid analysis and low sample consumption [1]. However, the fabrication process and valve control of the integrated chip were complicated, and a bunch of tubing connection inevitably make the chip operation complex, resulting in exacerbating the data reproducibility. To overcome such limitations, here we developed a novel slidable and valveless PCR-CE microdevice by designing a separate movable PCR chamber that can be manually switched from the sample injection to PCR amplification to CE separation unit subsequently .

EXPERIMENTAL

The slidable PCR-CE microdevice consists of three layers: a 4-inch resistance temperature detector (RTD) fabricated glass wafer, a microchannel patterned glass wafer containing a sample loading and CE microchannel, and a 10×10 mm glass wafer that was etched in the center to form a PCR microchamber (1 μL) which is called a slidable chamber. The sample injection part and the sample loading channel in the CE were disconnected initially, but the disconnected microchannel could be linked when the slidable chamber was placed on the right position. Schematics of the assembled microdevice and each layer are shown in Figure 1.

The operational process for the PCR cocktail loading, PCR amplification, and CE is shown in Figure 2. Prior to the slidable chip operation, the channel wafer and slidable chamber were treated with decyltricholorosilane to prevent non-specific adsorption of DNA on the glass wafer. The slidable chamber was installed on the channel wafer with the sample-injection region connected (Figure 2A). Upon loading a PCR cocktail targeting protein A (101 bp) gene of *S. aureus* at the sample inlet, the PCR cocktail was injected into the slidable PCR chamber by suction at the waste outlet. After injection of the PCR cocktail, mineral oil was dispensed around the slidable chamber to form an oil layer between the channel wafer and a slidable chamber which helped to prevent evaporation of a PCR cocktail during the movement and PCR thermal cycling. The slidable chamber was moved to the PCR unit where the RTD was positioned close to the PCR chamber (Figure 2B). Using a cooling fan and an external film heater that was attached on the bottom of the RTD wafer, fast thermal cycling was performed to amplify the target gene. Due to the oil layer, the slidable chamber was fixed steadily during the PCR. After PCR amplification, the slidable PCR chamber was moved to the CE region (Figure 2C) with the sample injection channel connected. Typical micro-CE operation consisting of the sample loading, backbiasing, and the separation was conducted to detect the resultant PCR amplicons. The fluorescence signal in the slidable chamber was checked at each step by using a confocal scanning laser microscope, and the equivalent fluorescent intensity was maintained, meaning there is no significant loss in the sliding process.

Figure 3 shows the portable PCR-CE genetic analyzer that is composed of the miniaturized chip operation hardware, a four-color portable fluorescence detector and a laptop computer. The miniaturized chip operation hardware (dimension of 24 × 21 × 10 cm) was equipped with a cooling fan for PCR thermal cycling, an electronic control board (PCE-e, Nanoscope Systems Inc., Korea), high-voltage power supplies (AA12-P4, Ultravolt, USA) for CE, a pneumatic pump (60615, Thomas, USA) and solenoid valves (GV010E1, KOGANEI, Japan) (Figure 3B). The miniaturized hardware communicates with PC with the RS-232 protocol and the command from PC is received and calculated by the FPGA based control board. Figure 3C shows the schematic diagram of the portable fluorescence detector. The collimated beam produced from a fiber-coupled diode laser (FiberTEC 488TM , Blue Sky Research) is reflected by a dichroic beam splitter (488nm BrightLine, Semrock) to an objective (UPLFLN 20X, Olympus) and focused on the channel of the microdevice. The fluorescent signal is collected by the

same objective and passes through the dichroic beam splitter into a diffraction grating (600 lpmm, Wasatch Photonics). The fluorescence signals are collected at 8 kHz sampling rate by the DAQ board (National Instrument) and displayed as an average value of 200 samples for noise suppression.

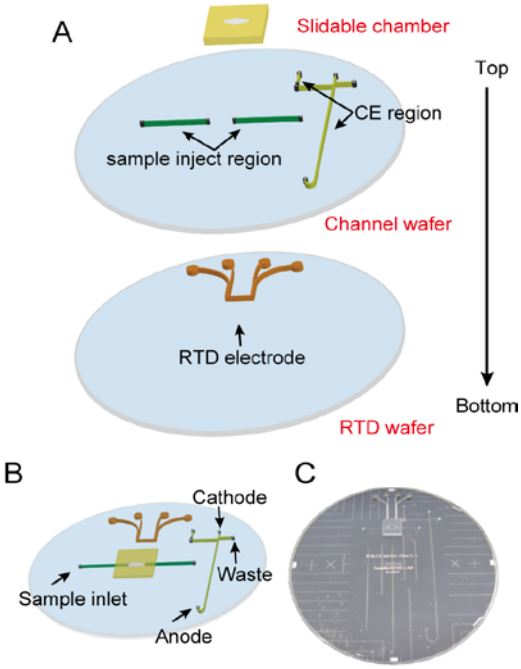

Figure 1. (A) Schematics of each layer (a RTD wafer, a channel wafer, and a slidable PCR chamber) for the integratedslidable PCR-CE microdevice. (B) The assembled structure and (C) photograph of the slidable PCR-CE microchip.

