A HIGH-SPEED HIGH-PERFORMANCE FULLY INTEGRATED RT-PCR MICROCHIP

Nari Han, Ki-Ho Han
Inje University, South Korea

ABSTRACT

This paper presents a fully integrated RT-PCR microchip, including genetic functionalities of mRNA extraction, cDNA synthesis, and DNA amplification. By using lateral magnetophoresis and magnetic oligo-dT beads, mRNA from blood lysate was extracted within 1 min. In an on-chip integrated DNA microchamber, cDNA was synthesized with the extracted mRNA with an external thermal control system and target genes were then amplified. By detection of human housekeeping genes of β-actin and GAPDH, feasibility of the RT-PCR microchip was verified. Furthermore, sensitivity of the RT-PCR microchip was evaluated by reducing down blood volume of 100 to 0.1 μl

KEYWORDS

cDNA synthesis, Lateral magnetophoresis, Magnetic oligo-dT beads, RNA extraction, RT-PCR microchip.

INTRODUCTION

Extraction of high-quality RNA from crude biological sample is critical to obtain accurate RT-PCR performance. Because RNA is however readily destroyed by ubiquitous RNases, it is difficult to extract high-quality RNA without contamination and degradation by RNases. To solve the problem, our group previously reported a reverse transcription (RT)-microchip [1], which was realized with the high-speed mRNA microextraction method [2] and the DNA microchamber. Whole RT-PCR process with the RT-PCR microchip has been accomplished by using a commercially available PCR machine for DNA amplification. As an advanced form, this paper introduces a fully integrated RT-PCR microchip, which can perform the whole RT-PCR process such as mRNA extraction, cDNA synthesis, and DNA amplification in a single microchip (Figure 1).

EXPERIMENT

The RT-PCR microchip was fabricated by a bottom glass, with an inlaid ferromagnetic wire array, and a top PDMS replica, formed the microchannel and DNA microchamber (Figure 2). The ferromagnetic permalloy (NiFe) wires were electroplated onto the glass substrate evaporating Ti/Cu/Cr seed layer. The SU-8 mold was then fabricated using a lithographic technique. PDMS prepolymer and a curing agent were thoroughly mixed at 10:1. The mixture was then poured on the SU-8 mold and cured at 85℃ for 40min. After curing, PDMS replica was peeled from the SU-8 mold. Before bonding the PDMS replica and the glass substrate, input and output access ports were punched in the replica. The PDMS replica was then attached to the bottom glass via plasma bonding using a Tesla coil. It consists of three inlets (sample, buffer, and reagent), two outlets (waste, DNA), RNA extraction microchannel containing inlaid ferromagnetic wire array, and the DNA microchamber, as shown in Figure 3(A). When an external magnetic field was applied, the magnetic oligo-dT beads with bound mRNA were laterally drawn along the ferromagnetic wires (Figure 3(B)) and flowed into the DNA microchamber (Figure 3(C)).

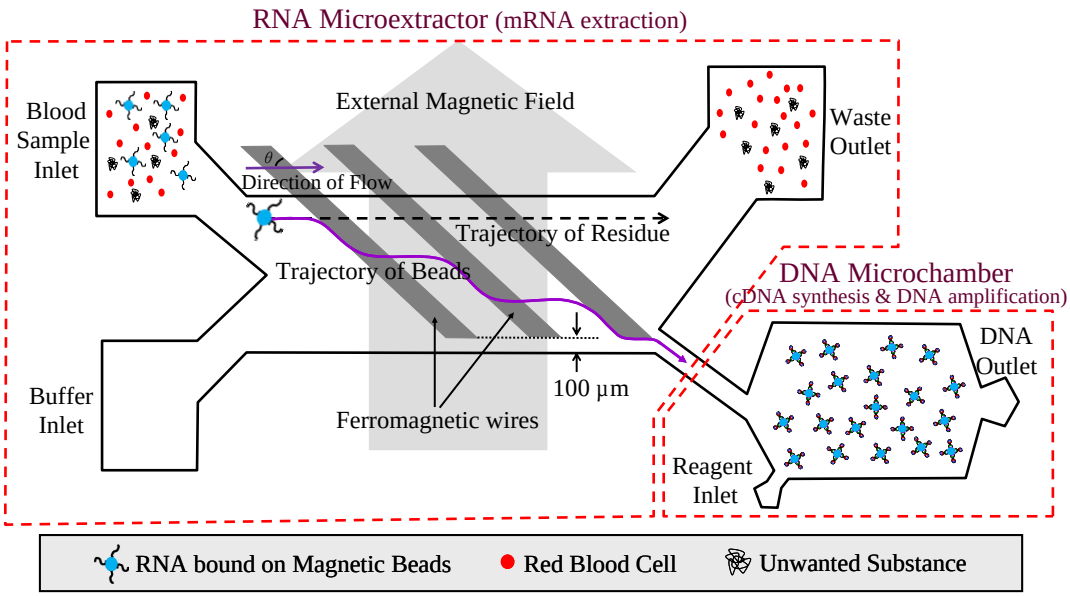

Figure 1. Architecture of the RT-PCR microchip, including the RNA microextractor and the DNA microchamber

978-0-9798064-5-2/μTAS 2012/$20©12CBMS-0001

The overall mRNA extraction procedure completed just within 1 min, which is compared with approximately 1 h of conventional methods. After RT-PCR reagent is injected through the reagent inlet, the extracted mRNA binding on the magnetic beads was synthesized to cDNA in the DNA microchamber. The target genes were then amplified in the DNA microchamber.

