NUCLEIC ACID EXTRACTION MICRODEVICE AND ITS MICROFLUIDIC PROTOCOL OPTIMIZATION

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ABSTRACT
This paper presents design, fabrication and microfluidic protocol optimization of a nucleic acid (NA) extraction microdevice having a silicon dioxide (SiO_2) surfaced simple microchannel for application in the integrated microsystem for infectious disease diagnostics.

KEYWORDS: Viral RNA Extraction, Protocol Optimization, Solid Phase Extraction, Point-of-Care Microsystem

INTRODUCTION
Since McCormick [1] introduced a method for deoxyribonucleic acid (DNA) extraction, known as solid-phase extraction (SPE), SPE using silica material [2] has become an essential technology for integrated point-of-care (POC) microsystem [3]. Recently, Price *et al.* provided an overview of these techniques [4], however it is still not easy to find the optimal solution for the microfluidic operational conditions to conduct the SPE in microstructure. Thus, this paper focuses on microfluidic protocol optimization through the experimental parameter study of SPE process on-chip.

DESIGN, FABRICATION AND PACKAGING
Figure 1 and 2 shows conceptual diagram of integrated microdevice and its microelectrofluidics packaging for SPE experiment. In fact, illustrated integrated microdevice (Fig.1) contains SPE section as well as microRT-PCR chamber for integrated process of viral RNA detection from blood sample. The fabricated microchip as well as microelectrofluidic plastic packaging is shown in Fig.3. The geometry of the silicon microchannel is 300μm-depth with 250μm-width having over 1cm^2 of total surface area.

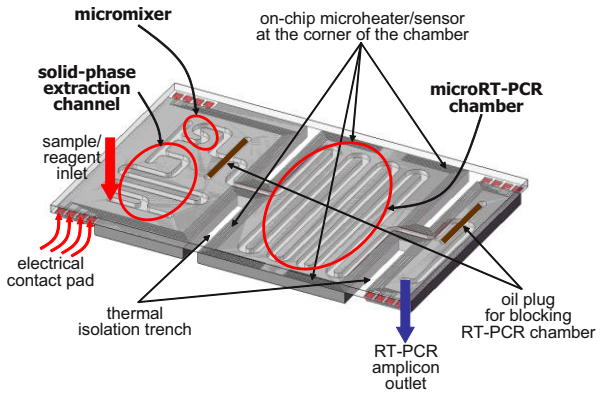


Figure 1: Conceptual drawing of the monolithically integrated microfluidic device for automated microscale viral RNA sample preparation process from blood sample. Shown integrated microchip contains SPE channel on the left hand side as well as microRT-PCR chamber on the center part of the chip for application to the viral RNA detection from blood sample.

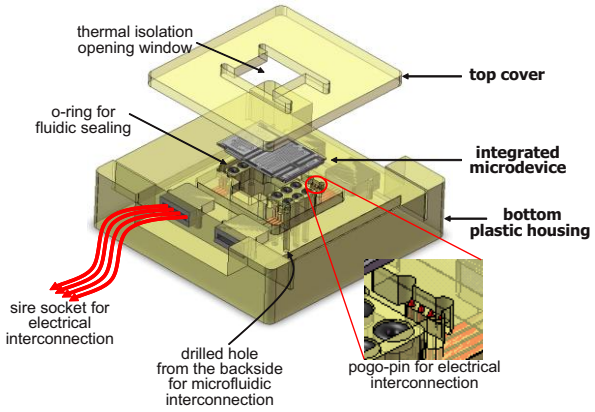
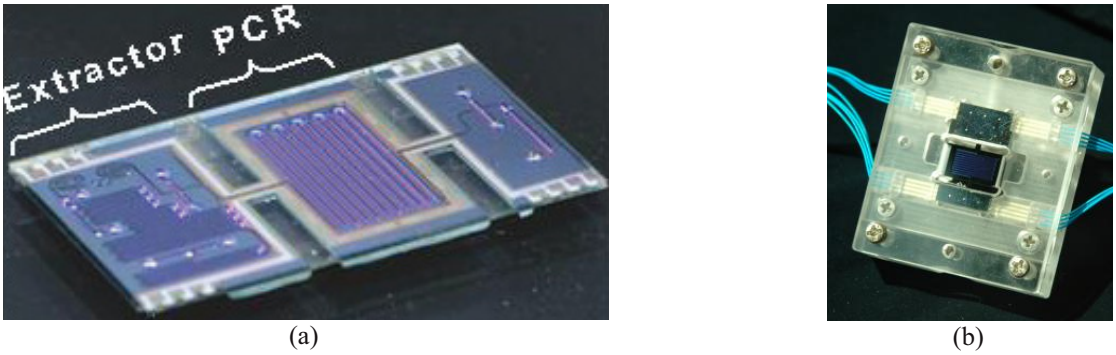


Figure 2: Perspective view of microelectofluidic packaging for integrated micro sample preparation module.



