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REVIEW

Application of microfluidic technologies on COVID-19 diagnosis and drug discovery



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Abstract The ongoing coronavirus disease 2019 (COVID-19) pandemic has boosted the development of antiviral research. Microfluidic technologies offer powerful platforms for diagnosis and drug discovery for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) diagnosis and drug discovery. In this review, we introduce the structure of SARS-CoV-2 and the basic knowledge of microfluidic design. We discuss the application of microfluidic devices in SARS-CoV-2 diagnosis based on detecting viral nucleic acid, antibodies, and antigens. We highlight the contribution of lab-on-a-chip to manufacturing point-of-care equipment of accurate, sensitive, low-cost, and user-friendly virus-detection devices. We then investigate the efforts in organ-on-a-chip and lipid nanoparticles (LNPs) synthesizing chips in antiviral drug screening and mRNA vaccine preparation. Microfluidic technologies contribute to the ongoing SARS-CoV-2 research efforts and provide tools for future viral outbreaks.

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1. Introduction

Coronavirus disease 2019 (COVID-19), caused by novel severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has led to an unprecedented worldwide pandemic. The World Health Organization (WHO) data suggests over 600 million confirmed cases and over 6.4 million deaths as of 4 September 2022. Compared with the case fatality rate of the 1918 influenza pandemic (2%) and the 1957 influenza pandemic (0.6%), SARS-CoV-2 is higher (about 3.3%). Furthermore, the spread rate of SARS-CoV-2 is 40-fold higher than that of SARS-CoV, which makes it more difficult to control¹. Thus, collaborating measures must be implemented to contain an emerging pandemic virus or slow its spread worldwide.

SARS-CoV-2 is an RNA virus containing a 30 kb genomes, encoding four structural proteins, including the spike protein (S), envelope protein (E), membrane protein (M), and nucleocapsid protein (N)^{2–4} (Fig. 1). Viral replication requires other auxiliary genes, including open reading frame 1a (ORF1a), ORF1b, RNA-dependent RNA polymerase (RdRp), and hemagglutinin esterase (HE)³. With the appearance of many variants of SARS-CoV-2, their propagation speed and virulence became more challenging to predict. Therefore, accurate, rapid, long-term detection strategies, frequent updating of therapies, and efficient protection are needed to control the spread of viruses. These require multidisciplinary solutions to achieve coordinated diagnosis and drug discovery⁵.

Microfluidic technologies allow processing or manipulating small (10^{-9} – 10^{-18} L) amounts of fluids based on designed channels with dimensions of tens to hundreds of micrometers in the chip^{6–8}. Many operations like separation, reaction, detection of different ingredients, and culture of cells or tissues can be integrated into miniaturized microscale devices^{9,10}. Microfluidic devices have played an important role in quickly responding to infectious diseases by developing point-of-care devices for accurate and fast diagnosis and constructing instruments to reveal the pathogenic mechanisms and conduct drug screens^{11–13}. Microfluidic devices can be lab-on-a-chip^{14,15}, organ-on-a-chip^{9,16,17}, and microreactor^{8,18,19}, depending on their application for COVID-19 management. Based on their destination use, microfluidic chips can be manufactured from different types of materials employing diverse fabrication methods. In Table 1, we emphasize the most common materials and fabrication methods used for disposable devices and provide a brief description of the applications, advantages, and disadvantages of each material^{20–23}. Among them, polydimethylsiloxane (PDMS), paper, and polymethylmethacrylate (PMMA) are the most common

materials employed for SARS-CoV-2 detection and drug discovery.

In this review, we discuss the role of microfluidic technology in SARS-CoV-2 detection and drug discovery. Firstly, we reviewed three kinds of COVID-19 diagnosis strategies based on the nucleic acid, antibodies, and antigens of SARS-CoV-2, respectively, and investigated the advantages and disadvantages of those methods. Secondly, we demonstrated organ-on-a-chip's application in studying the molecular mechanism of SARS-CoV-2 and drug screening. Finally, we examined the role of the microfluidic chip in preparing mRNA delivery nanoparticles. In a word, microfluidic technologies offer a great chance to respond to the current COVID-19 pandemic and will play a significant role in future viral outbreaks.

2. Microfluidic technology for SARS-CoV-2 detection

2.1. Nucleic acid detection

Nucleic acid detection is currently the gold standard for SARS-CoV-2 detection of its capacity to measure the viral genomic parts directly. SARS-CoV-2 nucleic acid exists in the nasopharyngeal fluids of infected patients, and nucleic acid test helps prevent infectious spread between people and communities²⁴. Traditional methods include quantitative real-time polymerase chain reaction (qRT-PCR)^{25,26}, isothermal amplification reaction²⁷, and clustered regularly interspaced short palindromic repeats-associated systems (CRISPR–Cas) technology²⁸, which are used to detect SARS-CoV-2 RNA sequences²⁹. Among them, RT-PCR is the gold standard of nucleic acid detection for COVID-19 diagnosis. The procedure of RT-PCR begins with the isolation and conversion of viral RNA to complementary DNA (cDNA) and then the amplification of cDNA by Taq DNA polymerase. Finally, it ends with the quantification of the viral load. Although RT–PCR serves as a sensitive, precise, and specific viral detection method, limitations of RT–PCR tests still exist, including sample storage, outside aerosol contamination, long waiting time, large-scale instruments, and professional operators^{24,30}.

