HIGH-SPEED RNA MICROEXTRACTION TECHNOLOGY USING MAGNETIC OLIGO-dT BEADS AND LATERAL MAGNETOPHORESIS

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ABSTRACT

This paper presents a high-speed RNA microextractor for the direct isolation of RNA from blood lysate using magnetic oligo-dT beads. The extraction is achieved through lateral magnetophoresis, generated by a ferromagnetic wire array inlaid on glass substrate. This RNA microextractor separated more than 80% of magnetic beads with a flow rate up to 20 ml/h, and the overall extraction procedure was completed within 1 min. The absorbance ratio of RNA to protein (A_{260}/A_{280}) was > 1.7, indicating that the extraction technology yielded nearly pure RNA. The feasibility of this technique was further evaluated for its applicability to RT-PCR procedures by performing cDNA synthesis and PCR.

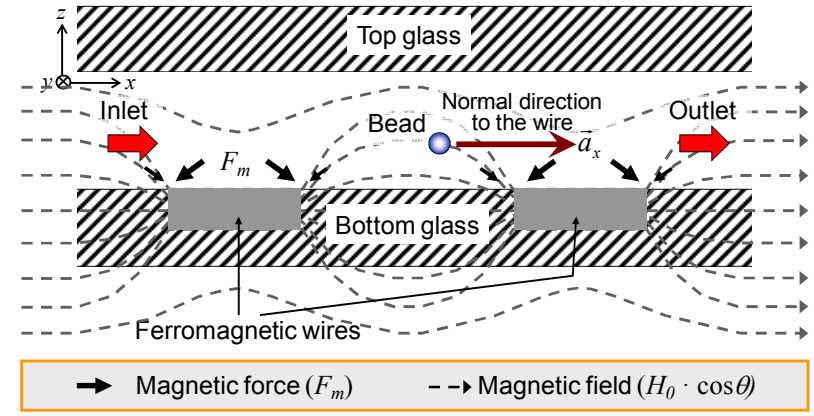
KEYWORDS: Extraction, Lateral magnetophoresis, Magnetic oligo-dT beads, RNA

INTRODUCTION

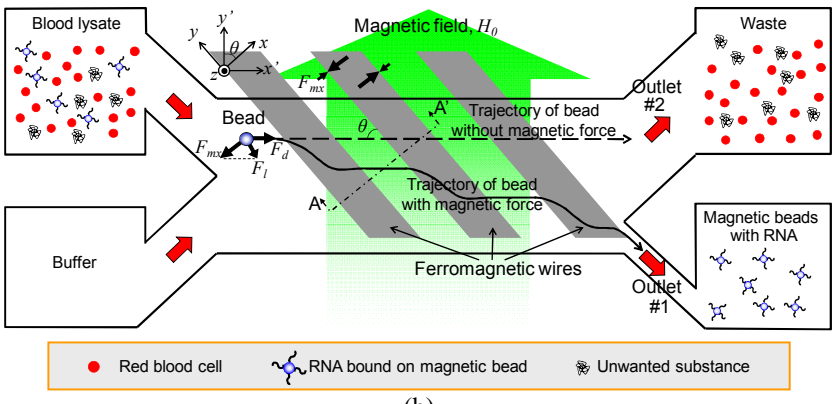
RT-PCR is a powerful genetic analysis technique for measuring gene expression. The extraction of high-quality RNA from crude biological samples is critical to obtain rapid and accurate RT-PCR performance. Conventional RNA extraction methods [1] expose purified RNA to the outside environment, risking contamination and degradation by RNases at any step in the extraction procedure. To avoid the limits, we introduce a high-speed RNA microextractor, which is a microchip-based device in a closed format to prevent RNase contamination and an integrated format for obtaining high-quality RNA without the need for highly trained personnel.

THEORY

Consider a high-gradient magnetic field generated near an inlaid ferromagnetic wire (Fig. 1(a)). As magnetic oligo-dT beads are passing over the ferromagnetic wire array placed at an angle of θ to the direction of flow (Fig. 1(b)), they experience magnetic force F_m with hydrodynamic drag force F_d. As a result, the magnetic beads are laterally driven into outlet #1 and other components in the crude lysate sample waste out through outlet #2 in Fig. 1(b). [2]



(a)



(b)

Figure 1: Working principle of the high-speed RNA microextractor. (a) The ferromagnetic wire array inlaid on the glass substrate with a high-gradient magnetic field; and (b) architecture of the high-speed RNA microextractor, including the ferromagnetic wire array placed at an angle of θ to the direction of flow.

