

Automated microfluidic system for RNA purification using a centrifugal force

Byung Hyun Park¹, Jae Hwan Jung¹, Hainan Zhang², Nae Yoon Lee^{*2} and Tae Seok Seo^{*1}

¹Department of Chemical and Biomolecular Engineering (BK21 program), Korea Advanced Institute of Science and Technology (KAIST), Daejeon, South Korea.

²Department of BioNano Technology, College of BioNano Technology, Gachon University, Seongnam-si, Gyeonggi-do, South Korea.

ABSTRACT

Herein, we demonstrated a simple, rapid and automated RNA purification system based on a centrifugal force using three passive capillary valves and a silica sol-gel matrix. We designed three reservoirs (sample, washing solution, elution buffer) which were connected with different dimensional microfluidic channels. By controlling RPM, we could dispense the RNA sample, washing solution, and elution buffer successively, so that the RNA was captured in the sol-gel solid phase, purified, and eluted. This novel rotary sample preparation system eliminates any complicated hardware and human intervention, and performs the RNA preparation with high speed and portability.

KEYWORDS

Nucleic acid purification, RPM control, Centrifugal force

INTRODUCTION

Nucleic acid purification is an essential step for many biological diagnostics. In particular, the on-site virus detection system requires the extraction of RNA/DNA from biological samples with ease and high rapidity. However, the conventional nucleic acid extraction method such as phenol extraction or ethanol precipitation is highly labor intensive and time-consuming due to numerous manual steps [1], and it is not adequate for the portable sample preparation. To address this issue, several microfluidic systems for the purification of nucleic acid were reported [2-3], but these microfluidic systems need the necessity of mechanical external syringe pumps to allow flow control and complex tube lines to connect the microfluidic system with syringe pumps, which is the bottleneck to realize an integrated micro-total-analysis-system. Therefore, more simple and automated microfluidic platform without external pumps is necessary. To this end, we have developed a centrifugal force based microdevice to easily purify the RNA with ease.

THEORY

To perform the automated RNA purification using the proposed microdevice, a driving force, namely a centrifugal force in our case, is required to overcome the capillary pressure derived from the hydrophobic surface and the narrow microchannel dimension. Since we designed the channel widths differently (120 μm for C_S , 40 μm for C_W , 20 μm for C_E in Fig. 1), there should be a unique critical burst RPM value for each microchannel to induce the flow of liquid in the reservoirs.

To predict the critical burst RPM, we adopted a simple model by balancing the pressure induced by a centrifugal force with the pressure induced by a capillary force that is given by the Young-Laplace equation .

$$\rho\omega_c^2\bar{r}\Delta r = 2\gamma\cos\theta\left(\frac{1}{d} + \frac{1}{w}\right) \quad (1)$$

where ρ is the liquid density, Δr is the length of liquid plug, \bar{r} is the mean radial position of the liquid plug, γ is the liquid surface tension, θ is the contact angle, d is the depth of the channel, and w is the width of the channel.

EXPERIMENT

The RNA purification microdevice consists of three layers as shown in Fig. 1. The middle PDMS layer patterned with microfluidic channels were fabricated by soft lithography. After punching the reservoirs, it was thermally bonded with the cover glass at the bottom. Then, a PDMS membrane was assembled on the PDMS layer. The dimensions of each microfluidic channel connected to three reservoirs were 120x50 μm (sample), 40x50 μm (washing solution), and 20x50 μm (elution buffer), respectively. The sol-gel matrix was synthesized as follows [4]. 2 mL tetramethyl orthosilicate (TMOS) was added to a solution of 0.44 g polyethylene glycol (PEG) in 10 mL of 0.01M acetic acid, and the mixture was stirred with a magnetic bar at 300 RPM in the ice bath for 45 min. Prior to introducing a hydrolyzed sol solution into the sol-gel chamber, the microdevice was exposed to UV ozone to form a hydroxyl functional group on the PDMS surface that enhances gelation reaction. 0.8 μL of the sol solution was injected into the sol-gel chamber via an injection hole using a pipette, and then the microdevice was incubated in a square dish at 40 $^\circ\text{C}$ for 12 h. The same procedure for sol-gel preparation was repeated twice to increase the packing of the sol-gel matrix in the microchamber.