In comparison, microfluidic devices can seal the chips' sample, reagent, and reaction system, dramatically reducing the outside aerosol contamination^{31,32}. In addition, microscale structure on microfluidic chips and fast heat transmission sharply decrease the reaction volume and the thermal cycle time, improving the nucleic acid amplification efficiency³³. Furthermore, lab-on-a-chip platforms highly integrate sets of microfluidic elements, which are dedicated to multiple operations of many laborious benchtop protocols without human intervention, thus able to perform complex jobs^{34,35}. For example, a plasmo-fluidic PCR chip with glass nanopillar arrays and gas-permeable microfluidic channels allows both automatic sample loading and microbubble-free PCR reaction. At the same time, the plasmonic nanopillar arrays result in ultrafast photothermal cycling. PCR results showed 306 s for plasmids expressing SARS-CoV-2 envelope protein, and the amplification efficiency is more than 91% (Table 2)³⁶. Another PCR system, named GeneSoC, has one heater for the reverse transcription (RT) reaction and two heaters for thermal cycling, and two micro blowers at both flow ends for the high-speed shuttle of the PCR solution, which achieves specific gene amplification within 15 min (Fig. 2A) (Table 2)³⁷.

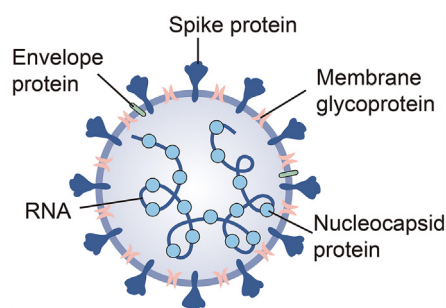


Figure 1 The structure of SARS-CoV-2.

Table 1 Summary on the materials of microfluidic devices.

Material	Application	Fabrication technology	Advantage	Disadvantage
Silicon	Point-of-care diagnostics Organ-on-chip devices	Photolithography Wet and dry etching Electron beam lithography	Availability, chemical compatibility, thermostability, ease of fabrication, design flexibility, semiconducting properties, possibility of surface modifications	Opacity, relatively high cost
Glass	Chemical reactions Synthesis of emulsions Polymeric nanoparticles Optical detection		Chemically inert, thermostable, electrically insulating, rigid, biologically compatible, allowing easy surface functionalization, higher resolution, thermal and chemical stability	Expensive and complex fabrication
Polydimethyl siloxane (PDMS)	Organ-on-a-chip Multi-channels Array of a special pattern High-throughput models	Soft photolithography	Cheap, easy to mold, good for prototyping, presenting optical transparency, gas permeability, biocompatibility, low autofluorescence, natural hydrophobicity, high elasticity.	Non-specific molecule adsorption, absorption of less hydrophobic molecules, incompatibility with many solvents, reagents, release of uncrosslinked small PDMS molecules, high cost.
Polymethyl methacrylate (PMMA)	Organ-on-a-chip Micro-physiological systems	Embossing Injection Molding Laser ablation Soft lithography	Better solvent compatibility, no small-molecule absorption, optically transparent, good mechanical properties, allowing surface modification	Poor thermal performance, poor resistance to chemical corrosion, complex processing
Perfluorinated polymers	Cell cultures High-precision assays Super-clean tools Valve and pump fabrications Synthesis devices		Thermo-processability, chemical inertness, compatibility with organic solvents, excellent antifouling properties	Lack of easy micropatterning and satisfactory elasticity
Cyclo-olefin polymers and copolymers (COPs/COCs)	Synthesis devices in which aggressive solvents are employed	Embossing	Optical transparency, enhanced chemical resistance, low water absorptivity, good electrical insulating properties, long-term stability of surface treatments, low level of impurities	Poor flexibility and lowly biocompatible
Epoxy resins	Organ-on-a-chip	3D printing	Enhanced stability at high temperatures, chemical resistance, transparency, very high resolution with small features.	High cost, poor flexibility, oxygen permeability
Paper	Rapid point-of-care diagnostic testing and medical screening	Wax patterning Alkyl ketene dimer printing Flexographic printing Shaping/cutting	Simplicity, accessibility, significant low costs, high porosity, high physical absorption, ease of manipulation and sterilization, potential for chemical or biological modifications, similarity to the native ECM, bio-affinity, biocompatibility, light weights, the ability to operate without supporting equipment, direct and <i>in situ</i> operation	Poor mechanical strength in a wet state, thickness requirements for achieving transparency

Table 2 Summary on microfluidic technology for SARS-CoV-2 nucleic acid detection.

Method	Fluid manipulation technique	Material	Immobilized antigen/antibody/gene	Detected biomolecules	Detector	Sensitivity/%	Specificity/%	Sample size/donor/standard	Limit of detection (LOD)	Detection time	Advantage	Ref.
Nanoplasmonic on-chip RT-PCR	Vacuum pump	PDMS	E gene	cDNA	Fluorescence	91	—	—	—	326 s	Rapid, small volume, efficient thermal cycling	36
GeneSoC	—	—	N gene	cDNA	Fluorescence	92	100	66 nasopharyngeal swab samples	10 copies/reaction	within 15 min	Simplicity and portability, capacity for high-speed and real-time monitoring, and the use of a single disposable tip per analysis.	37
Microfluidic nano-scale qPCR	—	—	SARS-CoV-1 N gene	cDNA	Fluorescence	94.50	81.30	182 clinical NP swab samples	Below 1 copy/ μ L	—	Ultra-sensitive detection, lower reagent consumption increased throughput, increased precision, increased confidence in test results, and the capacity to simultaneously test for multiple pathogens	42
Bio-mark TM	—	—	SARS-CoV-2 N gene	cDNA	Fluorescence	—	100	20 clinical nasopharyngeal swab samples, 92 + 74 purified RNA samples	7 copies/reaction	192 samples in less than 3 h	Analyzing a larger number of reactions per run, making the assay more cost-effective and less time-consuming.	43
Automated multiplex RT-PCR on chip	Siphon valve	PMMA	SARS-CoV-2 N gene	cDNA	Fluorescence	—	100	29 SARS-CoV-2 samples, and 1572 negative samples	20 copies/reaction	57 min	High-throughput sample-to-answer multiplex detection	33
Fully automated centrifugal RT-LAMP microfluidic system	Automated syringe pumps	PMMA, PDMS	SARS-CoV-2 N, E and ORF1ab genes	cDNA	Fluorescence	—	100 when 100 copies/ μ L	—	2 copies/reaction	Less than 70 min	Fully automated	44
Centrifugal LAMP microfluidic nucleic acid assay	Centrifugal forces	—	SARS-CoV-2 targeting N and ORF1ab sequence	cDNA	Fluorescence	—	100	54 samples	—	40 min	Capable of discriminating HCoV-229E, HCoV-OC43, HCoV-NL63, HCoV-HKU1, SARS-CoV, MERS-CoV, and SARS-CoV-2 simultaneously within 40 min	45
Two-stage isothermal amplification	Syringe pump	Clear resin	N gene, E gene and Orf1a gene of SARS-CoV-2 RNA	cDNA	Smartphone	—	—	Heat-inactivated SARS-CoV-2 and quantitative PCR control RNA	100 genome equivalent/mL	60 min	Simple readout, high sensitivity, and multiple analyte detection	47

