Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.
Integrated microdroplet array platform with temperature controller and micro-stirring for ultra-fast SARS-CoV-2 detection

Mengyun Zhou, Yong Luo, Lirong Wang, Chuan Fan, Tailin Xu*, Xueji Zhang**

Guangdong Key Laboratory for Biomedical Measurements and Ultrasound Imaging, School of Biomedical Engineering, Health Science Center, Shenzhen University, Shenzhen, Guangdong, 518060, PR China

ARTICLE INFO

Keywords:
Integrated Temperature controller Ultrasound Enhanced RT-LAMP SARS-CoV-2 detection

ABSTRACT

The outbreak of COVID-19 has created a huge challenge to global health systems. Experience in fighting the epidemic shows that the development of a rapid and sensitive POCT diagnostic platform for SARS-CoV-2 that can be deployed in situ is crucial to contain the outbreak. Here, we have developed a portable microdroplet detection platform that integrated temperature controller and micro-stirring for high-throughput and ultrafast COVID-19 diagnosis. Such a device uses a p-n junction (PN junction) as the temperature controller to adjust the temperature in a single microdroplet independently and precisely, ensuring the amplification of reverse transcription loop-mediated isothermal amplification (RT-LAMP). Meanwhile, the platform incorporates an ultrasonic micro-stirring unit, greatly increasing the interaction between RT-LAMP molecules and accelerating the amplification. The results show good linearity over a wide linear range (1 to 10^5 copies/μL) and low LOD (0.48 copy/μL). Our method reports in only 6.1 min for high-viral load samples, and combines with sample preparation, the total detection process could be done within 30 min. Such a portable and fully integrated microdroplet molecular diagnostic platform is a promising tool for point-of-care diagnosis of COVID-19 and other infectious diseases in resource-limited settings.

1. Introduction

The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic has caused a huge impact on human health and the economy worldwide (Ciotti et al., 2020; McGowan et al., 2020; Velavan and Meyer, 2020; Wu et al., 2020). The growing evidence indicates that many infected individuals with mild or no symptoms unknowingly spread the disease (Abdool Karim and de Oliveira, 2021; Karim and Karim, 2021). Large-scale screening of symptomatic and asymptomatic infected people and tracking their contacts is the most effective way to curb the epidemic (Ferretti et al., 2020; Harvey et al., 2021). Quantitative reverse transcriptase polymerase chain reaction (RT-qPCR) is a gold standard for COVID-19 diagnosis based on nucleic acid amplification tests (NAATs) (Carter et al., 2020; Esbin et al., 2020). However, RT-qPCR requires specialized equipment and a relatively long turnaround time, which are insufficient for rapid field diagnosis of pandemics in resource-poor areas (Valera et al., 2021; Yager et al., 2008; Yuce et al., 2021).

The development of point-of-care (POC) nucleic acid testing systems is expected to overcome the above shortcomings, which has recently attracted increasing attention (Gowri et al., 2021; Khodakov et al., 2021; Torres et al., 2021; Yadav et al., 2021). Loop-mediated isothermal amplification (LAMP) and other isothermal amplification methods enable efficient amplification of nucleic acids at a single temperature with a shorter detection time (<1 h) and show better tolerance to impurities than PCR, making them more suitable for POC diagnosis (Kevadiya et al., 2021; Yang and Chaput, 2021; Zhou et al., 2022; Taleghani and Taghipour, 2021). Under the action of Bst DNA polymerase, LAMP produces 10^9–10^10 DNA copies in 15–60 min at 60–65 °C, which has the advantages of simple operation, high specificity, and diverse readout method (Chaouch, 2021; Notomi et al., 2000; Pokhrel et al., 2020). Compared with recombinase polymerase amplification (RPA), rolling circle amplification (RCA), and strand displacement amplification (SDA), the LAMP shows excellent sensitivity and specificity (Oh et al., 2016; Nguyen et al., 2022). Furthermore, the Bst polymerase in LAMP has a higher tolerance to the inhibitors commonly found in clinical specimens (Francois et al., 2011; Nkouawa et al., 2010). Therefore, LAMP has a unique application in disease diagnosis and is
expected to achieve POCT for COVID-19 (Bokelmann et al., 2021; He et al., 2021; Juang et al., 2021; Li et al., 2022).

