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.

Journal Pre-proof

Advancing pathogen detection for airborne diseases

Tingting Zhai , Yuhan Wei , Lihua Wang , Jiang Li , Chunhai Fan

PII: S2667-3258(22)00424-1
DOI: https://doi.org/10.1016/j.fmre.2022.10.011
Reference: FMRE 431

To appear in: *Fundamental Research*

Received date: 28 June 2022
Revised date: 3 October 2022
Accepted date: 19 October 2022

Please cite this article as: Tingting Zhai , Yuhan Wei , Lihua Wang , Jiang Li , Chunhai Fan , Advancing pathogen detection for airborne diseases, *Fundamental Research* (2022), doi: https://doi.org/10.1016/j.fmre.2022.10.011

Perspective

Advancing pathogen detection for airborne diseases

Tingting Zhai[a], Yuhan Wei[a], Lihua Wang[b], Jiang Li[a,b,*], Chunhai Fan[a,*]

[a]School of Chemistry and Chemical Engineering, Frontiers Science Center for Transformative Molecules and National Center for Translational Medicine, Shanghai Jiao Tong University, Shanghai 200240, China

[b]The Interdisciplinary Research Center, Shanghai Synchrotron Radiation Facility, Zhangjiang Laboratory, Shanghai Advanced Research Institute, Chinese Academy of Sciences, Shanghai 201210, China

[*]Corresponding authors: Prof. Jiang Li, Shanghai Jiao Tong University, The Interdisciplinary Research Center, School of Chemistry and Chemical Engineering,Frontiers Science Center for Transformative Molecules and National Center for Translational Medicine, Shanghai 200240, China, *lijiang@zjlab.org.cn* (J. Li); *fanchunhai@sjtu.edu.cn* (C. Fan).

ABSTRACT

Airborne diseases including SARS, bird flu, and the ongoing Coronavirus Disease 2019 (COVID-19) have stimulated the demand for developing novel bioassay methods competent for early-stage diagnosis and large-scale screening. Here, we briefly summarize the state-of-the-art methods for the detection of infectious pathogens and discuss key challenges. We highlight the trend for next-generation

technologies benefiting from multidisciplinary advances in microfabrication, nanotechnology and synthetic biology, which allow sensitive, rapid yet inexpensive pathogen assays with portable intelligent device.

Keywords: infectious diseases; pathogens assay; biosensors; synthetic biological circuits

During the recent decades, we have witnessed the outbreaks of several severe airborne diseases including bird flu, SARS, MERS, and the ongoing Coronavirus Disease 2019 (Covid-19, caused by the coronavirus SARS-CoV-2) [1,2]. Particularly in the last two years, the SARS-CoV-2 variant of concern (VoC) Omicron, with dramatically enhanced infectivity and immune escape ability, has raised a new wave of pandemic, imposing a heavy burden on individuals, healthcare systems and societies worldwide, including in China [3]. Early-stage diagnosis and frequent large-scale screening have proved critical to controlling the spread of such highly contagious pathogens.

A variety of bioassay methods have allowed the diagnosis of airborne diseases by detecting pathogenic biomarkers (e.g., pathogenic nucleic acids or antigenic proteins). However, as evidenced in the present global pandemic of COVID-19 [1], the deployed methods have been inadequately satisfactory for the urgent needs of screening potentially infected patients. Owing to the cutting-edge advances in nanotechnology, synthetic biology, and microfluidic technology, a new generation of detection methods has emerged, holding promise in dealing with highly contagious

diseases in resource-limited settings. In this perspective, we discuss recent progress in the detection methods for airborne pathogens and provide an outlook on future development.

1. State-of-the-art methods for early-stage detection of pathogens

1.1. Nucleic acid detection

Polymerase chain reaction (PCR)-based methods. PCR can amplify specific nucleic sequences exponentially via repeated cycles of thermal denaturation and renaturation, thus presenting ultra-high sensitivity and specificity, while enabling the detection of very few or even single copies of target nucleic acids in complex samples [4]. Nowadays, quantitative PCR (qPCR) or real-time PCR has been the gold standard for pathogenic nucleic acid analysis. Its combination with reverse transcription (qRT-PCR) has been adopted as the first-line assay for confirming the infection of RNA viruses including SARS-CoV-2. However, conventional qPCR relies on expensive instruments run by professionals in specialized laboratories. A typical qPCR assay costs 2-6 h (largely due to the time-consuming temperature changing and heat transfer), not to mention the delay caused by the overwhelmed detection capacity during pandemics. Thus, traditional PCR has been unsatisfactory for the demand for rapid, large-scale screening.