Nucleic acid isothermal amplification analyzer	Centrifugal force	Polycarbonate	N gene	cDNA	Fluorescent detector	—	—	201 preclinical samples, 14 positive samples, and 614 clinical samples	50 copies/ μ L	90 min	High sensitivity, good specificity, strong robustness, and excellent repeatability	48
Lateral flow recombinase polymerase amplification assay	Gravitational force	PMMA	N gene, ORF1ab, E gene	cDNA	Naked eye	97	100	37 positive samples and 17 negative samples	1 copy/ μ L	30 min	Low-cost, rapid, high specificity and high sensitivity detection	49
Combines CRISPR-based diagnostics	Syringe pump	—	Spike gene	cDNA	Fluorescent detector	98.4	99	2088 patient specimens	100 copies/ μ L	5 h	High-throughput, multiplexed and microfluidic diagnostic	51
Digital CRISPR/Cas-assisted assay	Pipette	Polycarbonate	N gene	cDNA	Fluorescent detector	—	—	2 positive samples, 1 negative sample, and influenza sample	20 genome equivalent/mL	20 min	Rapid, and high sensitivity detection without RNA extraction	50
Electric field-driven microfluidics for rapid CRISPR-based diagnostics	Isotachophoresis	Glass	N gene, E gene, Rnase P gene	cDNA	Inverted epifluorescence microscope	—	96.9	40 negative swab samples	10 copies/ μ L	35 min	Minimal consumption of reagents, and rapid detection	52
RNA-triggered Cas13a catalysis system	Syringe pump	PDMS	Open reading frame 1a (O) gene and nucleocapsid (N) gene	cDNA	Fluorescence microscopy	—	100	SARS-CoV-2 RNA	6 copies/ μ L	60 min	Avoiding the need for expensive thermocyclers and professional technicians, simplicity, single-molecule quantitation capability, and broad applicability	53
Droplet digital PCR platform	Pipette	Polypropylene	N gene, ORF1ab	cDNA	A smartphone and a portable trans-illuminator	—	—	Template sample	3.8 copies/20 μ L	90 min	Low-cost, and rapid detection without bulky and expensive micropumps and optical detectors	54
Direct reverse-transcription-loop-mediated isothermal amplification	Syringe pump	PMMA	As1e, N, and E genes	cDNA	A smartphone with a 488 nm LED light	—	—	4 clinical respiratory virus samples	20 copies/ μ L	33 min	High sensitivity, accuracy, low-cost, and rapid detection	55
Amplification-free CRISPR/Cas12a-based diagnostic technology	Pipette	PMMA	ORF1ab gene	cDNA	Smartphone	—	100	11 samples from BEI resources	50 copies/ μ L	71 min	Visible readout, amplification-free detection without using any optical hardware	56

—, not applicable.