Herein, we demonstrate a fully integrated portable microdroplet array detection platform that combines a temperature controller with the contactless micro-stirring for high-throughput, ultrafast detection of SARS-CoV-2 based on enhanced RT-LAMP (Fig. 1). Bi$_2$Te$_3$ and Sb$_2$Te$_3$ are thermoelectric materials with advantages of long lifetime, good stability, small size, and high thermoelectric conversion efficiency (Perez-Marín et al., 2014; Poudel et al., 2008; Zhu et al., 2021). We combine Sb$_2$Te$_3$/Bi$_2$Te$_3$ p-n junction (PN junction) with micropillar array to ensure rapid and accurate temperature control of individual microdroplets. This capability ensures the stability and accuracy of RT-LAMP in microdroplets. Meanwhile, the integrated ultrasonic micro-stirring controllably generates microstreaming in the microreactor to rapidly mix and disperse reagents, thereby significantly shortening the detection time through the enhanced RT-LAMP. Ultimately, the green fluorescence of the RT-LAMP products can be easily excited via a blue flashlight and captured by a smartphone camera. The convenient result readout method lays the foundation for its field deployment. Such a platform has great potential in POCT for rapid, reliable, and high-throughput diagnosis of COVID-19 and other infectious diseases.

2. Experimental

2.1. Enhanced RT-LAMP reaction

Primers and target sequences are shown in Table S1. The RT-LAMP mixture was 10 μL containing 5 μL Master Mix (2X), 0.2 μL fluorescent dye (50X), 1 μL primer mix (10X) (16 μM FIP/BIP, 2 μM F3/B3, and 8 μM LF/LB), 2.8 μL nuclease-free water, and 1 μL target sample. The mixtures were added onto micropillars through a pipette with low-adsorption hydrophobic tips followed by mineral oil to avoid aerosol contamination and reduce evaporation. Then ultrasound with a frequency of 10$^6$ Hz was turned on for 10 s to mix the solution. Meanwhile, the temperature controller was turned on and maintained the temperature at 65 °C for 30 min. Throughout the RT-LAMP, the ultrasound was open for 10 s every 60 s, while the control group was without ultrasound treatment. The RT-qPCR control assays were performed using the Fast SARS-CoV-2 RT-qPCR kit according to the manufacturer’s instructions. For more information, please see the Supporting Information.

2.2. Spiked saliva sample testing

To simulate clinical samples, we artificially prepared samples by randomly spiking SARS-CoV-2 pseudovirus to saliva samples collected from healthy volunteers. Then the extracted RNA was divided into two parts, one for RT-LAMP and the other for RT-qPCR analysis. The fluorescent images of the platform were captured by an iPhone13 (Apple Inc., CA, USA) with a blue flashlight. The mean fluorescence intensity was then measured by a color-extracting app called Color Picker (available in both Android and iOS app stores).

3. Results and discussion

3.1. Fabrication and characterization

A fully integrated detection platform was prepared as described in the experiment and mainly consists of three parts: a micropillar array for anchored microdroplets as microreactors, an independent temperature controller for each microreactor, and an ultrasonic unit for on-demand mixing and dispersion. Due to the hydrophobic and high-viscosity properties of the PDMS, the micropillar array exhibits excellent performance in individual microdroplet management (Fan et al., 2021). The micropillar array firmly holds the microdroplets when flipping at 45, 90, and 180°, demonstrating the exceptional stability of such a platform in complex environments (Fig. S1).

The PN junction embedded in the micropillar has good temperature control performance for each microdroplet. As shown in Fig. 2a, the heating process is fast, reaching a stable temperature within 10–15 s and then remaining stable. The elevated temperature of microdroplets is positively linearly proportional to the increased voltage (Fig. 2b). The heating rate under different voltages all reach more than 3 °C/s after calculation (Fig. 2c). Meanwhile, the four laser on/off cycles exhibit
outstanding temperature conversion stability of the PN junction (Fig. 2d). Thermal infrared images in Fig. 2e–f indicate that each PN junction in the platform has independent and uniform temperature-control performance. The simulation in Fig. 2g shows the heating process of a single microdroplet under a PN junction (0.4 V), which is consistent with the thermal infrared images of the heating process. The microdroplet was rapidly heated to ~65 °C in 10 s and then maintained stably (Fig. 2h). Therefore, the temperature control unit with independent temperature-control capability, excellent heating rate, and good stability guarantee the RT-LAMP in microdroplets.