To overcome the limitations, one approach is to use miniaturized devices with high thermal conductivity in combination with small-volume samples, which allows rapid temperature change [5]. Some strategies achieve fast thermocycling by

transferring the reactants across different temperature zones via e.g., flow control [6], Rayleigh-Bénard convection [7], or rotation [8]. Another possible route is to drive DNA denaturation-renaturation with means such as electrochemically controlled pH switching [9] and photothermal conversion [10]. In addition, artificial intelligence (AI) has been harnessed to predict results with fewer amplification cycles, which can help shorten the assay time [11]. These strategies hold the potential for developing ultra-fast PCR assays.

Assays based on isothermal nucleic acid amplification. The development of isothermal nucleic acid amplification strategies provides promising alternatives to PCR. These strategies generally utilize both the polymerizing and unwinding activity of DNA polymerases for successive DNA synthesis and replacement without the need for heat denaturation [4]. Representative strategies include strand displacement amplification (SDA), nicking enzyme amplification reaction (NEAR), rolling circle amplification (RCA), recombinase polymerase amplification (RPA), loop-mediated isothermal amplification (LAMP), and nucleic acid sequence-based amplification (NASBA). Although the robustness and accuracy need further verification, some of them have been adopted in authorized, commercially available test kits, aiming at point-of-care (POC) nucleic acid assays.

1.2. Immunoassays

Immunoassays are biomolecular detection methods based on specific antigen-antibody interactions, which have long been employed for the detection of

biomarkers including antigenic proteins from pathogens, or antibodies and cytokines from patients. Conventional immunoassays such as lateral-flow immunoassays (e.g., immunogold strips) are suitable for rapid self-tests, thus have been widely deployed in the COVID-19 pandemic, allowing coarse screening of infection in nasal swabs in less than 15 min [12]. In comparison, enzyme-linked immunosorbent assays (ELISA) or chemiluminescent immunoassays (CLIA) relying on professional equipment can generate more accurate results, thus are more suitable for clinical serological diagnosis in hospital. In general, immunoassays can be carried out under ambient conditions, thus are amenable to POC assays that can be completed in minutes. However, the sensitivity and specificity of immunoassays are inherently constrained, largely due to the cross-reactivity of antigen-antibody interactions, and the lack of ways to effectively and selectively amplify target proteins. One promising solution is to use devisable nanostructures as scaffolds to engineer the spatial arrangement of multiple copies of detection ligands (e.g., aptamers, peptides, antibody fragments), which may better match the patterns of antigens on the pathogens [13,14]. Due to the multivalency effect, the nano-patterned ligands may provide much higher affinity against the target pathogens compared to conventional monovalent/bivalent ligands, thus holding promise for amplification-free detection of pathogens at early stages.

2. Advancing pathogen assays with biosensors and microarrays

2.1. Biosensors

Biosensors are devices mimicking natural molecular receptors, which generate

signals specific to target biomarkers with minimal laboratory-based processes. Particularly, electrochemical biosensors can translate molecular recognition events into electronic signals amenable to cost-effective, portable electronic devices. Besides, they can offer high sensitivity even without molecular amplification, holding promise for the rapid analysis of infectious pathogens. For example, a graphene-based field-effect transistor (FET) immunological biosensor has been fabricated for direct detection of SARS-CoV-2 spike proteins in nasopharyngeal swabs with a LOD of ~242 copies/mL [15]. More recently (Fig. 1a), the reagent-free sensing of SARS-CoV-2 viral particles and antigens in 5 min has been achieved by using an electrochemical sensor functionalized with kinetically responsive antibody probes [16].

To improve the performance of biosensors for nucleic acid assays, much endeavor has been made to rationally engineer the biosensing interfaces at the nanometer scale. A representative approach is to employ nanostructured microelectrodes with high curvature and high surface area to maximize molecular recognition efficiency [17], which has allowed PCR-free viral nucleic acid detection in whole blood [18]. Another approach proposed by our group is to employ DNA framework nanostructures to precisely program the spatial arrangement and conformation of probe molecules on the interfaces, which allows significant improvement in sensitivity and selectivity [19]. Recently, a DNA framework-empowered interface has been exploited for direct detection of

SARS-CoV-2 RNA from clinical samples within 4 min without the need for RNA extraction and amplification, which achieves a LOD (down to ~0.02 copies per μL) comparable to the conventional PCR (Fig. 1b) [20]. Overall, these electrochemical biosensors with nanostructured interfaces have shown potential in developing POC assays with ultra-high sensitivity independent of expensive equipment. Despite the progress, the quality control of such biosensors needs further optimization for mass production and practical application.