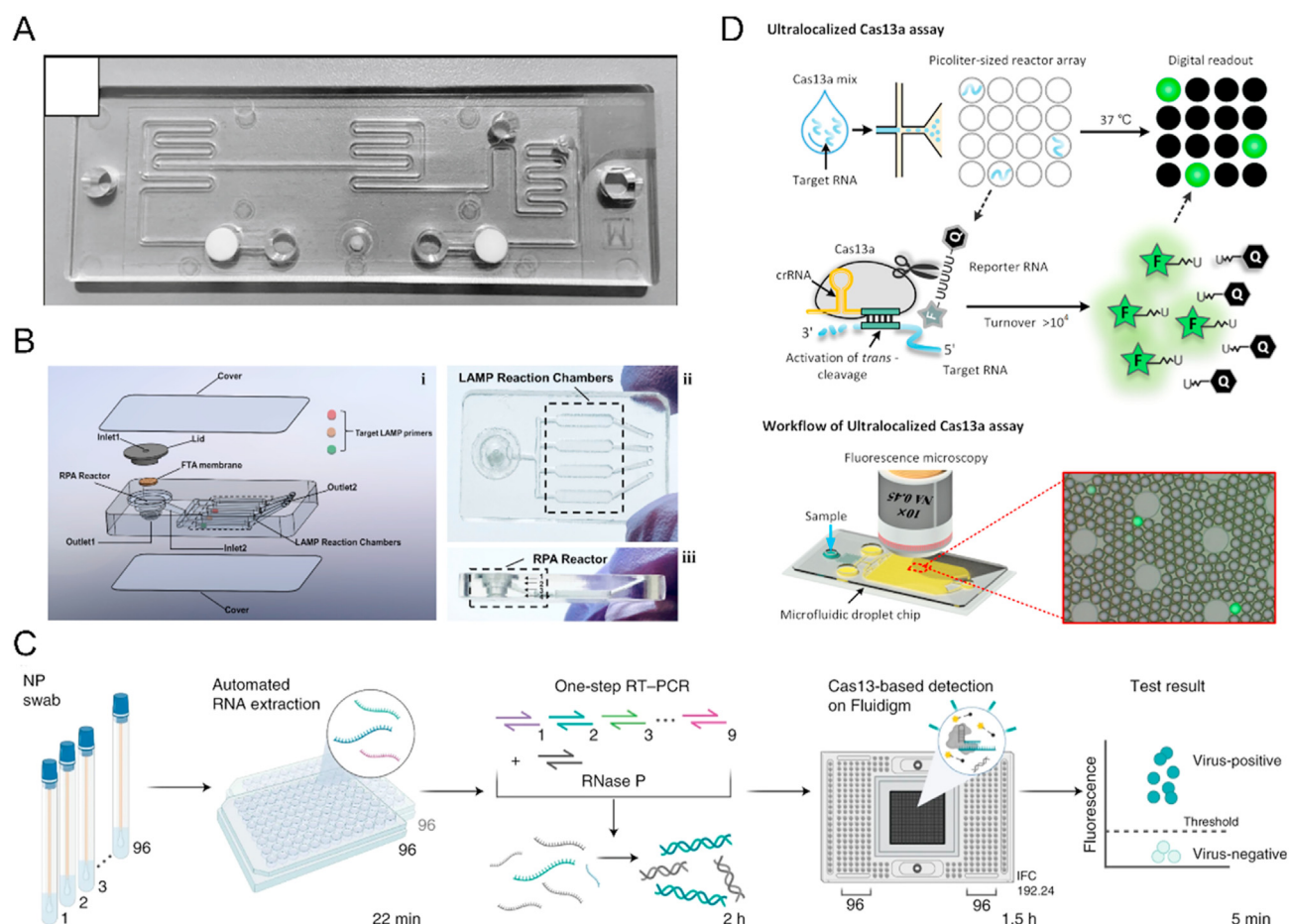


Figure 2 Example of microfluidic technology for SARS-CoV-2 nucleic acid detection. (A) A high-speed but low-throughput RT-qPCR system for the detection of SARS-CoV-2. Reprinted with the permission from Ref. 37. Copyright © 2021 Elsevier. (B) A 3D printed integrated microfluidic chip for multiplexed colorimetric detection of SARS-CoV-2 in wastewater. Reprinted with the permission from Ref. 47. Copyright © 2020 Elsevier. (C) Multiplexed CRISPR-based microfluidic platform for identification of SARS-CoV-2 variants. Reprinted with the permission from Ref. 51. Copyright © 2022 Springer Nature. (D) An ultralocalized Cas13a assay enables universal and nucleic acid amplification-free single-molecule RNA diagnostics. Reprinted with the permission from Ref. 53. Copyright © 2021 American Chemical Society.

Although qRT-PCR provides acceptable sensitivity for early infection identification, the possibility of false-negative or false-positive findings is one of the most critical clinical factors for pandemic transmission³⁸. Such findings occur when the viral content is low, as evident in asymptomatic or weakly positive individuals³⁹. Henceforth, timely diagnosis is required to avoid such outcomes^{40,41}. Xie et al.⁴² developed a three-step microfluidic qPCR method that could incorporate reverse transcription and targeted cDNA pre-amplification to address the issue of false-negative results while simultaneously keeping high sensitivity in the RT-PCR technique. They could enable detection below 1 copy/ μ L as a nanoscale PCR method (Table 2). Fassy et al.⁴³ also included the pre-amplification step enabling the detection of seven transcript copies per reaction for N genes (Table 2). Moreover, as the integration, simplicity, bubble easy removal, and hundreds of individual units driven by a single spindle motor, centrifugal microfluidic technology has been widely used in SARS-CoV-2 nucleic acid amplification reaction (Table 2)^{33,44,45}.

Unlike RT-PCR, isothermal amplification reaction does not need heating or cooling, simplifying the outside condition. Among them, loop-mediated isothermal amplification (LAMP) technology

has high sensitivity, rapid response speed, simple operation, and easy readout⁴⁶. More and more methods based on LAMP and microfluidic chips for SARS-CoV-2 detection have been established. For example, a device integrated nucleic acid extraction, two-stage isothermal amplification, and colorimetric detection on the chip to achieve sensitive multiplexed colorimetric detection. The authors detected SARS-CoV-2 with sensitivities of 100 genome equivalent (GE)/mL and 500 colony-forming units (CFU)/mL, respectively, in wastewater within 1 h (Fig. 2B) (Table 2)⁴⁷. Other isothermal amplification methods, including nucleic acid sequence-based amplification (NASBA) (Table 2)⁴⁸ and recombinase polymerase amplification (RPA) (Table 2)⁴⁹, have been integrated with lab-on-a-chip and used to detect SARS-CoV-2 pathogens.

With the rise of CRISPR–Cas technology in nucleic acid sensing and infectious pathogens diagnosis, more and more studies have proved its elegant detection mechanism, including fast assay time and low reaction temperature⁵⁰. Such devices are further integrated with microfluidic technology to achieve more powerful detection. A cost-effective virus and variant detection platform, which combines CRISPR-based diagnostics and

Table 3 Summary on microfluidic technology for SARS-CoV-2 antibodies detection.

