FULLY INTEGRATED ROTARY GENETIC ANALYSIS SYSTEM

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ABSTRACT

In this study, a fully integrated and portable Rotary Genetic Analyzer has been developed to analyze the gene expression of influenza A virus. The Rotary Genetic Analyzer is made up of four parts: a disposable microchip, three thermal blocks for temperature control, a servo-motor for precise and high-rate spinning of the chip, and a miniaturized optical fluorescence detector. The microdevice was designed for performing microglass bead-based RNA template purification and then Rotary reverse transcriptase polymerase chain reaction (RT-PCR). Target gene amplification was real-time monitored using the integrated Rotary Genetic Analyzer system.

KEYWORDS

Genetic analysis, rotary RT-PCR, sample pretreatment, influenza A virus

INTRODUCTION

Early diagnostics has emerged as a critical issue due to the variety of human epidemics or pandemics such as influenza A virus, foot-and-mouth disease, and severe acute respiratory syndrome (SARS), which have threatened human being continuously throughout the world [1]. However, conventional methods for genetic analysis are not suitable for early diagnostics because of time-consuming process, lack of portability, and use of expensive instrumentations and large volume of samples. To overcome the limitations, many researchers have investigated the fully integrated microsystem with 'sample-in-answer-out' capability for on-site detection [2]. Despite the excellent demonstration of the previous reports, the complicated design and fabrication of the integrated chip and the complex chip operation system still need to be resolved to improve the data reproducibility. To address this issue, we propose a novel fully integrated rotary genetic analysis system which employs a simple chip design and enables automatic sample pretreatment and RT-PCR operation without need of an external pump for fluid control. Recently, our group reported a rotary reverse transcriptase polymerase chain reaction (RT-PCR) system and a rotary RNA sample pretreatment microdevice [3, 4]. Thus, we combined the two microsystem (rotary RT-PCR and rotary RNA purification) to realize a full integrated Rotary Genetic Analyzer in this study for on-site influenza A viruses detection with high speed.

THEORY

The integrated Rotary Genetic Analyzer is shown in Figure 1a. The Rotary Genetic Analyzer is made up of four parts including a disposable microchip (Figure 1b), three thermal blocks embedding in the rotary stage for temperature control (Figure 1c), a servo-motor for precise and high-rate spinning of the chip (Figure 1d), and a miniaturized optical fluorescence detector (Figure 1e). The RT-PCR microchip is placed on the custom-made rotary stage and fixed on the rotary axis at the center of the rotary stage. An in-house LabVIEW graphical interface (National Instruments, Austin, TX, USA) was used to automatically control the rotation of the chip and the RT-PCR through DAQ boards.

The chip was equipped with the sample, washing buffer and elution buffer reservoirs and the each reservoir was connected with the different dimension of the microchannels. Due to the hydrophobic surface and the different channel size, we could load the sample, washing buffer and elution buffer subsequently simply by changing the RPM. Burst RPMs of each channel are theoretically calculated by balancing the centrifugal pressure with the capillary pressure.

$$\Delta P = \rho \omega_c^2 \bar{r} \Delta r \quad \textit{(Centrifugal pressure)}$$

$$= 2\gamma \cos\theta \left(\frac{1}{d} + \frac{1}{w}\right) \quad \textit{(Capillary pressure)}$$

EXPERIMENT

Thermal blocks made of duralumin were used as a heater for thermal cycling. Thermal blocks were equipped with a film heater and a resistance temperature detector (RTD). A RTD film (RdF Corporation, NH, USA) made of platinum was sandwiched with two metal blocks and the film-type heater (MINCO™, MN, USA) was attached on the bottom of the thermal block (Figure 1f). The home-made thermal blocks were installed onto a recessed area on the top of the rotary stage which is made of Teflon due to the property of insulation and heat resistance, and the temperature of each block was corresponded to the thermal cycling temperature, namely 95 ℃ (denature), 58 °C (annealing), and 72 ℃ (extension). After installing the RT-PCR microchip, the upper Teflon cover which has a block-shaped hollow was moved downward to tightly seal the Rotary stage to prevent the loss of heat and maintained the uniform temperature distribution on each heat block.

A disposable RT-PCR microchip (50 × 20 × 1.125 mm) consists of a solid-phase extraction based sample pretreatment unit and 2 μL of the PCR chamber as shown Figure 2a. The microchip (1 mm thickness) was fabricated using a patterned polycarbonate sheet by a CNC milling machine, which was bonded with a polycarbonate film (125 μm thickness) by a thermal bonding method at 140° C with 1 MPa of pressure for 15 min (Figure 2b). Microglass

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beads with 150~212 μm diameter were introduced into the sample pretreatment chambers (500 μm depth) and held in place by a weir structure (100 μm depth) for constructing a solid-phase extraction system as shown in Figure 2c. The rotary hole of the microdevice was fitted into the shaft of the centrifugal system, and the rotation speed was exactly controlled by the software. Since the channel width and depth connected to the sample reservoirs were different (580 μm (width) × 500 μm (depth) for RNA solution, 250 μm × 200 μm for washing solution, and 200 μm × 50 μm for elution solution), the RNA solution, the washing buffer, and the elution buffer were sequentially injected into the bead chamber by simply tuning the RPM. As an elution buffer, 2 μL of RT-PCR cocktail without RNA template was used. The purified RNA sample was moved to the PCR chamber with the RT-PCR cocktail, and then RT-PCR was performed to amplify the target gene which was monitored by an optical fluorescent detector above the extension block.