3.2. Enhanced RT-LAMP

The side view of the single microreactor of the integrated detection platform is shown in Fig. S2. The ultrasonic mixing unit works by converting the input electrical signal into acoustic waves through the piezoelectric transducer and then transmitting it through the micropillar to the microdroplet above, producing microstreaming to achieve contactless mixing and stirring. Due to the intermittent open-operation mode, the ultrasound does not cause short-term changes or fluctuations in the temperature of microdroplets (Fig. S3). Meanwhile, the ultrasonic frequency we used is insufficient to generate the ultrasonic cavitation without damaging the target sequence/ primer and enzyme activity (Arai et al., 2022). It is worth mentioning that we effectively avoid the problem of aerosol contamination by covering the microdroplet with mineral oil.

Ultrasonic technology has been proven to accelerate the mixing of molecules in microdroplets (Bachman et al., 2020; Huang et al., 2019). To reduce the strong non-specific adsorption of ssDNA on the PDMS surface, hydrophilic treatment is necessary through plasma and the change of contact angles is present in Fig. S4 (Zhou et al., 2012). As shown in Fig. 3a, ultrasound-induced microstreaming enables rapid mixing and dispersion of reagents in microdroplets, thus greatly accelerating the RT-LAMP reaction by enhancing the interaction between molecules. The laboratory-based ultrasonic generating device is larger as shown in Fig. S5, which was minimized into a palm-sized device in this work to make the platform more suitable for POCT. The corresponding circuit design diagram and the physical picture of the portable ultrasonic device (~7.5*6.5*2.5 cm) are presented in Fig. 3b-c.

As a proof of concept, the SARS-CoV-2 pseudovirus was used to replace the real SARS-CoV-2. We examined analytical performance by analyzing the N gene of serial diluted SARS-CoV-2 pseudovirus RNA at
different concentrations. The ultrasound-induced microstreaming significantly shortens the threshold time at different concentrations (Fig. 3e). The threshold time was reduced from 11.9 min to 6.1 min at a concentration of $10^5$ copies/μL. Meanwhile, real-time fluorescence curves show that the threshold time of the ultrasound-treated group was decreased from 27.1 min to 18.3 min (1 copy/μL) (Fig. S6). This result was also confirmed by fluorescence images in a single microdroplet (Fig. 3d). The standard curve of RT-LAMP exhibits good linearity ($R^2 = 0.97$) over a wide range of concentrations (1 to $10^5$ copies/μL), with a LOD of 0.48 copy/μL (Fig. 3f). The LOD is calculated by three times the standard deviation of the blank (Chiavaioli et al., 2017). The enhanced RT-LAMP on the platform exhibits high reproducibility with relative standard deviations (RSD) of 8.74%, 7.14%, 6.97%, 5.22%, 5.29%, and 3.43%, respectively (Fig. 3f). We also demonstrated that the method effectively distinguished other coronavirus variants highly similar to SARS-CoV-2, such as SARS-CoV (Fig. 3g). In addition, RT-qPCR was used to verify the results of RT-LAMP. The results showed that the threshold cycle (Ct) of RT-qPCR exhibited good linearity with different concentrations (Fig. S7). As shown in Fig. S8, the results between RT-LAMP and RT-qPCR showed a correlation coefficient of 0.92, indicating considerable agreement.

3.3. High-throughput detection of SARS-CoV-2 in saliva

Furthermore, we evaluated the analytical performance of the platform in clinical samples by randomly adding SARS-CoV-2 pseudovirus to healthy donor saliva. The schematic diagram of the detection process is shown in Fig. S9. 96 saliva samples were analyzed in both the method and RT-qPCR to validate the accuracy in real clinical samples. The fluorescence image on the platform captured by a smartphone is shown in Fig. 3i. The results indicated that our method exhibited a similar number of SARS-CoV-2 positive samples as RT-qPCR (49 vs. 50) with a low false negative of 2% (Fig. S10). The Tt and Ct of positive samples with a correlation coefficient of 0.92 were displayed in Fig. 3h, which supports the feasibility of this method with good accuracy in clinical sample diagnosis. Therefore, combined with the advantages of lyophilized LAMP reagents in terms of tolerance to ambient temperature and long storage time, such a platform is expected to provide sensitive and accurate POCT diagnosis of SARS-CoV-2 in saliva.