Method	Fluid manipulation technique	Material	Immobilized antigen/antibody/gene	Detected biomolecule	Detector	Sensitivity/%	Specificity/%	Sample size/donor/standard	Limit of detection (LOD)	Detection time	Advantage	Ref.
Monolithic chips integrating a microfluidic chain reaction	Paper capillary pump	Transparent resin	N	Anti-N Abs	Naked eye or smartphone integrated with a folded origami box	—	—	—	0.3 ng/mL	—	Frugal, versatile, bona fide lab-on-a-chip, sensitive, quantitative and reproducible.	70
Microfluidic DA-D4 point-of-care test (POCT)	Pipette pump	POEGMA	S1, N, RBD	Anti-S1, anti-N, anti-RBD Abs	Fluorescent detector	100 (anti-S1 & anti-RBD) 96.3 (anti-N)	100	46 plasma samples from 31 positive patients and 41 negative samples.	0.12 ng/mL	60 min	Easy to use, quantitative, high specificity and sensitivity, capable of measuring antibody kinetics and seroconversion directly from unprocessed blood or plasma, capable of detecting IP-10, low sample volume requirement, low cost.	71
Microfluidic nanoimmunoassay platform based on MITOMI	Pneumatic valves	PDMS	His-tagged S	Anti-S IgG	Nikon ECLIPSE Ti microscope equipped with a LED fluorescent excitation system and a Cy3 filter set	98	100	289 positive and 134 negative samples	1 nmol/L IgG	—	High sensitivity and specificity, high-throughput, negligible reagent consumption, ultra-flow volume blood sampling	72
Semi-automatic serological assay	Valve pump	PDMS	S, S1, RBD and N	Anti-S/S1/RBD/N IgG/IgN	Inverted fluorescence microscope	95	91	100 serum samples from 66 positive patients	1.6 ng/mL	2.6 h	Low-cost, low sample/reagent consumption, multiple analyte detection, and high throughput.	73
Single molecule arrays	Commercial products	Commercial products	S ligated micron-sized beads	Anti-S Abs	Simoa HD-1 analyzer	—	—	28 plasma samples from 10 positive patients	—	—	High throughput, rapid detection, and high specificity	74
An immunoassay based on dual-encoded beads and rolling circle amplification	Syringe pump	PDMS	S, N, RBD	Anti-S, anti-N, anti-RBD Abs	Fluorescent detector	100	100	11 positive samples and 11 negative samples	—	—	High throughput, high sensitivity, tunable detection range	75

(continued on next page)

Table 3 (continued)

Method	Fluid manipulation technique	Material	Immobilized antigen/antibody/gene	Detected biomolecule	Detector	Sensitivity/%	Specificity/%	Sample size/donor/standard	Limit of detection (LOD)	Detection time	Advantage	Ref.
Decentralized, instrument-free microfluidic device	Capillary force	PDMS	S-RBD ligated magnetic beads, secondary Abs against human IgG ligated polystyrene beads	Anti-S RBD IgG	Direct visual inspection	99	100	91 vaccinees	13.3 ng/mL	20 min	High specificity, low-cost, user-friendly interfaces, power-free operation, and quantification by visual inspection	76
Sandwich-based enzyme-linked immunosorbent assay on electrodes	DC pump	Grey resin	SI, N, RBD	Anti-SI, anti-N, anti-RBD Abs	Electrochemical biosensors	100	100	3 saliva samples from actively infectious patients	2.3 copies/ μ L	50 min	Low-cost, self-contained, simple and sensitive readout for both viral RNA as well as host antibodies, and user-friendly	77

-, not applicable.

commercially available Fluidigm microfluidics with a streamlined workflow for clinical use, performed on 525 patient specimens in an academic setting and 166 specimens in a clinical setting. Besides, the platform also enables the identification of 6 SARS-CoV-2 variant lineages, including Delta and Omicron, and evaluated it on 2088 patient specimens, which achieved high-throughput surveillance of multiple viruses and variants simultaneously (Fig. 2C) (Table 2)⁵¹. Similarly, to integrate commercially available microfluidic digital chip with CRISPR/Cas12a-assisted RT-PCR, the assay achieved rapid detection: within 15 min for qualitative detection and 30 min for quantitative detection. It also showed a high signal-to-background ratio, broad dynamic range, and high sensitivity—down to 1 genome equivalent (GE)/ μ L of SARS-CoV-2 RNA and 20 GE/ μ L of heat-inactivated SARS-CoV-2 (Table 2)⁵⁰. A selective ionic focusing technique based on an appropriate electric field gradient and a microfluidic chip was utilized to purify target RNA from raw nasopharyngeal swab samples automatically. It detected SARS-CoV-2 RNA in about 35 min by LAMP and CRISPR-Cas12 assay from both contrived and clinical nasopharyngeal swab samples (Table 2)⁵². For droplet microfluidics, the RNA-triggered Cas13a catalysis system in cell-like-sized droplet reactors enhanced target and local reporter concentrations simultaneously. It achieves >10,000-fold enhancement in sensitivity compared to the bulk Cas13a assay and enables absolute digital single-molecule RNA quantitation (Fig. 2D) (Table 2)⁵³.

More and more researchers have combined microfluidic devices with smartphones to achieve automated image analysis free of optical detectors. Chen et al.⁵⁴ used a flattened pipette tip with an elliptical cross-section, which extended a high aspect-ratio microfluidic chip design to pipette scale for rapid (<5 min) generation of several thousand monodispersed droplets for digital PCR. A smartphone can image positive droplets, and the detection of limit is 3.8 copies per 20 μ L (Table 2). Nguyen et al.⁵⁵ developed an internet of things-based device that could complete a series of operations on a miniaturized chip (Table 2). The results were processed by a microprocessor and displayed on the phone. The method based on RT-LAMP achieved a limit of detection of 20 genome copies/ μ L, and the clinical sample of SARS-CoV-2 was successfully analyzed with high sensitivity and accuracy. Yin et al.⁴⁷ integrated RPA and LAMP on a 3D-printed microfluidic chip, and by taking advantage of smartphone connectivity, test results could be reported and tracked. Silva et al.⁵⁶ presented a cellphone-based amplification-free system with CRISPR/CAS-dependent enzymatic (CASCADE) assay. It relied on mobile phone imaging of a catalase-generated gas bubble signal within a microfluidic channel and achieved high accuracy (AUC = 1.0; CI: 0.715–1.00) (Table 2).