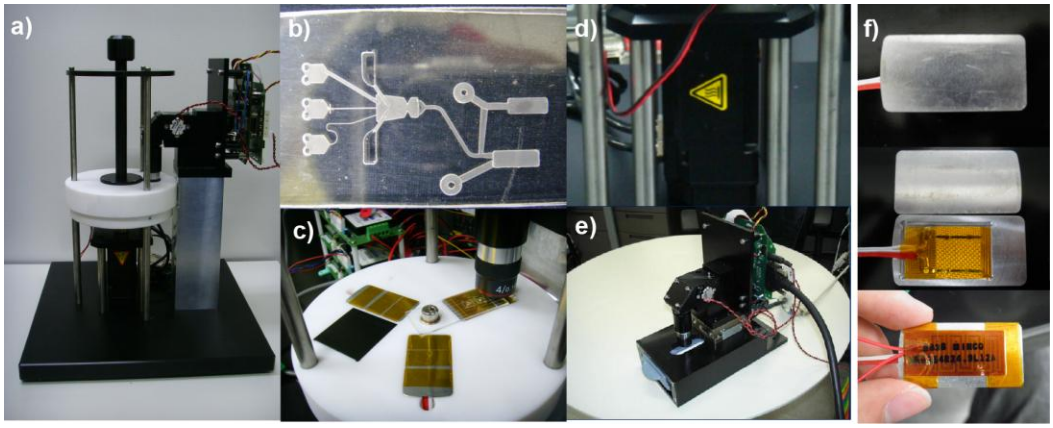

Figure 1. a) Image of the fully integrated Rotary Genetic Analyzer b) A disposable plastic PCR microchip c) A Rotary stage d) A servo-motor e) A fluorescence detector f) A thermal block; top view (top), RTD(middle), film heater (bottom).

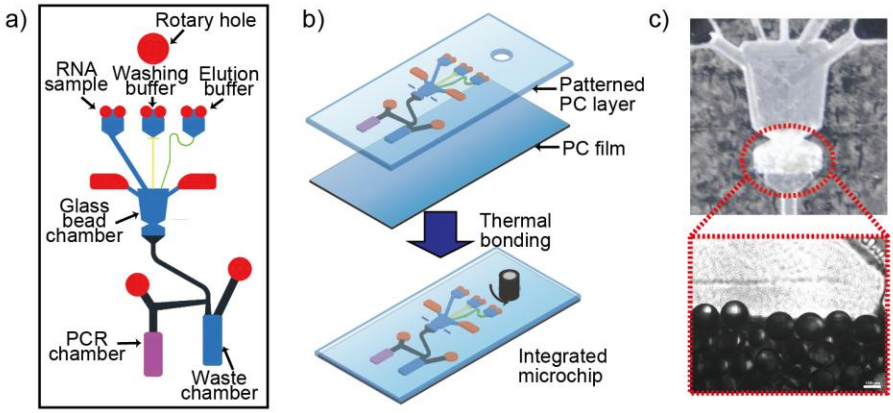

Figure 2. a) Schematic illustration of a Rotary PCR microchip b) Thermal bonding of the microchip; two layers (a patterned polycarbonate (PC) layer and a PC film) c) Packed microglass beads in the sample pretreatment chamber and an optical image of the beads (scale bar: 100 μm).

RESULTS AND DISCUSSION

Figure 3a shows that three samples were subsequently loaded into the bead chamber from (i) to (iv): the influenza A H3N1 viral RNA sample (800 RPM for 10 sec), a washing buffer (1400 RPM for 30 sec), and PCR cocktail (3500 RPM for 30 sec). After loading the washing buffer, the residual solutions in the bead chamber and the microchannel were completely removed by increasing the RPM to 2000 RPM. Since the siphon structure is introduced to an elution channel, the elution solution could be moved to the bead chamber at higher 3500 RPM. In Figure 3b, the oil was initially loaded to the PCR chamber (i), and the sample solution (ii) and the washing buffer (iii) moved to the waste chamber in a successive order. At this moment, the waste chamber was fully filled with the sample and washing solution. Finally, the PCR cocktail containing RNA sample was placed in the PCR chamber. The clear isolation of a PCR cocktail (a green solution) in the PCR chamber was shown in Figure 3c. Due to the density difference, the PCR cocktail containing RNA templates was positioned at the bottom and the mineral oil was on the top that help to prevent evaporation of a PCR cocktail during RT-PCR process.