RESULTS AND DISCUSSION

To verify the feasibility of the present RT-PCR microchip, human housekeeping genes of β-actin (Figure 4(A)) and GAPDH (Figure 4(B)) were amplified using blood lysate. Its sensitivity was also evaluated by detecting human β-actin with reduced blood volume. The experimental results showed that the present RT-PCR microchip can be used to successfully detect the β-actin gene from just a 0.1-μl blood volume (Figure 5). It explains that the proposed RT-PCR method using the RT-PCR microchip is much higher sensitive than conventional methods using the silica column matrix and the magnetic oligo-dT beads [1]. The proposed RT-PCR method has also advantages such as minimizing usage of reagent volume and reducing process time of the whole RT-PCR (Table 1). Consequently, by using the RT-PCR microchip, the whole RT-PCR process (involving mRNA extraction, cDNA synthesis, and DNA amplification) could be done in easy and fast without exposure of RNA to the environment, thereby achieving highly sensitive genetic assays. As future works, through an integration with the following genetic analysis functionality (*e.g.* capillary electrophoresis), the present RT-PCR microchip could be fully automated, thereby leading a global standardization in genetic analysis.

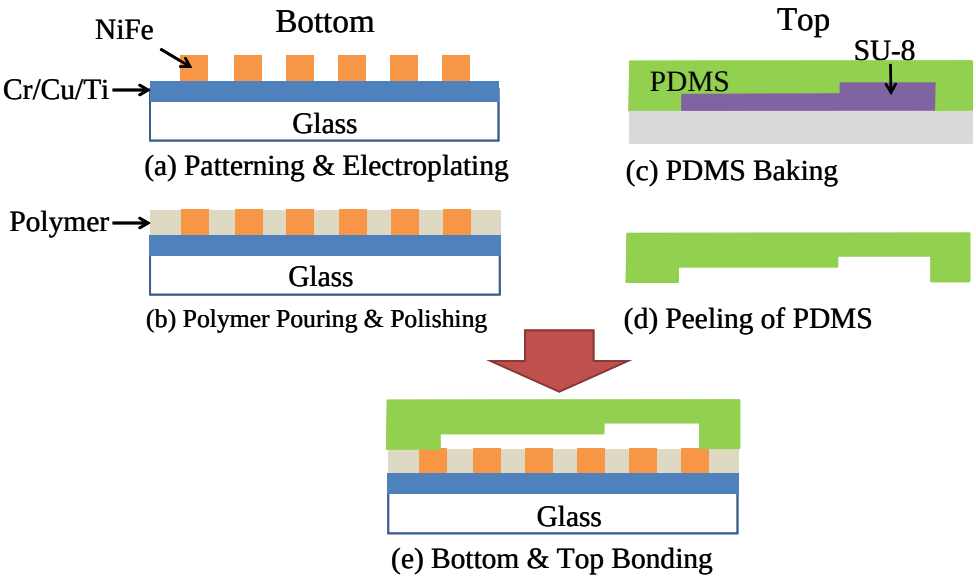

Figure 2. The fabrication process of the RT-PCR microchip

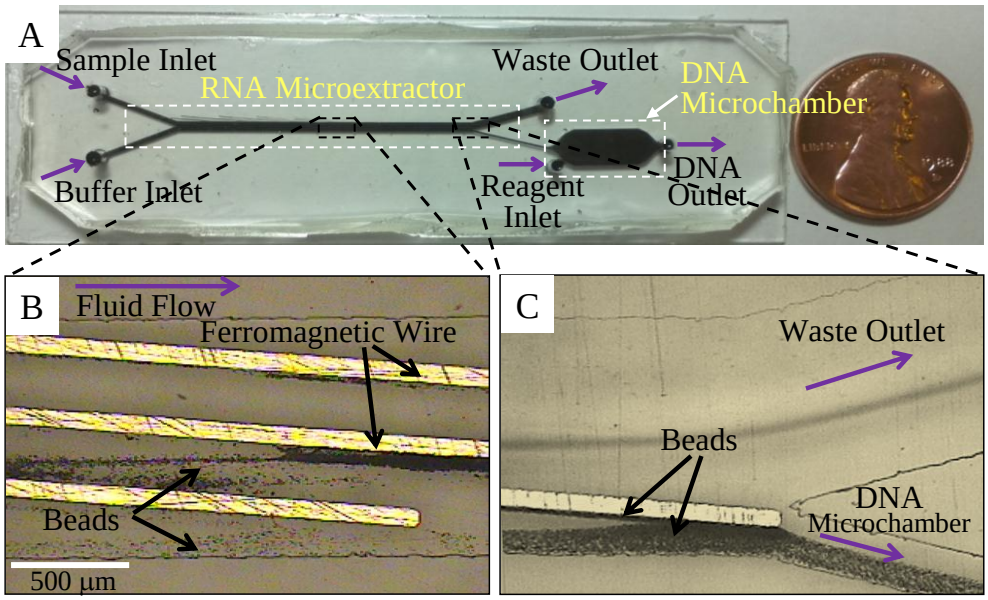