Paper-based POC tests also play a crucial role in the current COVID-19 pandemic. Considering the strength of paper, it has been widely used to develop paper-based devices for SARS-CoV-2 detection. Among these, Lateral flow assay (LFA) is a highly mature paper-based diagnostic technology. Researchers and manufacturers have invested the most effort and cost in developing COVID-19 diagnostic kits using LFA⁵⁷. The conventional LFA test detects the target molecule on an absorbent membrane with detection molecules aligned to form the test and control lines. The signal is analyzed qualitatively in a visual or semi-quantitative reading, a very simple, rapid, and portable analytical platform⁵⁸. In 2000, Wang et al.⁵⁹ designed an amplification-free nucleic acid immunoassay, implemented on a lateral flow strip for the fluorescence detection of SARS-CoV-2 ORF1ab, E, N

gene in less than 1 h and achieved 100% of sensitivity and 99% specificity. In the same year, Zhu et al.⁶⁰ devised a multiplex RT-LAMP coupled with a nanoparticle-based lateral flow biosensor. Limit of detection (LOD) was 12 copies per reaction, and the sensitivity and specificity of SARS-CoV-2 ORF1ab and N gene were 100%. Subsequently, a lateral flow-based RT-LAMP detection of SARS-CoV-2 using enzymatic incorporation of biotin-labeled dUTP enhanced the sensitivity and decreased the detection time to 15 min⁶¹. In 2022, Li et al.⁶² integrated isothermal amplification, CRISPR cleavage, and lateral flow detection in a single closed microfluidic platform, enabling contamination-free visual detection and achieving excellent sensitivity (94.1%), specificity (100%) and accuracy (95.8%) for COVID-19 samples.

2.2. Antibody detection

Although RT-PCR is the gold standard for COVID-19 identification, it could not provide evidence of past infection, as nucleic acid only increases highly at acute stage^{63,64}. On the contrary, serological assays detecting antibodies against SARS-CoV-2 not only indicate the previous infection but also help to track the immune response of the host^{65,66}. Furthermore, serological analysis is an effective supplement to nucleic acid testing in COVID-19 epidemiological studies and vaccine development^{67,68}. Traditional methods for COVID-19 management, including enzyme-linked immunosorbent assays (ELISA) and chemiluminescent immunoassays (CLIA), are currently available. However, ELISA

and CLIA depend on large laboratory instruments, professional skills, and complex operation⁶⁹. A large amount of sample and reagent consumption also increases the cost of tests. Compared with the methods above, serological assays based on lab-on-a-chip achieve more convenient and rapid detection of COVID-19-associated antibodies.

Lab-on-a-chip assays, which allow a high degree of integration of all operations of the conventional laboratory on micro-size chips, have performed user-friendly assays for COVID-19 antibodies analysis. The essential step to complete multiple actions independently is reagent storage on the chip. Yafia et al.⁷⁰ introduced the microfluidic chain reaction (MCR) as the conditional, structurally programmed propagation of capillary flow events (Table 3). With MCR, the authors automated a protocol for SARS-CoV-2 antibodies detection in saliva by continuous subsampling and analysis of coagulation-activated plasma with parallel operations, including timers, iterative cycles of synchronous flow, and stop-flow operations. Another assay introduced a device fabricated by inkjet printing antibodies detection reagent as stable and spatially discrete capture spots. It is an entirely self-contained immunoassay platform and dramatically simplifies the detector design and assay readout (Fig. 3A) (Table 3)⁷¹.

Microfluidic technology also supports high-throughput analysis to facilitate large-scale detection of SARS-CoV-2. A microfluidic nano immunoassay for detecting anti-SARS-CoV-2 IgG antibodies in 1024 samples per device is a low-cost, ultralow-volume whole blood sampling method based on two commercial

