SANDIA BIOSCIENCE

SAND2013-9088C

A World-to-Digital Microfluidic Interface for Whole Blood RNA Preparation

Mais J. Jebrail[1], Anupama Sinha[2], Samantha Vellucci[1], Ronald F. Renzi[3], Cesar A. Rios[1], Carmen Gondhalekar[1], Kamlesh D. Patel[3] and Steven S. Branda[1]

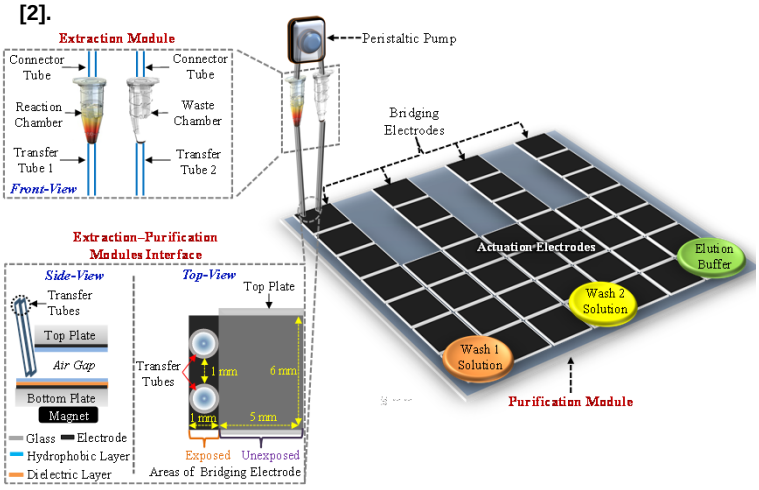
Departments of Biotechnology and Bioengineering[1], Systems Biology[2], Advanced Systems Engineering and Deployment[3], Sandia National Laboratories (Livermore, CA, USA)

ABSTRACT

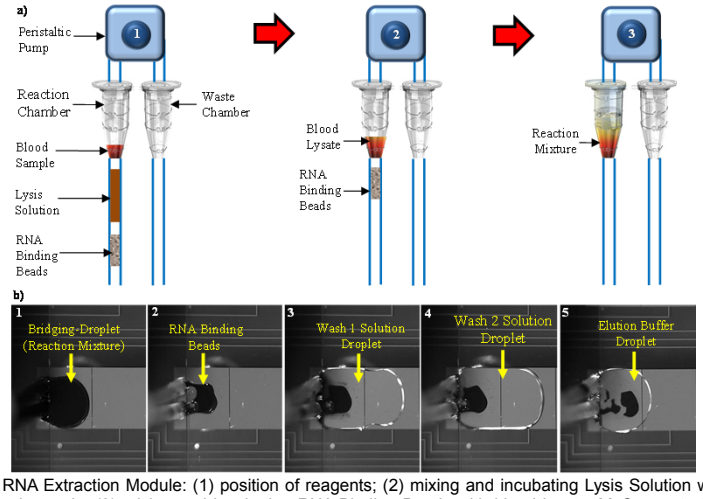
A critical limitation of many microfluidic analytical devices is the disparity between macroscale physiological samples (e.g., whole blood, serum, tissue extract, etc.), and the microscale sample volumes actually processed by microfluidic devices. We have developed a novel world-to-micrlfluidic interface mating an external module (for large-volume sample intake and extraction) to digital microfluidics (DMF)[1] (for microscale purification and enrichment). To demonstrate the capabilities of the new method, we implemented the extraction of total RNA from real-world volumes of whole blood samples and concentration on the microscale.

RESULTS

➢ **World-to-microfluidic system comprises three distinct regions: macroscale Extraction Module, DMF-based Purification Module and Extraction–Purification modules Interface. To couple the modules, the Extraction Module was lowered until the open ends of its Transfer Tubes made contact with the Bridging Electrode (patterned on bottom DMF plate) at an ~70˚ angle and adjacent to air gap between the DMF plates [2].**

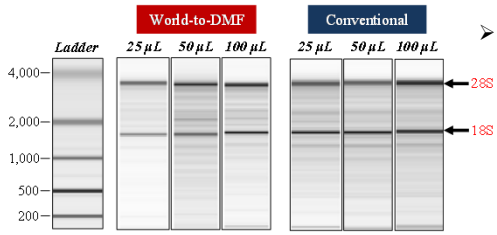


➢ Total RNA extraction from real-world volumes (25, 50 and 100 µl) of blood samples.