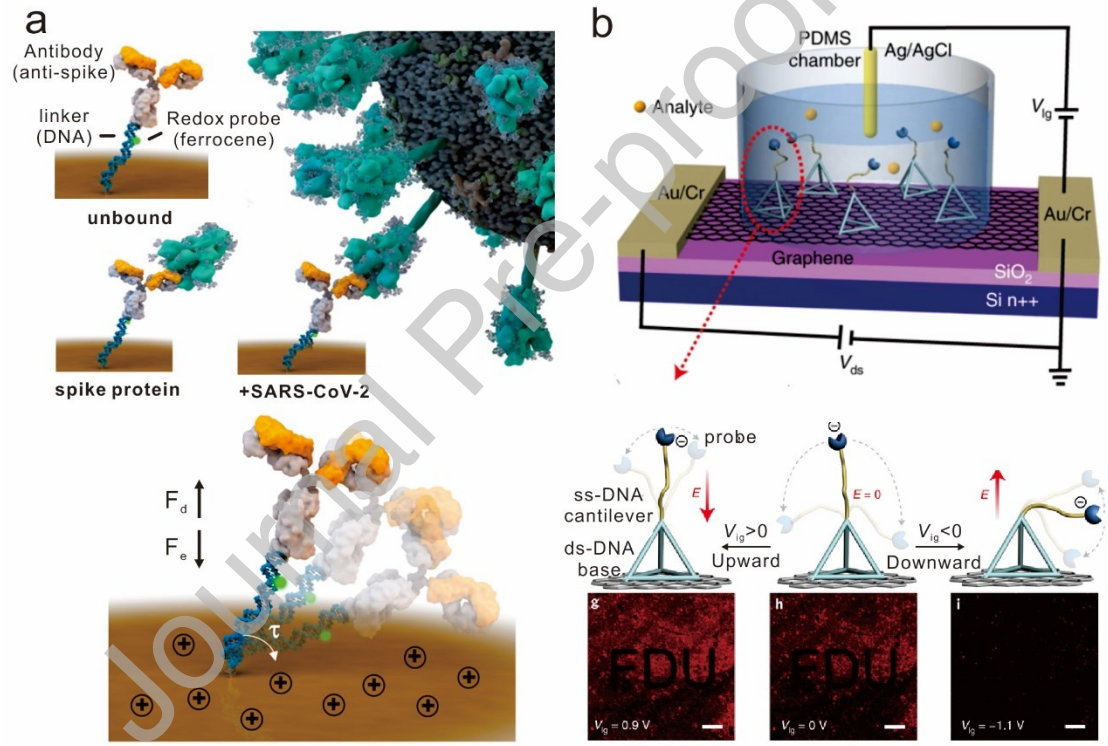

Fig. 1. Electrochemical biosensors for pathogen assays. (a) Schematic of reagent-free sensing of viral particles using an electrochemical strategy to monitor the kinetics of a DNA-antibody complex [16]. (b) An ultrasensitive FET nucleic acid sensor with the probe interface programmed by tetrahedral DNA nanostructures [20].

2.2. Microarrays

Biosensing microarrays (or chips) can be regarded as the arrays of biosensors allowing hundreds or even thousands of parallel tests on a miniaturized (e.g., square centimeter-scale) area, which have played important roles in high-throughput applications such as studies on pathogenic genomics, pathogen-host interactions and digitalized bioassays (e.g., digital PCR [21] and digital ELISA [22], for the absolute quantification of a single analyte). However, the sensitivity of solid-phase microarrays is constrained by the limited reaction efficiency on the solid-liquid interface, especially when the sensing area is very small for each unit. In this regard, the combination of microfluidic/microdroplet systems and microbeads/nanoparticles would allow homogenous/near-homogenous reactions on chips with improved sensitivity [23]. For example, a single-molecule ELISA system based on these techniques enables the detection of target proteins in serum with a LOD ($<10^{-15}$ M) much superior to the conventional ELISA [22]. Recently, next-generation sequencing technologies (e.g., nanopore sequencing) in combination with microarrays have allowed fast sample preparation, long-sequence read, and high-throughput sequencing for metagenomic studies, which can quickly identify novel pathogens or variants in complex environments [24,25], meanwhile acquire their genomic information for rapid development of target-specific detection methods [1].