For on-chip RNA purification, 5 μL of a sample solution containing a synthetic RNA or biological samples (off-chip purified H1N1 viral RNA or a real H1N1 virus lysate sample), 5 μL of the washing solution, and 5 μL of the elution buffer were loaded in the sample reservoir, washing reservoir, and elution reservoir, respectively (Fig. 1). The microdevice was fixed on the rotary system, and the centrifugal force-driven RNA extraction process was executed. The rotation procedure was as follows: loading the sample solution at 1600 RPM for 15 s, incubating the

RNA sample in the sol-gel chamber at +960~960 RPM for 2 min, loading the washing solution at 2000 RPM for 45 s, removing the waste at the R_O , loading the elution buffer at 2500 RPM for 2 min, and finally recovering 5 μL of the purified RNA in the R_O . 2 μL of the eluted solution was used for quantification by a Nanodrop spectrophotometer (ND-1000, Wilmington, DE) to determine the RNA capture yield in the sol-gel chamber, and 2 μL of RNA solution was employed for RT-PCR

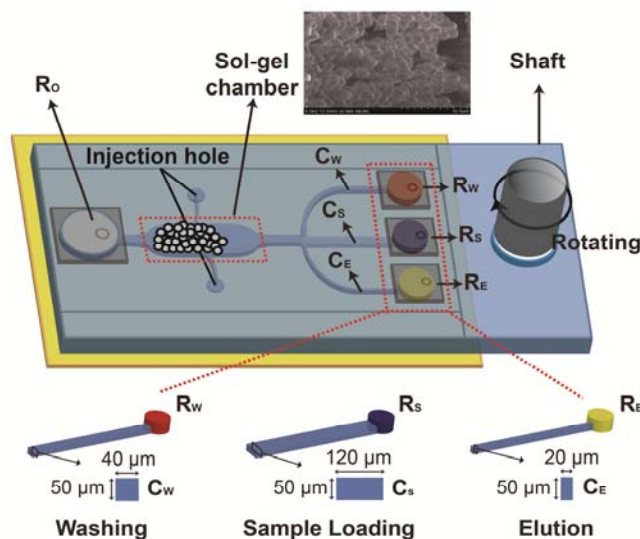


Figure 1: Schematic image of the sample pretreatment microdevice. (R_S : sample reservoir; R_W : washing solution reservoir; R_E : elution buffer reservoir; R_O : outlet reservoir; C_S :120 μm width channel, C_W : 40 μm width channel, C_E : 20 μm width channel)

RESULT AND DISCUSSION

To demonstrate the effectiveness of sequential flow of liquids by controlling RPM, 5 μL of aqueous dye solutions were loaded in the R_W (red), R_S (blue), R_E (yellow), respectively. Experimentally, we gradually increased the RPM to determine the critical RPM values for loading the sample, washing solution, and elution buffer. The theoretical versus experimental critical burst rotational speed was plotted in Fig. 2. The experimental burst RPM was matched with the theoretical one with the standard deviation of 12.7% for the sample loading, 6.8% for the washing solution loading and 7% for the elution buffer loading.

Then we examined the RNA capture efficiency in our microdevice using the FITC-labeled synthetic RNA. Fig. 3 shows the fluorescence image of the sol-gel after the FITC-labeled synthetic RNA loading and washing step. The uniform fluorescence signal implies that the successful homogenous synthesis of sol-gel matrix and the efficient RNA capture in the solid matrix. The fluorescence intensity of Fig. 3 (left) was 1303, while that of Fig. 3 (right) was 197, indicating that ~84% of the fluorescence signal was reduced after the elution. This value is comparable with the RNA capture yield of ~80% measured by Nanodrop spectrophotometer.

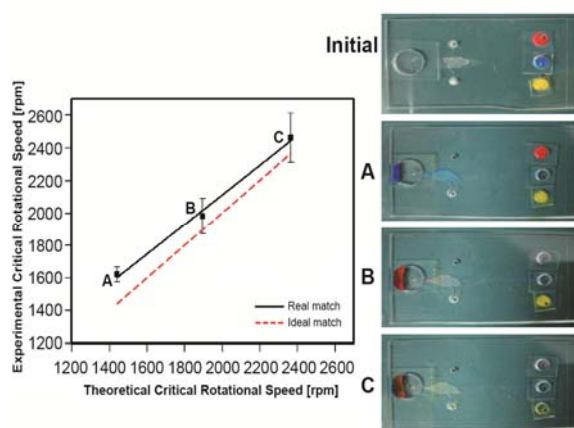


Figure 2. Theoretical versus experimental critical rotational speed. RPM control subsequently eluted (A) the blue solution at 1600 rpm for 15s in R_S , (B) the red solution at 2000 rpm for 60s in R_W , and (C) the yellow solution at 2500 rpm for 120s in R_E .

