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DOCTORAL THESIS

The Development of All-in-one POCT Platforms for Nucleic Acid Extraction and Testing Based on Digital Microfluidics

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ABSTRACT

Nucleic acid testing has become a mainstream technology for molecular diagnosis. Traditional methods require complex procedures, lengthy detection cycles, and the requirement for skilled technicians. To address the limitation, the integration of nucleic acid testing with microfluidic technologies has been investigated. In this thesis, the integration of nucleic acid analysis with digital microfluidics was investigated and all-in-one platforms were established which allowed for sample processing, amplification, and detection on a single device, offering a new avenue for point-of-care diagnosis.

Firstly, a novel DMF device was developed to facilitate LAMP reactions. The chip contains a bottom plate that is made of a PCB substrate and patterned with copper electrodes. It incorporated a dielectric layer of polyimide film and was covered with a FluoroPel hydrophobic layer. With the addition of 0.1% Tween 20 as a surfactant and an actuation voltage of 260 Vrms, various reagents and samples were efficiently actuated on the device. The droplet dispensing on this chip demonstrated high accuracy and precision, with minimal variation in droplet volume, showing a CV value of 6.26%. Furthermore, the DMF chip included a thermal module capable of providing heating for LAMP reactions, creating a controlled isothermal environment within the confined microfluidic setup. This feature eliminated the need for external heating sources, allowing for easier integration with other systems. Dry reagents were utilized for LAMP amplification, enabling parallel detection of different genes with minimal manual intervention. The dry primers showed remarkable stability, with an estimated shelf life of 106 days at room temperature. This long-term stability is particularly advantageous for point-of-care testing (POCT) applications in ambient conditions.

Secondly, the DMF device was combined with the LAMP techniques (DMF-LAMP) for both quantitative and qualitative multiplex detection. In chapter three, the real-time LAMP method was integrated with the DMF device to achieve quantitative detection of multiple genes. The common foodborne pathogens including *E. coli*, *S. typhimurium*, *L.monocytogene*, and *S. aureus* were employed as the detection targets. Dehydrated primers of the target genes were rehydrated on the specified reaction sites on the DMF chip, allowing parallel amplification of multiple genes in 2 μL droplets through a single test. The dried primers showed long-term stability of 106 days under room temperature, which is especially beneficial to use in POCT scenarios. DMF-LAMP method presented good sensitivity for multiplex detection, achieving a limit of detection (LOD) of 10^2 copies of genomic DNA per reaction. The sensitivity was demonstrated to be comparable or even higher sensitivity than the conventional LAMP method while demonstrating superior efficiency for multiplex detection compared to the qPCR method at a similar cost. And the entire process, from sample to result, can be completed in approximately 50 min. The threshold time of the LAMP reaction exhibited a linear correlation with the DNA concentration, thus enabling the quantitative analysis of multiple genes. In certain situations, for instance, in initial pathogen screening or presence/absence testing, a simple qualitative assessment can efficiently determine the presence of the target without requiring precise quantification.

To cater to qualitative applications, a DMF device was designed to couple with colorimetric LAMP reaction, enabling rapid and straightforward quantitative detection. The neutral red dye was employed as the colorimetric indicator of color change generated by LAMP amplification. The on-chip colorimetric LAMP reaction was performed in a 5 μL droplet. Simultaneous amplification of multiple genes could be completed in only 20 min. The endpoint detection method with highly concentrated dye was optimized for visual detection with the naked eye. The result demonstrated good sensitivity and specificity of the proposed method, with a LOD of 10 copies/μL DNA per reaction. The applicability of the method was verified through the diagnosis of shrimp diseases. Results showed good agreement with the standard qPCR method. Meanwhile, a universally adaptable image-based analytical method was proposed. With a smartphone for image capture, the method enabled accurate identification of positive results from negative ones under different lighting conditions. The simplicity, affordability, and applicability of the visual detection and image processing analysis make this platform well-suited for rapid quantitative detection of target genes. The incorporation of colorimetric LAMP reaction into the platform addresses the need for rapid and accessible diagnostics. The results are visually detectable with the naked eye, making it user-friendly for non-specialized individuals and enhancing its suitability for resource-limited and on-site testing scenarios. However, the sample preparation procedures, such as the DNA extraction steps, were not incorporated into the DMF-LAMP platforms, which increased the overall complexity of the testing process.

Lastly, the research was focused on the integration of the DNA extraction process into the DMF-LAMP system. Fully integrated platforms were established which incorporated all necessary procedures of nucleic acid detection, including cell lysis, DNA extraction, and LAMP amplification, so called all-in-one platforms. The on-chip DNA extraction method utilizes magnetic bead-based technology within a hybrid channel-DMF structure. Notably, the on-chip extraction exhibits high efficacy, particularly at low input concentrations. For instance, the test with 10^3 CFU/mL of *E. coli* cell suspension showed significantly higher recovery compared to conventional manual extraction using a DNA extraction kit ($P < 0.05$). The recovery rate remains robust within the concentration range of 10^3-10^6 CFU/mL, varying from 88.43% to 95.83% based on the input gDNA concentrations. To address the need for rapid and accessible diagnostics, colorimetric LAMP reaction was integrated into the platform. The results were visually detectable with the naked eye, making it user-friendly for non-specialized individuals, thus enhancing its applicability in resource-limited and on-site testing scenarios. The entire process, from sampling to result, was fully automated on the chip, and the time required is approximately 60 min. The platform exhibits impressive sensitivity in simultaneously detecting common foodborne pathogens, achieving a LOD within the range of 10^2-10^3 CFU/mL. Furthermore, the integrated device can be configured for real-time LAMP detection, allowing simultaneous quantification of multiple pathogens within the dynamic range of 10^3-10^6 CFU/mL.

Overall, these microfluidic platforms designed for nucleic acid testing hold promising potential for efficient and streamlined molecular diagnostics. Future

improvements will focus on extending the platform for a broader range of applications, such as POCT clinical diagnosis.