3. Synthetic biology-empowered pathogen assays

The advances in synthetic biology have allowed us to repurpose and engineer biological components into customized circuits that can work in cell-free settings,

which provide new opportunities for isothermal pathogen assays. Recently, there is a growing interest in using CRISPR (clustered regularly interspaced short palindromic repeats)-Cas (CRISPR-associated proteins) systems for isothermal nucleic acid assays [26]. CRISPR-Cas systems as the naturally existing adaptive immune systems in microbes, allow specific recognition of target DNA/RNA without heat denaturation. They can also achieve isothermal signal amplification based on high-turnover nucleic acid cleavage, rather than on nucleic acid replication. For example, CRISPR-Cas12 and CRISPR-Cas13 systems can mediate indiscriminate cleavage of collateral DNA/RNA strands upon RNA-guided DNA/RNA recognition [27,28]. Thus, in the presence of the target nucleic acids, a reporter DNA/RNA (simultaneously labeled with a fluorescence dye and a quencher) can be cleaved and release a fluorescent signal (Fig. 2a). These systems have been demonstrated for the nucleic acid assay of SARS-CoV-2 [26], which can be carried out on cost-efficient test papers, producing visual readouts in a short sample-to-answer time. Recently, a CRISPR-Cas9 system has been adapted for multiplexed RNA detection [29] (Fig. 2b), which employs trans-activating CRISPR RNAs to specifically bind the target RNAs and guide the Cas9 cleavage of corresponding reporter DNAs, allowing discrimination of SARS-CoV-2 and its variant with single-base resolution in patient samples.

Another representative approach for synthetic biology-empowered assays is based on synthetic riboregulators (also called "toehold switches"). A typical riboregulator is an engineered RNA motif placed on the 5' untranslated region of an

mRNA (Fig. 3c), which allows structural reconfiguration in response to a specific nucleic acid sequence, activating the translation of the downstream reporter gene (generating a reporter protein). These functions are all carried out under physiological conditions, and hence hold promise in isothermal nucleic acid assays [30]. Recent studies have achieved single-nucleotide specificity, enabling the discrimination of SARS-CoV-2 variants [31].

Compared to PCR, the signal amplification efficiencies of the CRISPR-Cas and riboregulator systems are still very limited. But, they can be seamlessly cascaded with other established isothermal nucleic acid amplification strategies (e.g., NASBA, RPA, and LAMP) [30], enabling adequate sensitivity for direct detection in clinical samples. By generating different reporting proteins, these synthetic circuits can readily adapt to diverse readout modes [30], such as paper-based colorimetric platforms, electrochemical biosensors, or even glucosemeters [32]. These advantages together facilitate the application of these cutting-edge methods in resource-constrained settings. Moreover, the synthetic circuits can be integrated with molecular logic calculations in response to multiple nucleic acid inputs, which allow more accurate identification of target pathogens and their subtypes [33]. However, for POC application, how to keep the long-term stability of the reagents (the enzymes and RNA molecules) in these methods under ambient conditions is worth considering in further development of these methods.