To quantify the RNA purification yield in the microbead chamber, we set up the calibration curve by using the serially diluted influenza A H3N2 viral RNAs (Figure 4a), and performed the RNA capture on the proposed microdevice. The M and H3 genes were successfully amplified from the extracted influenza A H3N2 RNA that was confirmed in the agarose gel electrophoresis (Figure 4b). The RT-PCR was performed on the Rotary Genetic

Analyzer, and the significant fluorescence intensity difference between 0 and 40 cycles was detected due to the produced amplicons (Figure 4c). The whole process of the sample pretreatment and RT-PCR could be accomplished in 30 min on the fully integrated Rotary Genetic Analyzer system.

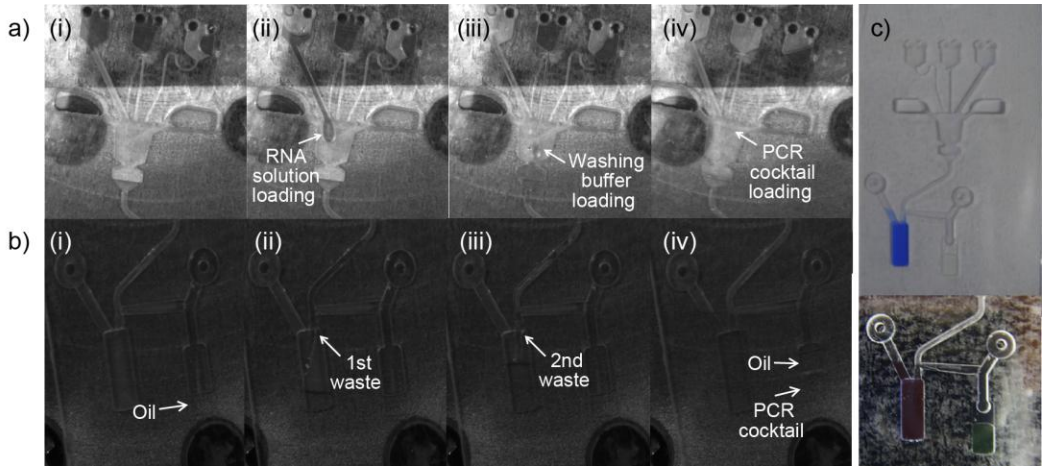

Figure 3. a) Flow control :i) RNA sample, a washing buffer, and an elution buffer in the reservoirs, ii) RNA sample loading at 800 RPM for 10 sec, iii) washing buffer loading at 1400 RPM for 30 sec, iv) PCR cocktail loading at 3500 RPM for 30 sec b) Isolation of the PCR cocktail containing target RNA: i) Oil loading to the PCR chamber, ii) 1st waste from RNA solution loading to the waste chamber, iii) 2nd waste from washing buffer loading, iv) PCR cocktail loading to the PCR chamber c) An optical image of an isolated solution in the waste and PCR chamber.

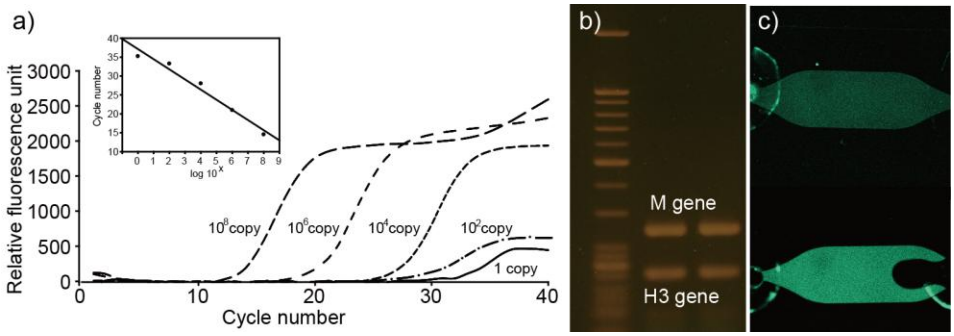

Figure 4. a) Real-time PCR calibration curve for influenza A H3N2 virus detection. The used RNA copy number was from 1 copy to 10^8 copy. (Inset: A linear regression of the cycle number versus the logarithm of copy number.) b) Agaros gel electrophoresis image for detecting H and H3 gene amplicons c) Fluorescence signal of the PCR chamber at 0 cycle (top) and 40 cycle (bottom).

CONCLUSION

We have demonstrated a fully integrated and portable Rotary Genetic Analyzer for detection of the gene expression of influenza A virus, which has 'Sample-in-answer-out' capability including sample pretreatment, rotary amplification, and optical detection. Target gene amplification was real-time monitored using the integrated Rotary Genetic Analyzer system.

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