EXPERIMENTAL

The high-speed RNA microextractor was fabricated using two Borofloat™ glass slides and SU8-to-glass adhesive bonding (Fig. 2(a)). It consists of two inlets, two outlets, and a piecewise curved ferromagnetic nickel wire array inlaid on the glass substrate. The sample and buffer inlets are used for injecting crude lysate sample mixed oligo-dT magnetic beads and PBS buffer solution, respectively. The outlet #1 was designed for extracting the beads and the outlet #2 for removing the rest of the lysate components. To enrich beads, the width of outlet #1 was designed to be 200 µm, one quarter if the width of outlet #2 (800 µm). The width and thickness (50 µm and 10 µm, respectively) of the ferromagnetic wire were designed to generate a high gradient magnetic field. To reduce aggregation and binding of the magnetic beads on the sidewall of the microchannel with the permanent magnet, the ferromagnetic wire was designed as a piecewise curve with three bend angles of 5.7°, 7.1°, and 11.3° relative to the direction of flow, and patterned 100 µm from the sidewall of the microchannel (Fig. 2(b)).

RESULTS AND DISCUSSION

With no external magnetic field, the magnetic beads flow into outlet #2 (Fig. 3(a)) along with the rest of the lysate. On the other hand, when an external magnetic field was applied, the magnetic beads were drawn laterally and flowed into outlet #1, while the remainder of the lysate still flowed into outlet #2 (Fig. 3(b)). Figure 4(a) shows the separation efficiency of beads extracted from outlet #1 for various sample flow rates from 10 to 25 ml/h. The result shows that the high-speed RNA microextractor separated more than 80% of the magnetic beads with a flow rate up to 20 ml/h, and the overall extraction procedure was completed within 1 min, compared with conventional column-type RNA purification methods requiring about 1 h to complete. The average absorbance ratio of RNA to protein (A_{260}/A_{280}) (Fig. 4(b)) was greater than 1.7, indicating that the extracted RNA was sufficiently pure for RT-PCR procedures. Figure 5 shows representative gel-electrophoresis data of the RT-PCR for human β-actin gene (219-bp) product using RNA isolated from blood lysate using the high-speed RNA microextractor. Consequently, the experimental results verified that the high-speed RNA microextraction technology is a practical method for easy, rapid, and high-precision RT-PCT using minimal reagent volumes without requiring highly trained personnel.

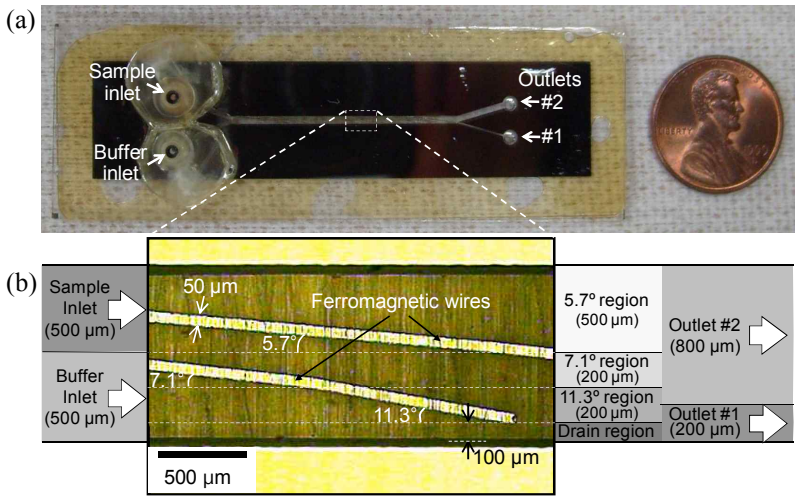


Figure 2: Photographs of the fabricated high-speed RNA microextractor and the enlarged microchannel.

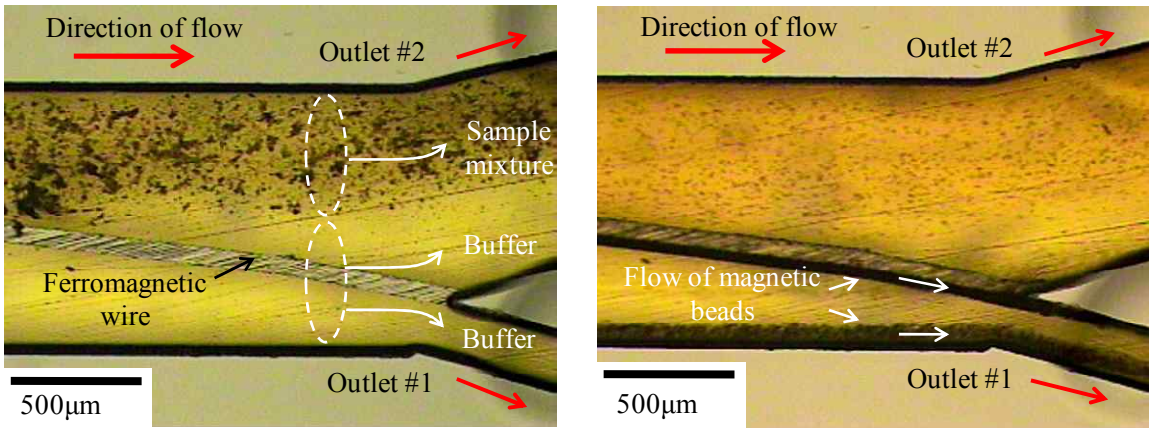


Figure 3: Sample and buffer solutions passing through the microchannel of the RNA microextractor (a) without an external magnetic field; and (b) with an external magnetic flux of 0.14 T at sample and buffer flow rates of 15 ml/h, respectively.