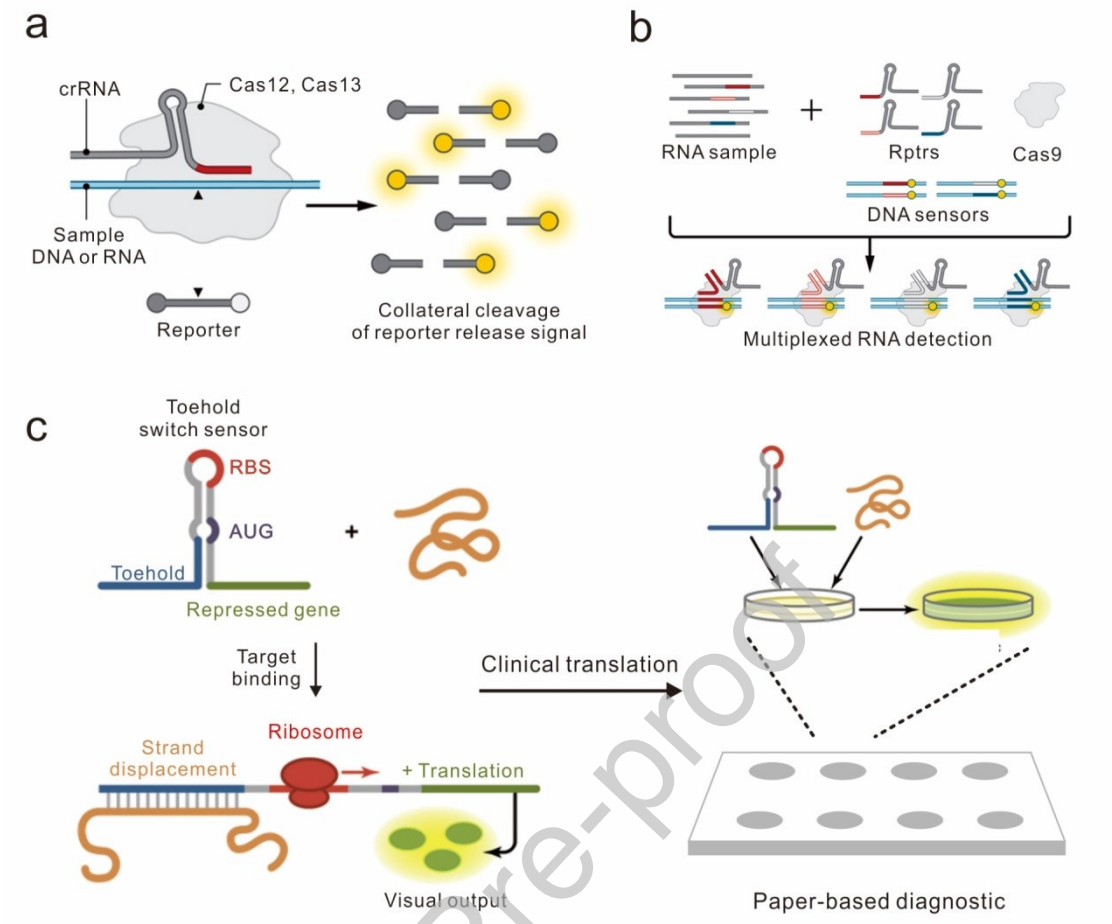

Fig. 2. Pathogen assays with synthetic biology. (a) Schematic of CRISPR-based nucleic acids detection with collateral cleave activity, Cas12 or Cas13 system, and (b) the novel CRISPR-Cas9 system using reprogrammed tracrRNAs (Rptrs) for multiplexed RNA detection [26]. (c) Schematic of a riboregulatory nucleic acid sensor. Its structural configuration is responsive to the target RNA, allowing switchable translation of the reporter proteins. This system can be translated onto test papers by freeze-drying [30].

4. System integration for real-world detection of pathogens

The cutting-edge methods for pathogen detection in real samples still face several challenges. One major challenge is the matrix effects arising from the interferences by

the complex constituents in real samples, which may result in false results or compromised sensitivities. To ensure the accuracy of the methods in real samples, multistep procedures with different reagents are often involved, which rely on cumbersome operations such as sample extraction, transfer, mixing, and separation. For example, for detection in blood samples, dilution and/or plasma/serum separation are desired to reduce the matrix effects; for nucleic acid assays in real samples, the use of lysing reagent to isolate nucleic acids from the pathogens is usually required.

Microfluidic platforms driven by external power such as centrifuging, electronic/magnetic fields, and pumps have been developed to integrate multiple reactions. For example, in an immunoassay microfluidic system, bubbles pumped into the channels are utilized to drive multiple reagents separately and successively onto the interfaces for reactions at different stages, enabling automatic detection of H1N1 mimetics within minutes [34]. However, the requirement for external equipment to power and control the microfluidic systems still restricts their field application. Recently, self-powered or power-free microfluidic systems (actuated by, e.g., vacuum [35], gravity [36], and/or lateral flow [37]) have been developed for detection independent of cumbersome peripherals. Recently, a monolithic chip utilizes microfluidic chain reactions powered by an on-chip paper pump to implement an eight-step ELISA protocol autonomously [38], allowing quantitative detection of antibodies against the N protein of SARS-CoV-2 in saliva. In addition, heterogeneous sensing interfaces for different types of analytes can be integrated into a unified

microarray, which enables "all-in-one" assays of multiple biomarkers including nucleic acids and proteins [19,39], holding promise for facile yet comprehensive diagnosis.

The development of mobile smart devices (e.g., smartphones) has also facilitated signal processing, data analysis, and generation of readable conclusions. Recently, smart devices with customized apps have been combined with bioassay chips for diverse infectious diseases including COVID-19 [39-41]. Further, the development of high-speed mobile networks (e.g., 5G networks), big data, artificial intelligence (AI) and cloud computing is enabling remote medical observation, analysis, prognosis, and management, which is helpful for patients in resource-restricted settings, and can relieve the burden on hospital infrastructure during pandemics (Fig. 3).