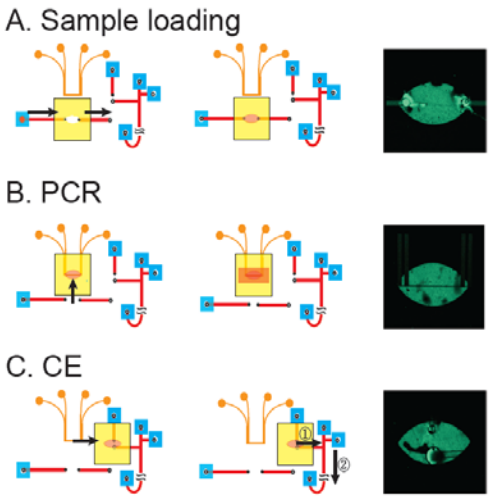

Figure 2.Operation process for (A) sample loading, (B) PCR amplification, and (C) CE separation on the slidable PCR-CE chip and (right panel) the fluorescence image of the slidable chamber at each step.

RESULTS AND DISCUSSION

We performed pathogen detection targeting protein A gene of *S. aureus* as a model to evaluate the high-performance of the slidable PCR-CE on the portable system. A PCR cocktail was injected into the slidable PCR chamber from the sample inlet to the waste outlet without bubble problem in the chamber, and the PCR thermal cycling was executed for 80 min in the PCR region with the portable genetic analyzer system. After thermal cycling, the slidable chamber was placed in the CE part, and the resultant amplicons were separated in 6-cm CE channel. The CE separation was completed in 5 min and the fluorescence signal was detected at the end of the CE channel. As shown in Figure 4, the target gene (101 bp) was clearly observed

in the electropherogram without any side-product peak. We performed the limit of detection experiment on the slidable PCR-CE microdevice, and found that 10 pg of DNA template was necessary to identify the pathogen in the proposed portable genetic analyzer system.

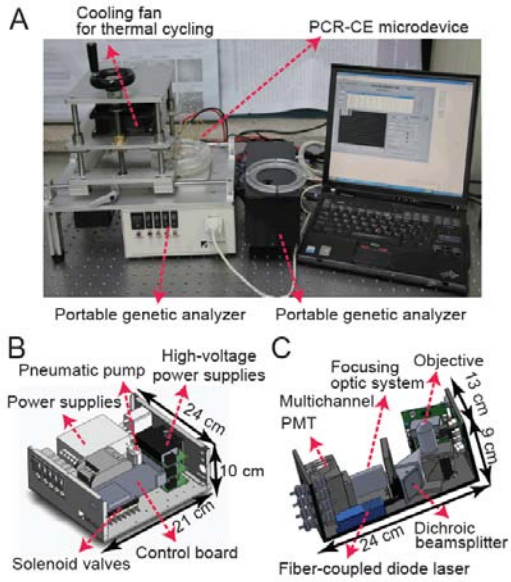

Figure 3. (A) Photograph of a portable PCR-CE genetic analyzer system. (B) Scheme of the portable chip operation hardware.(C) Scheme of the portable fluorescence detector.

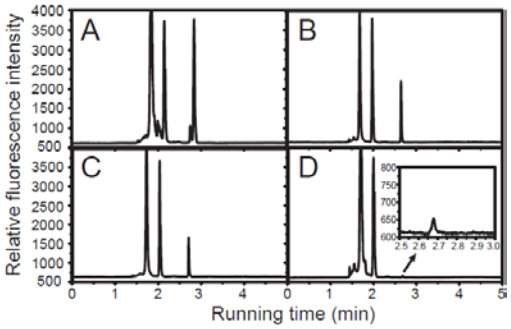

Figure 4. Detection limit of the slidable PCR-CE microdevice to identify S. aureus. The amount of the used template was (A) 10 ng, (B) 1 ng, (C) 100 pg, and (D) 10 pg.

CONCLUSION

An integrated valveless slidable PCR-CE microdevice has been developed for pathogen detection. The slidable PCR-CE reaction was completed in 85 min on a portable genetic analyser microsystem. The proposed integrated microdevice enables us to perform genetic analysis without any external valve or pump system that greatly simplified the chip fabrication and chip operation process compared with the conventional PCR-CE device.

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