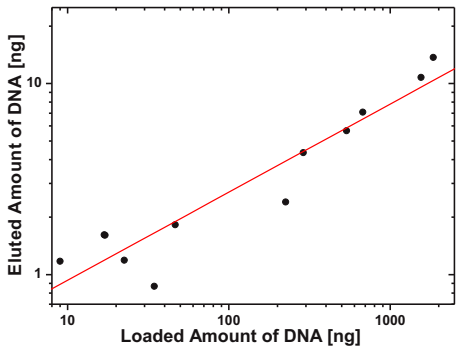
(a) (b)

Figure 3: Photographs of the integrated micro sample preparation module: (a) monolithically integrated microfluidic device for NA extraction with RT-PCR; (b) microelectrofluidic packaging for integrated sample preparation module.

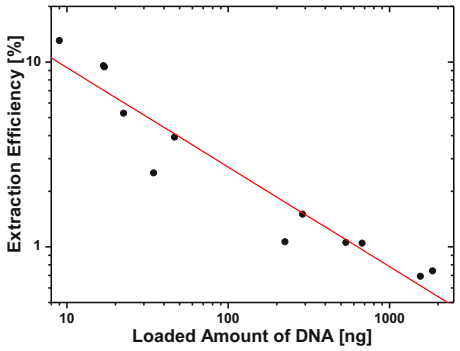
EXPERIMENTAL RESULTS AND DISCUSSION

Microfluidic protocol of SPE consists of a sequential process of blood sample flowing for viral RNA binding, washing by AW1 and AW2 buffers (from Qiagen Viral RNA mini kit), respectively, and elution of RNA by flowing distilled water. For the quantitative analysis of extraction efficiency, known amount of DNA in buffer solution is used.

Figure 4 to 8 illustrate all the empirical relationships of the extraction efficiency with respect to the eluted amount of DNA, binding flow rate, elution flow rate, sample pH value, and elution sequence, respectively. The binding capacity of the simple microchannel structure shows 2~20ng of DNA (Fig.4(a)) and increasing this amount would cause the surface area to be saturated, thereby decreasing the extraction efficiency (Fig.4(b)). The binding flow rate (Fig.5) and elution flow rate (Fig.6) seems not to severely affect the extraction efficiency. However, since only 10μl of elutant is used for the downstream RT-PCR process in the integrated microfluidic device (Fig.1), we decide to keep the flow rate of elution as 20μl/min. Optimized condition for the pH value of loading sample (Fig.7) is found to be 6.57 among three tested values of 5.72, 6.57, and 8.08. Also it is found that the first 10μl would be the most useful fraction for downstream processes as compared to other fractions (Fig.8). Optimized microfluidic protocol is summarized in Table 1. Finally, with the optimized protocol, it is demonstrated that the dengue serotype II (DEN2) viral RNA, with initial virus amount of 8pfu spiked into the 70μl of blood, is successfully extracted by performing following RT-PCR amplification and gel electrophoresis (Fig.9).



(a)



(b)

Figure 4: Empirical relationship of: (a) eluted amount of DNA (ng); and (b) extraction efficiency with respect to the loaded amount of DNA (ng), respectively.

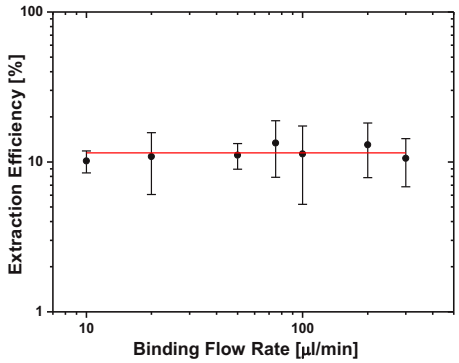


Figure 5: Empirical relationship of the extraction efficiency with respect to the varying binding flow rate (μl/min).