Figure 3. Photomicrographs of (A) the fabricated RT-PCR microchip; (B) and (C) the enlarged microchannel

Table 1. Comparison of process times for RT-PCR procedures based on the silica matrix column (written as "Column"), magnetic oligo-dT beads ("Bead"), and "RT-PCR microchip" methods

Product	Protocol of RT-PCR	Conventional Methods		Proposed Method
		Column	Bead	RT-PCR Microchip
Blood Lysate	RBC Lysis	40 min	10 min	-
	WBC Lysis	1 min	1 min	1 min
	Homogenize	5 min	-	-
RNA Extraction	Preparation of Beads	-	3 min	3 min
	RNA Binding	-	5 min	5 min
	RNA Extraction	10 min	20 min	< 1 min
	Elution	3 min	-	-
Total Time for RNA Extraction		59 min	39 min	< 10 min
cDNA	cDNA Synthesis	1 h	1 h	1 h
PCR	DNA Amplification	1 h 30 min	1 h 30 min	1 h 30 min
Total Time for RT-PCR Process		3 h 21 min	3 h 9 min	< 2 h 40 min

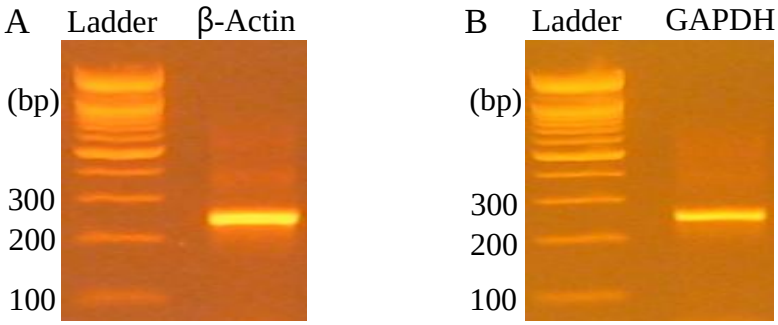

Figure 4. Gel electrophoresis photograph of (A) β–actin gene (244 bp) and (B) GAPDH gene (250 bp)

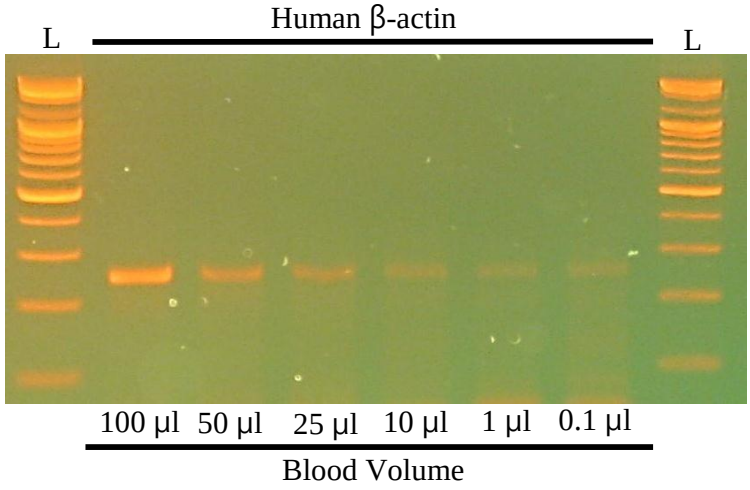

Figure 5. Gel electrophoresis photograph of β–actin gene (244 bp) at various blood volumes of 100 to 0.1 µl

REFERENCES

[1] H. Lee, K.-H. Han, *A high-speed high-performance reverse transcription microchip*, MicroTAS2011, Seattle, USA (Oct.2-6) pp.747-749 (2011).

[2] H. Lee, J. Jung, S.-I Han and K.-H. Han, *High-speed RNA microextraction technology using magnetic oligo-dT beads and lateral magnetophoresis*, Lab on a Chip, vol.10, pp.2764-2770 (2010).

CONTACT

Ki-Ho Han +82-55-320-3715 or mems@inje.ac.kr