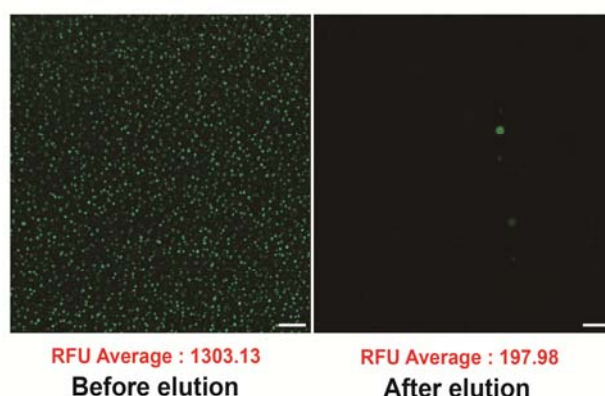


Figure 3. The fluorescence images of the sol-gel chamber before (left) and after (right) elution step, Quantitative analysis of the fluorescence intensity showing the capture yield of ~80%

Next, we employed the off-chip based purified influenza H1N1 RNA in order to mimic the real RNA sample purification and to confirm that the recovered RNA can be used as a template for gene expression in the RT-PCR. Fig. 4 shows that H1 gene (102 bp) and M gene (160 bp) were successfully amplified from the H1N1 RNA template in all cases. Even 0.0625 ng/ μ L concentration produced clearly two bands in the agarose gel electrophoresis.

Finally, we carried out the RNA purification by directly using the virus lysate to demonstrate the capability of our microdevice for the RNA extraction from a real sample. As shown in Fig. 5, the product bands of both the H1 and M gene were successfully obtained, and the signal from the off-chip purified RNA (1 lane in Fig. 5) was comparable to that obtained from the on-chip purified RNA (2 lane in Fig. 5).

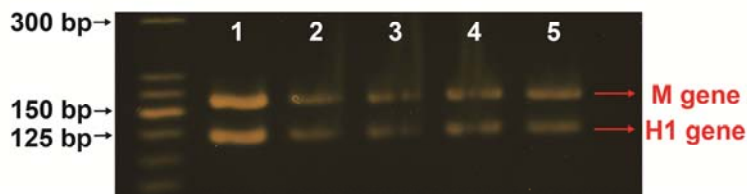


Figure 4. Automatic extraction of the purified H1N1 viral RNA. The serially diluted RNA sample was extracted and amplified using RT-PCR. (1: 1ng/ μ L, 2: 0.5ng/ μ L, 3: 0.25ng/ μ L, 4: 0.125ng/ μ L, 5: 0.0625ng/ μ L)

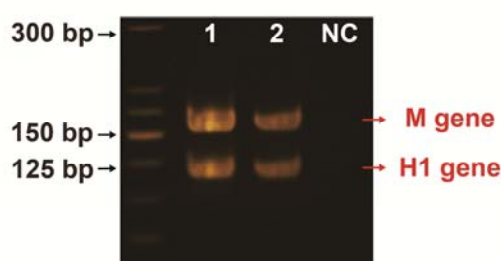


Figure 5. RNA extraction using real H1N1 virus sample and the RT-PCR results (1: Off-chip purified H1N1 RNA RT-PCR products, 2: On-chip purified H1N1 RNA RT-PCR products, NC: negative control)

CONCLUSION

In this study, we have demonstrated a highly simple, rapid and automatic RNA purification microsystem by using a rotary platform and a sol-gel incorporated microdevice. Simple RPM control could elute selectively the designated solution depending on the channel dimension. The entire process of RNA purification could be executed in 5 min with ~80% capture yield. Further integration of a DNA/RNA sample pretreatment unit into the PCR amplification microdevice is under way on the rotary platform to realize a 'sample-in-answer-out' genetic analysis microsystem.

ACKNOWLEDGEMENT

This work was supported by the R&D Program of MKE/KEIT [10035638], the Innopolis Daegu of MKE, and Technology Development Program for Agriculture and Forestry, Ministry for Agriculture, Forestry and Fisheries, Republic of Korea.

REFERENCES

- [1] P. Chomczynski, N. Sacchi, Single step method of RNA isolation by acid guanidine thiocyanate-phenol-chloroform extraction, *Anal. Biochem.*, **162**, 156, (1987).
- [2] K. A. Hagan, J. M. Bienvenue, C. A. Moskaluk, and J. P. Landers, Microchip-Based Solid-Phase Purification of RNA from Biological Samples, *Anal. Chem.*, **80**, 8453, (2008).
- [3] B. C. Satterfield, S. Stern, M. R. Caplan, K. W. Hukari, and J. A. A. West, Microfluidic Purification and Preconcentration of mRNA by Flow-Through Polymeric Monolith, *Anal. Chem.*, **79**, 6230, (2007).
- [4] Q. Wu, J. M. Bienvenue, B. J. Hassan, Y. C. Kwok, B. C. Giordano, P. M. Norris, J. P. Landers and J. P. Ferrance, Microchip-Based Macroporous Silica Sol-Gel Monolith for Efficient Isolation of DNA from Clinical Samples, *Anal. Chem.*, **78**, 5704-5710, (2006).

CONTACT

T. S. SEO, Tel: +82-42-350-3973; seots@kaist.ac.kr