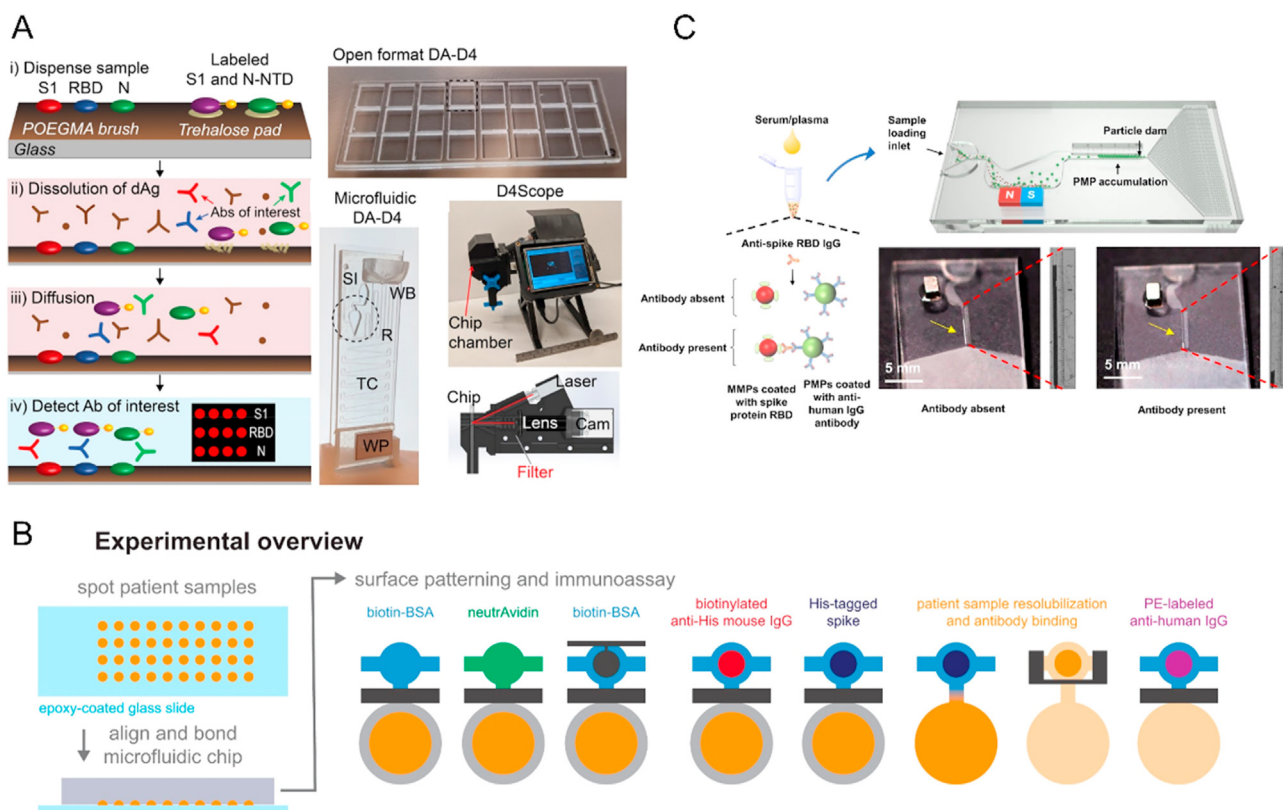


Figure 3 Example of microfluidic technology for SARS-CoV-2 antibodies detection. (A) A point-of-care test for multiplexed, quantitative serological profiling of COVID-19. Reprinted with the permission from Ref. 71. Copyright © 2021 American Association for the Advancement of Science. (B) A high-throughput microfluidic nano immunoassay for detecting anti-SARS-CoV-2 antibodies. Reprinted with the permission from Ref. 72. Copyright © 2021 Proceedings of the National Academy of Sciences. (C) Microfluidic particle dam for direct visualization of SARS-CoV-2 antibody levels. Reprinted with the permission from Ref. 76. Copyright © 2022 American Association for the Advancement of Science.

devices and repurposed a blood glucose test strip. The glucose test strip permits the collection, shipment, and analysis of 0.6 μL of whole blood easily obtained from a simple finger prick. The microfluidic device is a standard two-layer PDMS device consisting of a flow and a control layer. Fluids in the flow layer can be manipulated with pneumatic valves formed by the control layer. The device contains 1024-unit cells, each consisting of an assay and a spotting chamber (Fig. 3B) (Table 3)⁷².

Similarly, a high-throughput microfluidic device can parallel assess the reactivities of four kinds of SARS-CoV-2 antibodies from up to 50 sera samples. The device contains an array of 200 microchambers (4 rows and 50 columns) with a volume of 5.5 nL each. Each microchamber was surrounded by valves and a button valve in the center. All the reagents were loaded into microchambers by valve control and automated computer operation. The assay achieved a sensitivity of 95% and a specificity of 91% (Table 3)⁷³. Gilboa et al.⁷⁴ used Single Molecule Arrays (Simoa) to develop an assay to assess antibody neutralization with high sensitivity and multiplexing capabilities based on antibody-mediated blockage of the ACE2-spike interaction (Table 3). The assay did not require live viruses or cells and could be performed in a biosafety level 2 laboratory within 2 h. Our group⁷⁵ also reported a multiplexed strategy for chip-based sandwich immunoassays integrated with color/size dual-barcoded beads and rolling circle amplification, which achieved simultaneous antibodies and inflammatory biomarkers detection, with the detection range spanning 10 orders of magnitude (Table 3).

Furthermore, detection based on chips permits direct readout without relying on any detection module like a decentralized, instrument-free microfluidic device that directly visualizes SARS-CoV-2 antibody levels. Magnetic microparticles (MMPs) and polystyrene microparticles (PMPs) can bind to SARS-CoV-2 antibodies simultaneously. In a microfluidic chip, this binding reduces the incidence of free PMPs escaping from magnetic separation and shortens PMP accumulation length at a particle dam. This visual, quantitative result enables application in either sensitive mode or rapid mode (Fig. 3C) (Table 3)⁷⁶. Additionally, simultaneous detection of the RNA of SARS-CoV-2 and serological host antibodies to the virus would facilitate the determination of the immune status of COVID-19 patients. A 3D-printed microfluidic chip that simultaneously detects SARS-CoV-2 RNA in saliva and anti-SARS-CoV-2 immunoglobulins in saliva or blood plasma *via* multiplexed electrochemical outputs within 2 h. Low-cost point-of-care microfluidic electrochemical sensors for performing multiplexed diagnostics would facilitate the widespread monitoring of COVID-19 infection and immunity (Table 3)⁷⁷.

Under an urgent need to develop a sensitive and specific assay for rapid detection of antibodies to SARS-CoV-2 infection within minutes, especially in medically underserved areas, readout enabled by a smartphone is more popular and valuable. An electrochemical method based on aerosol jet nano-printed reduced-graphene-oxide-coated 3D electrodes and smartphone-assisted readout achieved a limit-of-detection of 2.8×10^{-15} and 16.9×10^{-15} mol/L of S1 and RBD antibodies⁷⁸. Another electrochemical capillary-flow immunoassay can quantify IgG antibodies targeting anti-N antibodies down to 5 ng/mL in 10 μL of human whole blood samples in under 20 min. The device can be coupled to a near-field communication potentiostat operated from a smartphone, confirming its true POC potential⁷⁹. Moakhar et al.⁸⁰ reported a cost-effective multiplexed fluidic device (NFluidEX), which integrated nano gold electrodes, a multiplexed fluidic-impedimetric readout, built-in saliva collection/preparation, and

smartphone-enabled data acquisition and interpretation. It achieved 100% sensitivity and 100% specificity for RBD IgG and IgM.