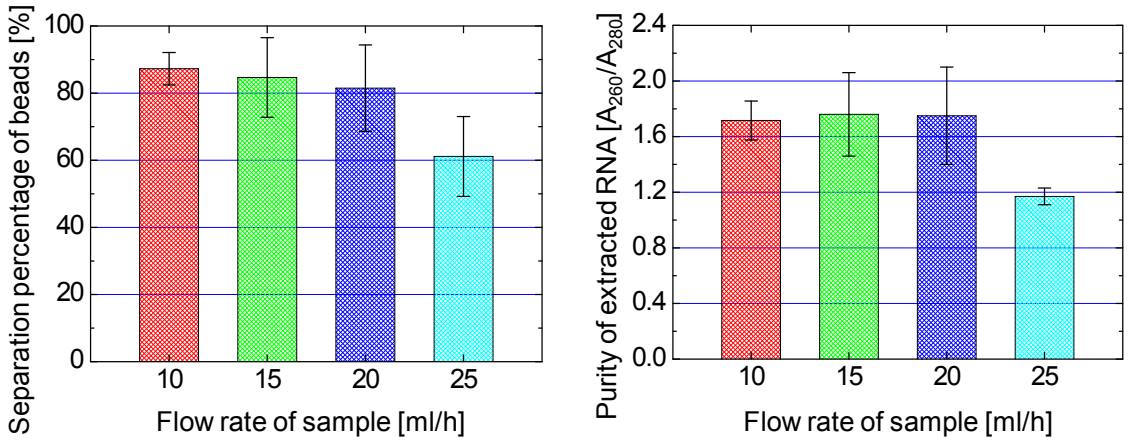


Figure 4: (a) Relative separation percentage of the magnetic beads extracted from outlet #1 with various the sample flow rates; and (b) purity of the RNA preparation for various the sample flow rates. The error bars represent one standard deviation calculated from three data sets.

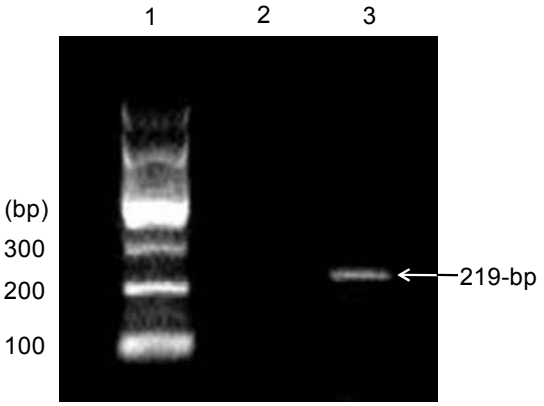


Figure 5: RT-PCR results using human blood lysate RNA extracted from the RNA microextractor.

CONCLUSION

In this study, we introduced a high-speed RNA microextractor for isolating RNA from human blood lysate using magnetic oligo-dT beads and lateral magnetophoresis. The experimental results showed that the microextractor continuously separated more than 80 % of the magnetic beads from human blood lysate at sample flow rates up to 20 ml/h; notably, the procedure was completed in just 1 min. RNA spectrophotometric measurements showed that the absorbance ratio of RNA to protein (A_{260}/A_{280}) was > 1.7, indicating that the purity of the RNA preparation was sufficient for RT-PCR procedure. In addition, through subsequent cDNA synthesis and PCR with the extracted RNA preparation, we further verified that the microextractor was effective for RT-PCR. We believe that the present RNA microextraction technology may provide a practical method for easy, rapid, and high-precision RT-PCR using minimal reagent volumes without the need for highly trained personnel. As the RNA microextractor can be readily integrated with other downstream functional compartments (e.g., cDNA synthesis and PCR), the RNA microextractor could be used for developing microscale automated platforms for genetic analysis systems.

ACKNOWLEDGEMENTS

This work was supported by Basic Science Research Program (Grant No. D00771) and by Mid-career Researcher Program (Grant No. 2009-0083956) through the National Research Foundation (NRF) of Korea funded by the Ministry of Education, Science and Technology (MEST).

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[2] "Lateral-driven continuous magnetophoretic separation of blood cells," J. Jung, K.-H. Han, Applied Physics Letters, **93**, 223902 (2008).

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