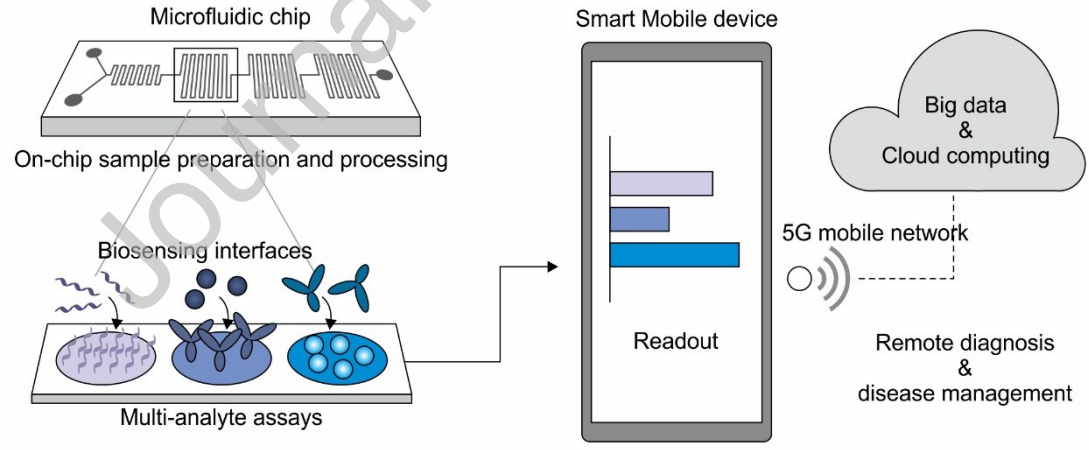

Fig. 3. Outlook of next-generation biosensing platform integrating automatic sample preparation on a chip, multi-biomarker microarray, smart device, mobile network, artificial intelligence, cloud computing, etc., holding promise for rapid yet precise

diagnosis of infectious diseases.

5. Outlook

Despite the progress in advancing bioassays for diverse pathogen-related biomarkers, challenges remain in satisfying the demands in public health emergencies, particularly in the recent pandemic. Although some believe that Omicron is less fatal compared to previous VoCs, recent studies suggest that Omicron has still conferred substantial excess mortality in a short time, even in a highly vaccinated and increasingly immune population, due to the enhanced transmissibility and immune escape capability [42]. Meantime, growing evidence shows that COVID-19 may result in long-term residual effects (long COVID) that should not be underestimated [43]. And, given the large infection population and the intrinsic high mutation rate of RNA viruses, the emergence of new dangerous variants is almost inevitable. Thus, it is desirable to preventively monitor potentially pathogenic microorganisms in human and environmental samples by combining the above-mentioned technologies such as AI, big data, and metagenomic sequencing. Although challenges remain, it is worth pursuing such an integrated system with high accuracy, speed, and throughput whereas miniaturized size and affordable cost, which thus can be widely deployed among communities, enabling proactive, rapid-response, and intelligent disease control measures in the future.

Acknowledgments

This work was financially supported by the National Natural Science Foundation

of China (21991134, 91953106), and Science Foundation of the Shanghai Municipal Science and Technology Commission (19JC1410300, 21dz2210100).

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.

References

[1] F. Wu, S. Zhao, B. Yu, et al., A new coronavirus associated with human respiratory disease in China, Nature 579 (7798) (2020) 265-269.

[2] Z. Zhao, Y. Wang, L. Qiu, et al., New Insights from Chemical Biology: Molecular Basis of Transmission, Diagnosis, and Therapy of SARS-CoV-2, CCS Chemistry 3 (1) (2021) 1501-1528.

[3] X. Zhang, W. Zhang, S. Chen, Shanghai's life-saving efforts against the current omicron wave of the COVID-19 pandemic, Lancet 399 (10340) (2022) 2011-2012.

[4] Y. Zhao, X. Zuo, Q. Li, et al., Nucleic acids analysis, Sci. China Chem. 64 (2) (2021) 171-203.

[5] P. Neuzil, C.Y. Zhang, J. Pipper, et al., Ultra fast miniaturized real-time PCR: 40 cycles in less than six minutes, Nucleic Acids Res. 34 (11) (2006) e77.

[6] M.U. Kopp, A.J. de Mello, A. Manz, Chemical amplification: Continuous-flow PCR on a chip, Science 280 (5366) (1998) 1046-1048.

