Digital microfluidic system for LAMP-based detection of *Trypanosoma brucei* using molecular beacon probes

Liang Wan[1*], Tianlan Chen[1, 2], Jie Gao[1], Cheng Dong[1, 2], Yanwei Jia[1†], Pui-In Mak[1, 2] and Rui P. Martins[1, 2, 3]
[1]*State-Key Laboratory of Analog and Mixed-Signal VLSI, University of Macau, Macao*
[2]*Department of ECE, Faculty of Science and Technology, University of Macau, Macao*
[3]*On Leave from Instituto Superior Técnico, Universidade de Lisboa, Portugal*
[†]*Corresponding author: yanweijia@umac.mo*

Digital microfluidics (DMF) is an emerging technology in the microfluidic field to manipulate individual droplet on an electrodes array by electro-wetting forces.[1] Its feature of electric driving and miniature footprint has promised it as a perfect technology for point-of-care (POC) diagnostics.[2] In molecular diagnostics, Loop-mediated isothermal amplification (LAMP) works at a constant temperature,[3] significantly simplifies the requirement on reaction equipment. However, the widely accepted DNA detection using a double-stranded DNA dye after LAMP is less specific, given the possibility of primer-dimer formation.

In this work, we introduce a digital microfluidic system for LAMP detection of pathogen nucleic acid using a molecular beacon probe. A heat sink with real-time temperature sensor to provide the thermal regulation for the biochemical reaction was integrated into the operation unit generating actuation voltage for DMF chip. The DMF chip had a two-plate sandwich structure with the bottom plate patterned with electrodes and coated with dielectric and hydrophobic layers, and a conductive top plate drilled with holes for sample loading. A fluorescence microscope was used to monitor the signal of reaction. In order to increase the specificity of LAMP reaction, a low-T_m molecular beacon probe was designed on the single-stranded “loop” structure on the by-product of LAMP reaction. In the proof-of-principle detection, 2 μL of LAMP reaction samples containing purified *Trypanosoma brucei* DNA was loaded onto the DMF chip, lowering the sample consumption for more than 10 times compared with conventional LAMP off-chip. The amplification was finished in 30 min on-chip. The melting curve analysis was accomplished within 8 min, much shorter than a conventional qPCR machine which would take about 30 min.

In summary, the DMF system has been proved to be feasible for nucleic acid detection based on LAMP and molecular beacon probes of disease pathogen with high accuracy. The system structure is also open for further integration of sample preparation and fluorescence detection for POC diagnostics.

References

1 K. Choi, A. Ng, R. Fobel, A. Wheeler, *Annual Review of Analytical Chemistry*, 2012, **5**, 413–440.
2 A. Ng, M. Lee, K. Choi, A. Fischer, J. Robinson, A. Wheeler, *Clin Chem*, 2015, **61**, 420–429.
3 T. Notomi, H. Okayama, H. Masubuchi, T. Yonekawa, K. Watanabe, N. Amino, T. Hase, *Nucleic Acids Res,* 2000, **28**, e63.