4. Conclusions

In summary, we have demonstrated a fully integrated microdroplet array platform with excellent temperature control ability and contactless micro-stirring performance that ensures and enhances RT-LAMP amplification in microdroplets for ultra-fast and high-throughput SARS-CoV-2 diagnosis. As summarized in Table S3, our results were similar to RT-qPCR with a LOD of 0.48 copy/μL and the total detection time could be as short as 40 min compared with other methods based on RT-LAMP/RT-RPA, which supports the potential of this platform for practical application in screening and diagnosis. This proof-of-concept assay platform demonstrates the essential capabilities required for...
rapid sensitive assays suitable for use in resource-constrained point-of-care settings or screening sites compared with RT-qPCR. Compared with antibody-based methods for rapid detection at home, our method is more suitable for detecting infection cases at the early stage of infection, but the convenience of this detection platform needs to be further improved. In addition, some improvements can be made to further promote the user-friendliness and resource requirements of current devices. For example, how to conduct sample pretreatments such as sample lysis and nucleic acid extraction on this platform is an urgent problem to be explored next. We believe that this smartphone-based platform or similar applications in the future have the potential to rapidly expand screening capabilities for COVID-19 and other diseases, thereby promptly containing the pandemic.

CRediT authorship contribution statement

Mengyuan Zhou: Data curation, Methodology, Writing – original draft. Yong Luo: Methodology, Formal analysis. Lirong Wang: Investigation, Software. Chuan Fan: Software. Tailin Xu: Writing – review & editing, Funding acquisition. Xueji Zhang: Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

Acknowledgment

This work was funded by China Postdoctoral Science Foundation Grant (2021TQ0210), National Natural Science Foundation of China (21804007, 21890742), Shenzhen Stability Support Plan (20200806163622001), Shenzhen Key Laboratory for Nano-Biosensing Technology (ZDSYS2021011216140001), Shenzhen Overseas Talent Program.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bios.2022.114903.

References

Abdool Karim, S.S., de Oliveira, T., 2021. N. Engl. J. Med. 384 (6), 586–598.
Arai, T., Sato, T., Matsushita, T., 2022. Adv. Sci., 20220576.
Bachman, H., Chen, C., Rafo, J., Zhao, S., Yang, S., Tian, Z., Nama, N., Huang, P.H., Huang, T.J., 2020. Lab Chip 20 (7), 1238–1248.
Bokelmann, L., Nickel, O., Maricic, T., Pasbo, S., Meyer, M., Borte, S., Riesenberg, S., 2021. Nat. Commun. 12 (1), 1467.
Carter, L.J., Garner, I.V., Smoot, J.W., Li, Y., Zhou, Q., Savesson, C.J., Sasso, J.M., Gregg, A.C., Soares, D.J., Beskid, T.R., Jervey, S.R., Liu, C., 2020. ACS Cent. Sci. 6 (5), 591–605.
Chai, M., 2021. Rev. Med. Virol. 31 (6), e2215.
Chiavon, F., Gouveia, C.A.J., Jorge, P.A.S., Badini, F., 2017. Biosensors 7 (2), 23.
Citti, M., Ciccozzi, M., Terrinoni, A., Jiang, W.C., Wang, C.B., Bernardini, S., 2020. Crit. Rev. Clin. Lab. Sci. 57 (6), 365–388.
Edgar, L., Kendall, M., Zhao, L., Nurtay, A., Abeler-Dorner, L., Parker, M., Bonsall, D., Fraser, C., 2020. Science 368 (6491), eabb6936.
Fan, C., Luo, Y., Xu, T., Song, Y., Zhang, X., 2021. Nanoscale 13, 739–745.
Ferretti, L., Wymant, C., Kendall, M., Zhao, L., Nurtay, A., Abeler-Dorner, L., Parker, M., Bonsall, D., Fraser, C., 2020. Science 368 (6491), eabb6936.
Ferretti, L., Wymant, C., Kendall, M., Zhao, L., Nurtay, A., Abeler-Dorner, L., Parker, M., Bonsall, D., Fraser, C., 2020. Science 368 (6491), eabb6936.
Fan, C., Luo, Y., Xu, T., Song, Y., Zhang, X., 2021. Nanoscale 13, 739–745.