[7] M. Krishnan, V.M. Ugaz, M.A. Burns, PCR in a Rayleigh-Benard convection cell, Science 298 (5594) (2002) 793.

[8] J.H. Jung, S.J. Choi, B.H. Park, et al., Ultrafast Rotary PCR system for multiple influenza viral RNA detection, Lab Chip 12 (9) (2012) 1598-1600.

[9] Y. Zhang, Q. Li, L.J. Guo, et al., Ion-Mediated Polymerase Chain Reactions Performed with an Electronically Driven Microfluidic Device, Angew. Chem. Int. Ed. 55 (40) (2016) 12450-12454.

[10] J.H. Lee, Z. Cheglakov, J. Yi, et al., Plasmonic photothermal gold bipyramid nanoreactors for ultrafast real-time bioassays, J. Am. Chem. Soc. 139 (24) (2017) 8054-8057.

[11] H. Sun, L. Xiong, Y. Huang, et al., AI-aided on-chip nucleic acid assay for smart diagnosis of infectious disease, Fundam. Res. 2 (3) (2022) 476-486.

[12] R.W. Peeling, P.L. Olliaro, D.I. Boeras, et al., Scaling up COVID-19 rapid antigen tests: promises and challenges, Lancet Infect. Dis. 21 (9) (2021) e290-e295.

[13] P.S. Kwon, S. Ren, S.J. Kwon, et al., Designer DNA architecture offers precise and multivalent spatial pattern-recognition for viral sensing and inhibition, Nat. Chem. 12 (1) (2020) 26-35.

[14] C. Sigl, E.M. Willner, W. Engelen, et al., Programmable icosahedral shell system for virus trapping, Nat. Mater. 20 (9) (2021) 1281-1289.

[15] G. Seo, G. Lee, M.J. Kim, et al., Rapid detection of COVID-19 causative virus

(SARS-CoV-2) in human nasopharyngeal swab specimens using feld-effect transistor-based biosensor, ACS Nano 14 (4) (2020) 5135-5142.

[16] H. Yousefi, A. Mahmud, D.R. Chang, et al., Detection of SARS-CoV-2 viral particles using direct, reagent-free electrochemical sensing, J. Am. Chem. Soc. 143 (4) (2021) 1722-1727.

[17] J. Das, S.O. Kelley, High-performance nucleic acid sensors for liquid biopsy applications, Angew. Chem. Int. Ed. 59 (7) (2020) 2554-2564.

[18] W. Liu, J. Das, A.H. Mepham, et al., A fully-integrated and automated testing device for PCR-free viral nucleic acid detection in whole blood, Lab Chip 18 (13) (2018) 1928-1935.

[19] M. Lin, P. Song, G. Zhou, et al., Electrochemical detection of nucleic acids, proteins, small molecules and cells using a DNA-nanostructure-based universal biosensing platform, Nat. Protoc. 11 (7) (2016) 1244-1263.

[20] L. Wang, X. Wang, Y. Wu, et al., Rapid and ultrasensitive electromechanical detection of ions, biomolecules and SARS-CoV-2 RNA in unamplified samples, Nat. Biomed. Eng. 6 (3) (2022) 276-285.

[21] B.J. Hindson, K.D. Ness, D.A. Masquelier, et al., High-throughput droplet digital PCR system for absolute quantitation of DNA copy number, Anal. Chem. 83 (22) (2011) 8604-8610.

[22] D.M. Rissin, C.W. Kan, T.G. Campbell, et al., Single-molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations,

Nat. Biotechnol. 28 (6) (2010) 595-599.

[23] L. Mou, X.Y. Jiang, Materials for Microfluidic Immunoassays: A Review, Adv. Healthc. Mater. 6 (15) (2017).

[24] T. Charalampous, G.L. Kay, H. Richardson, et al., Nanopore metagenomics enables rapid clinical diagnosis of bacterial lower respiratory infection, Nat. Biotechnol. 37 (7) (2019) 783-792.

[25] D.P. Depledge, K.P. Srinivas, T. Sadaoka, et al., Direct RNA sequencing on nanopore arrays redefines the transcriptional complexity of a viral pathogen, Nat. Commun. 10 (1) (2019) 754.

[26] O.O. Abudayyeh, J.S. Gootenberg, CRISPR diagnostics, Science 372 (6545) (2021) 914-915.

[27] J.S. Chen, E.B. Ma, L.B. Harrington, et al., CRISPR-Cas12a target binding unleashes indiscriminate single-stranded DNase activity, Science 360 (6387) (2018) 436-439.