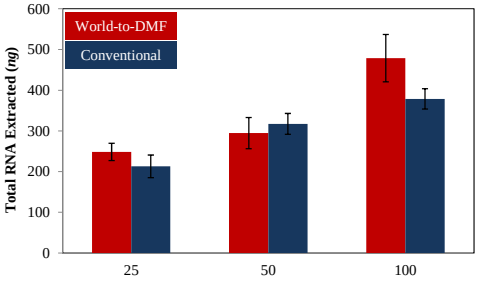


a) RNA Extraction Module: (1) position of reagents; (2) mixing and incubating Lysis Solution with blood sample; (3) mixing and incubating RNA Binding Beads with blood lysate. **b)** Concentrating and purifying RNA from hundreds of microliters of reaction mixture volume down to the microliter scale (~ 5 – 10 µL) on DMF.

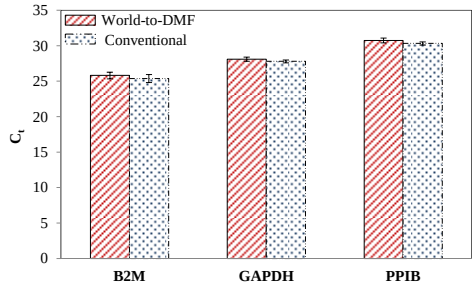
➢ Qualitative and quantitative analysis of extracted total RNA.



➢ Bioanalyzer gel-like images reveal sharp 28S and 18S ribosomal RNA bands in the 1,000–4,000 bp region indicative of intact total RNA. A260/280 ratio ≥ 1.8 (pure total RNA) and RNA Integrity Numbers ~ 7 (good-quality total RNA).



➢ Total RNA extraction from real-world volumes (25, 50 and 100 µl) of whole blood samples. The new method proved to be very efficient–RNA yields were analogues to those from conventional method, and in some cases better.



➢ Total RNA isolated from 50 µL of blood sample in qRT-PCR targeting three genes: beta-2 microglobulin (B2M), GAPDH (glyceraldehyde 3-phosphate dehydrogenase) and PPIB (Peptidylpropyl isomerase. As shown, target amplification was successful with highly consistent C_t values.

CONCLUSION

We introduced a new world-to-microfluidic interface in which an extraction module coupled to a DMF-based purification module. The new method is fast and automated relative to conventional methods (pipet, centrifuge, etc) and is capable of yielding good-quality total RNA – essential in many downstream molecular biology applications (e.g., real-time-PCR, microarray analysis, RNA-Sequencing, etc.). This work is an important first step in our efforts to develop fully automated methods for biomarker discovery (i.e., clinical transcriptomics).

REFERENCES

[1] M.J. Jebrail, M.S. Bartsch and K.D. Patel, Digital Microfluidics: A Versatile Tool for Applications in Chemistry, Biology and Medicine, *Lab on a Chip*, 12, 2452-2463, (2012).

[2] G.J. Shah, H. Ding, S. Sadeghi, S. Chen, C.J. Kim, R.M. van Dam, On-Demand Droplet Loading for Automated Organic Chemistry on Digital Microfluidics, *Lab on a Chip*, 13, 2785-95, (2013).

ACKNOWLEDGMENTS

MJJ thanks the Natural Sciences and Engineering Research Council (NSERC) of Canada and the Sandia Laboratory Directed Research and Development (LDRD) program for funding. Sandia National Laboratories is a multi-program la-boratory managed and operated by Sandia Corporation, a wholly owned subsidiary of Lockheed Martin Corporation, for the U.S. Department of Energy's National Nuclear Security Administration under contract DE-AC04-94AL85000. SAND Number: 2013-6814 A.

Sandia National Laboratories