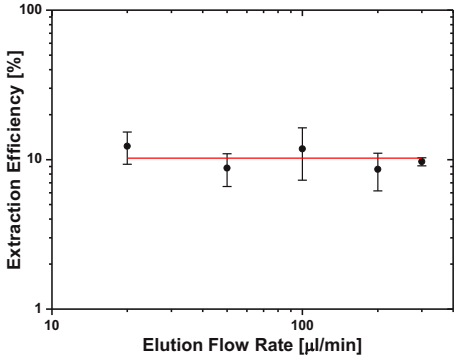


Figure 6: Empirical relationship of the extraction efficiency with respect to the varying elution flow rate (μl/min).

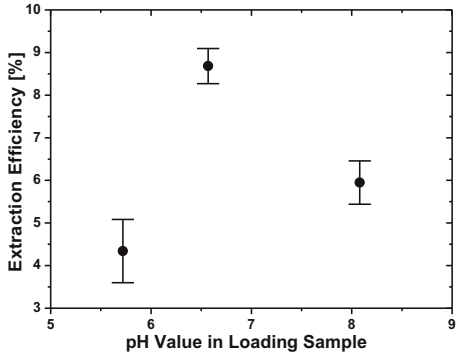


Figure 7: Empirical relationship of the extraction efficiency with respect to the varying pH value in loading sample.

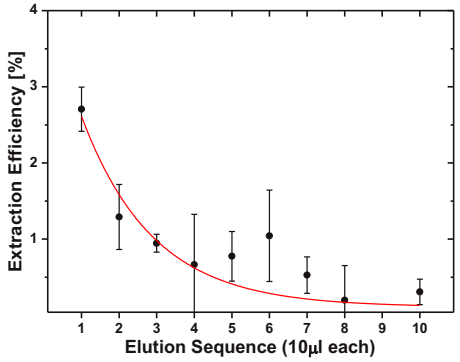


Figure 8: Empirical relationship of the extraction efficiency with respect to the elution sequence of collected each 10μl fraction.

Table 1. Optimized microfluidic protocol for SPE using silicon-based microchannel structure.

step	Flowrate (μL/min)	reagent	reagent volume (μL)	processing time
binding	150	loading sample*	320	2min 8sec
1st Wash	50	AW1	100	2min
2nd wash	50	AW2	100	2min
elution	20	water	10	30sec

*composition of loading sample: 70μL whole blood + 10μL virus + 80μL AVL buffer + 160μL ethanol

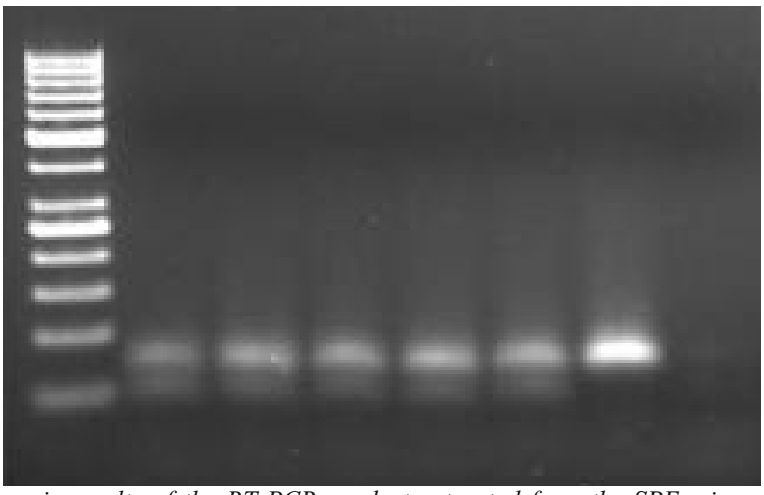


Figure 9: Gel electrophoresis results of the RT-PCR product extracted from the SPE microchannel of the integrated microfluidic device with 80μl of spiked-blood sample having 100pfu/ml concentrations of 69bps-sized dengue serotype 2 viral RNA: Lane 1 represents the 50bps-sized ladder; Lanes 2-6 are RT-PCR products from the elutant of first 10μl to the fifth 10μl, respectively. Lane 7 and 8 show the positive and negative controls, respectively.

CONCLUSION

Consequently, this work presents that simple SiO_2 surfaced microchannel has good potential for application in the integrated POC microsystem for infectious disease diagnostics, showing the capability of extracting viral DEN2 RNA from just finger pricked amount of blood sample in 7 minutes.

ACKNOWLEDGEMENTS

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