[28] J.S. Gootenberg, O.O. Abudayyeh, J.W. Lee, et al., Nucleic acid detection with CRISPR-Cas13a/C2c2, Science 356 (6336) (2017) 438-442.

[29] C.L. Jiao, S. Sharma, G. Dugar, et al., Noncanonical crRNAs derived from host transcripts enable multiplexable RNA detection by Cas9, Science 372 (6545) (2021) 941-948.

[30] X. Tan, J.H. Letendre, J.J. Collins, et al., Synthetic biology in the clinic: engineering vaccines, diagnostics, and therapeutics, Cell 184 (4) (2021) 881-898.

[31] F. Hong, D. Ma, K. Wu, et al., Precise and Programmable Detection of Mutations Using Ultraspecific Riboregulators, Cell 180 (5) (2020) 1-15.

[32] E. Amalfitano, M. Karlikow, M. Norouzi, et al., A glucose meter interface for point-of-care gene circuit-based diagnostics, Nat. Commun. 12 (1) (2021).

[33] D. Ma, Y. Li, K. Wu, et al., Multi-arm RNA junctions encoding molecular logic unconstrained by input sequence for versatile cell-free diagnostics, Nat. Biomed. Eng. 6 (3) (2022) 298-309.

[34] F. Yang, X. Zuo, Z. Li, et al., A bubble-mediated intelligent microscale electrochemical device for single-step quantitative bioassays, Adv. Mater. 26 (27) (2014) 4671-4676.

[35] E.-C. Yeh, C.-C. Fu, L. Hu, et al., Self-powered integrated microfluidic point-of-care low-cost enabling (SIMPLE) chip, Sci. Adv. 3 (3) (2017) e1501645.

[36] D. Ye, L. Li, Z. Li, et al., Molecular threading-dependent mass transport in paper origami for single-step electrochemical DNA sensors, Nano Lett. 19 (1) (2019) 369-374.

[37] R. Banerjee, A. Jaiswal, Recent advances in nanoparticle-based lateral flow immunoassay as a point-of-care diagnostic tool for infectious agents and diseases, Analyst 143 (9) (2018) 1970-1996.

[38] M. Yafia, O. Ymbern, A.O. Olanrewaju, et al., Microfluidic chain reaction of structurally programmed capillary flow events, Nature 605 (7910) (2022)

464-469.

[39] Y. Dou, J. Su, S. Chen, et al., A smartphone-based three-in-one biosensor for co-detection of SARS-CoV-2 viral RNA, antigen and antibody, Chem. Commun. 58 (41) (2022) 6108-6111.

[40] K. Ming, J. Kim, M.J. Biondi, et al., Integrated quantum dot barcode smartphone optical device for wireless multiplexed diagnosis of infected patients, ACS Nano 9 (3) (2015) 3060-3074.

[41] T. Laksanasopin, T.W. Guo, S. Nayak, et al., A smartphone dongle for diagnosis of infectious diseases at the point of care, Sci Transl Med 7 (273) (2015) 273re1.

[42] J.S. Faust, C. Du, C. Liang, et al., Excess mortality in Massachusetts during the Delta and Omicron waves of COVID-19, JAMA (2022) 10.1001/jama.2022.8045.

[43] A. Nalbandian, K. Sehgal, A. Gupta, et al., Post-acute COVID-19 syndrome, Nat. Med. 27 (4) (2021) 601-615.

Tingting Zhai received her bachelor's degree from Hunan University in 2013 and Ph.D. degree from Nanjing University in 2018. From 2018 to 2021, she was a postdoctoral fellow at Shanghai Jiao Tong University. She is currently an assistant

professor at the School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University. Her scientific interests focus on DNA nanotechnology-based biosensing and bioimaging.

Jiang Li obtained his Ph.D. degree from Sichuan University in 2009. After that, he joined the faculty at Shanghai Institute of Applied Physics (SINAP), Chinese Academy of Sciences (CAS). He is now a professor at Shanghai Advanced Research Institute, CAS. His research interests focus on DNA nanotechnology and nucleic acid analysis.

Chunhai Fan is a K. C. Wong Chair Professor at Shanghai Jiao Tong University (SJTU) and a member of Chinese Academy of Sciences (CAS). Before joining SJTU, he was a CAS Distinguished Professor at the Shanghai Institute of Applied Physics. He is a fellow of Royal Society of Chemistry (FRSC), an elected fellow of the International Society of Electrochemistry (ISE) and American Association for the Advancement of Science (AAAS). His research interests include biosensing, DNA nanotechnology, and DNA computing.