ABSTRACT
In this study, we demonstrated a novel method of reverse transcriptase-polymerase chain reaction (RT-PCR) using a rotary system for detecting influenza A virus subtype H1N1. The Rotary Genetic Analyzer consists of three parts including a disposable plastic PCR microchip, thermal blocks for temperature control, and a stepper motor for precise rotating of the chip. The microchip is spun on the thermal blocks automatically by LabVIEW program to perform the target gene amplification by using viral RNA templates. The RT-PCR amplicons were successfully produced in 22 min which were confirmed in the capillary electrophoresis (CE) microdevice demonstrating the ultrafast H1N1 detection.

KEYWORDS: Influenza A virus, Reverse transcriptase-Polymerase chain reaction, Rotary, Virus detection

INTRODUCTION
A variety of influenza viruses has prevailed throughout the world which was damaged economically and socially over the past 100 years with a frequency of dozens of years, and continuously threatened human being as well as livestock. Influenza A virus are zoonotic pathogen and infected among human beings and several animals such as birds, pigs, and horses [1]. Due to the well-connected transportation over the world, the epidemic is rapidly contagious as shown in the case of pandemic influenza A H1N1 in 2009. In addition, mutant virus caused by antigenic shift can appear which has high fatality and infectiousness over the world. Thus, early diagnosis of such pathogen is of paramount importance for biomedical examination and public healthcare screening [2, 3]. To address this issue, we have proposed a novel Rotary RT-PCR microsystem for rapid detection of influenza A H1N1 virus.

THEORY
The integrated Rotary genetic analyzer system is shown in Figure 1a. The Rotary RT-PCR genetic analyzer consists of three parts: thermal blocks for temperature control, a disposable plastic PCR microchip, and a stepper motor for precise rotating of the microchip. The RT-PCR microchip is placed on the custom-made rotary stage and fixed at the center of the rotary stage in controlling the rotation of the chip. A LabVIEW graphical interface (National Instruments, Austin, TX, USA) developed in-house was used to automatically control the system through DAQ boards (National Instruments). Three thermal blocks are embedded in the rotary stage for thermal cycling control and a stepper motor is installed under the rotary stage for rotating the microchip for fast RT-PCR reaction.

EXPERIMENTAL
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 1b). 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. The design of the electrical circuits for temperature control with a RTD and a heater was used by modifying the previous reports [4]. A 4 mA current source was supplied to the RTD through the outer set of leads, and the measured voltage across the RTD was collected and processed using an active low-pass filter, through the DAQ board. Temperature control was accomplished by a proportion/integrator/differentiator (PID) module with a LabVIEW program, which outputs electrical signals through the DAQ board to control the power supply of the film heaters. 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 was fabricated by using a conventional soft-lithography procedure. A SU-8 50 (MicroChem, MA, USA) photoresist was spun on a Si wafer and patterned by UV exposure through the mask layout designed by AutoCAD. PDMS pre-polymer and curing agent (Sylgard 184, Dow corning, MI, USA) were mixed with a 10:1 ratio and poured onto the SU-8 master. After curing at 65 °C for 1 h, the PDMS replica was obtained, and bonded with a cover slip glass (18 mm × 18 mm, Marienfeld, Germany) by plasma treatment for 1 min. The size of the microchip was 18 × 50 × 1 mm with a 5 mm diameter hole to be tuned in the central shaft, and the volume of the PCR chamber was 1 μL as shown in Figure 1c. Thickness of the PDMS replica was reduced to 1 mm for efficient heat transfer with the heat blocks during thermocycling.
The RT-PCR chamber composed of the PDMS-glass hybrid was placed on the thermal block for direct contact with a heater.

A stepper motor was purchased from National Instruments (NEMA 23, National Instruments) and installed underneath of the Rotary stage (Figure 1d). It was fixed by a shaft installed in the center of the stage and the rotating speed can be adjusted up to 2000 rpm. The designated rotational angle and the residence time were precisely controlled by the programmed LabVIEW software.

The microchip containing the RT-PCR cocktail was loaded on the Rotary stage and Rotary RT-PCR was performed as follows. The temperatures of the three thermal blocks were set according to the scheme in Figure 2a. First, reverse transcription was carried out at 50 °C for 30 min on the block #2, and initial activation step was conducted at 95 °C for 15 min on the block #1. After adjusting the temperature of block #1 to 94 °C and block #2 to 58 °C, the microchip was spun on the thermal block #1, #2, and #3 successively for PCR amplification as shown in Figure 2b. Block #1 was designated for denature process (94 °C), block #2 was for annealing process (58 °C), and block #3 was for extension process (72 °C). After 34 thermal cycling a final extension step was performed for 10 min at 72 °C. Finally, the PCR product was collected from the microchip and mixed with a 7 μL of Hi-DiTM formamide (Applied Biosystems, CA, USA). The mixture was heated for 3 min at 95 °C and snap-cooled on ice for denaturation of the amplicon before μCE analysis.

RESULTS AND DISCUSSION

For accurate temperature control, calibration of the thermal blocks was conducted. The calibration curve of RTD for each three thermal blocks shows the linear correlation between the temperature and the measured voltage (Figure 3a). Therefore, fitted thermal blocks on the stage were stably maintained with a desired temperature during PCR (Figure 3b). The microchip moved on the stabilized heat block continuously for thermal cycling process. The motor programmed by LabVIEW precisely located the microchip on the each thermal blocks and the time for moving the chip between the blocks was 50 ms.

![Figure 1: a) Image of the integrated Rotary RT-PCR system b) A disposable plastic RT-PCR microchip c) A stepper motor d) A thermal block; top view (left), RTD (middle), film heater (right).](image)

![Figure 2: a) Schematic illustration of the spinning microchip by the rotary system, b) Thermal cycling profile for the influenza A H1N1 virus.](image)

The influenza A H1N1 virus should give two peaks as shown in Figure 4: one is for H1 gene (102 bp) and the other is for M gene (295 bp). The generated two amplicons were analyzed on CE microdevice within 5 min. As shown in Figure 4a, two peaks were clearly displayed representing H1 gene and M gene, confirming influenza A virus subtype H1N1. We controlled the reaction time by tuning the time of denature(D)/ annealing(A)/ extension(E). When the D, A, and E was set to 60, 60, 60 sec (total 102 min), which are the same protocol with a conventional thermocycling method, the strong two peaks appeared in
the electropherogram (Figure 4a). The elution time of each peak was 300 sec for H1 gene and 500 sec for M gene, respectively. We reduced the time to 68 and 22 min (D/A/E = 30/60/30 sec and 10/20/10 sec, respectively) as shown in Figure 4b and c. Influenza A H1N1 virus confirming peaks were successfully displayed even 22 min, which is 4.5-fold reduced time compared with a conventional method.

**CONCLUSION**

We have demonstrated a novel Rotary RT-PCR system for ultrafast detection of influenza A virus subtype H1N1, which is successfully detected within 22 min Rotary amplification. This novel Rotary genetic analyzer platform enables us to perform on-site pathogen detection with high speed.

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