LFA was also used to detect SARS-CoV-2 antibodies. Chen et al.⁸¹ reported a lateral flow immunoassay (LFIA) that uses lanthanide-doped polystyrene nanoparticles (LNPs). Feng et al.⁸² used lanthanide, Eu(III) fluorescent microsphere to determine the solid phase immunochromatographic assay. It could detect anti-SARV-CoV-2 IgG within 10 min. However, these methods were qualitative/semi-quantitative analyses. To further improve the sensitivity, Zhou et al.⁸³ prepared highly luminescent quantum dot nanobeads (QBs) by embedding numerous quantum dots into a polymer matrix and then applied it as a signal-amplification label in LFIA. It allowed one order of magnitude improvement in analytical sensitivity compared to conventional gold nanoparticle-based LFIA. Moreover, Chen et al.⁸⁴ developed an ultra-sensitive surface-enhanced Raman scattering-based LFIA strip for simultaneous detection of anti-SARS-CoV-2 IgM and IgG. It used gap-enhanced Raman nanotags, in which 1-nm gaps between the core and shell produced the “hot spots” and provided about 30-fold enhancement compared to conventional nanotags.

2.3. Antigen detection

Because of the kinetics of antibody production in the body, serological antibodies need at least a week to reach a detectable concentration⁸⁵, which cannot quickly respond to the SARS-CoV-2 infection. Direct detection of viral antigens provided rapid evidence for COVID-19. Many microfluidic devices have enabled rapid, low-cost, and high-sensitive self-testing of SARS-CoV-2 antigens—a handheld microfluidic filtration platform combined ultrahigh throughput hydrodynamic filtration and sandwich immunoassay. The immunoassay used nano and microbeads to capture SARS-CoV-2 N proteins and exhibited high throughput separation (<30 s) and a low limit of detection (LOD) < 100 copies/mL (Fig. 4A) (Table 4)⁸⁶. A microfluidic magneto immunosensor based on a unique sensing scheme, utilizing dually-labeled magnetic nanobeads for immunomagnetic enrichment and signal amplification, can detect SARS-CoV-2 N antigen at 50 pg/mL in whole serum and 10 pg/mL in $5 \times$ diluted serum (Fig. 4B) (Table 4)⁸⁷.

Parallel viral antigen and antibody analysis is a fast and robust all-in-one point-of-care strategy for multiplexed detection of respiratory infections. A biosensing approach consists of a functionalized polycarbonate disc-shaped surface with microfluidic structures, where specific bio-reagents are immobilized in microarray format, and a portable optoelectronic analyzer. The device was used to quantify the concentration of viral antigens and specific immunoglobulins G and M for SARS-CoV-2 using 30 μL of a sample in 30 min (Table 4)⁸⁸. Similarly, a portable microfluidic ELISA technology profiled anti-SARS-CoV-2 S1 IgG in human serum with only 8- μL sample volume and accurately detected SARS-CoV-2 antigens (S1 and N proteins) with pg/mL level sensitivities in 40 min (Table 4)⁸⁹. The versatility of the POCT device paves the way for detecting other pathogens and analytes in the incoming post-pandemic world.

Deep-learning (DL)-based image processing has been combined with a smartphone to apply SARS-CoV-2 antigen detection. Shokr et al.⁹⁰ employed adversarial neural networks with conditioning to develop an easy-reconfigurable virus diagnostic platform that leveraged a smartphone-taken microfluidic chip photos dataset to generate image classifiers for different target pathogens on-demand rapidly. The generalizability of the system was

Table 4 Summary on microfluidic technology for SARS-CoV-2 antigens detection.

Method	Fluid manipulation technique	Material	Immobilized antigen/antibody/gene	Detected biomolecule	Detector	Sensitivity/%	Specificity/%	Sample size/donor/standard	Limit of detection (LOD)	Detection time	Advantage	Ref.
Handheld microfluidic filtration platform	Hand-held syringe	PDMS	Monoclonal antibodies against SARS-CoV-2 S and N protein	SARS-CoV-2	Naked eye detection	95.4 and 100	100	93 nasal swab samples	<100 copies/mL	15–30 min incubation	Without dependence on a fluorescence or optoelectronic detector, fast, naked-eye visible results, robust and easy operation	86
microfluidic Magneto immunosensor	Syringe pump	PET, PMMA	Capture antibodies against N protein	SARS-CoV-2 N protein	Electrochemical measurements	NR	—	11 samples	10 pg/mL	<1 h	Minimal sample and reagent consumption, simplified sample handling, and enhanced detection sensitivity	87
Serological assay and antigen assay biosensing on chip	Centrifugal force	—	SARS-CoV-2 N/RBD or anti-SARS-CoV-2	SARS-CoV-2 antibodies or antigens	Chemiluminescence	98–100	84–98	135 serum samples and 147 nasopharyngeal specimens	—	6 samples in 30 min	The first POCT technology using a consumer electronics device, coupling serological and viral antigen testing in parallel to detect respiratory infections	88
A portable microfluidic chemiluminescent ELISA technology	Liquid pump	Polystyrene	SARS-CoV-2 antibodies against S1 and N	SARS-CoV-2 S1 and N protein	Chemiluminescence	75	100	19 patient samples and the two negative controls	2 ng/mL for IgG, 4 pg/mL and 62 pg/mL for S1 and N protein	15 min for antibody and 40 min for antigen	Rapid, sensitive, and quantitative analysis of COVID-19 related markers	89

—, not applicable.

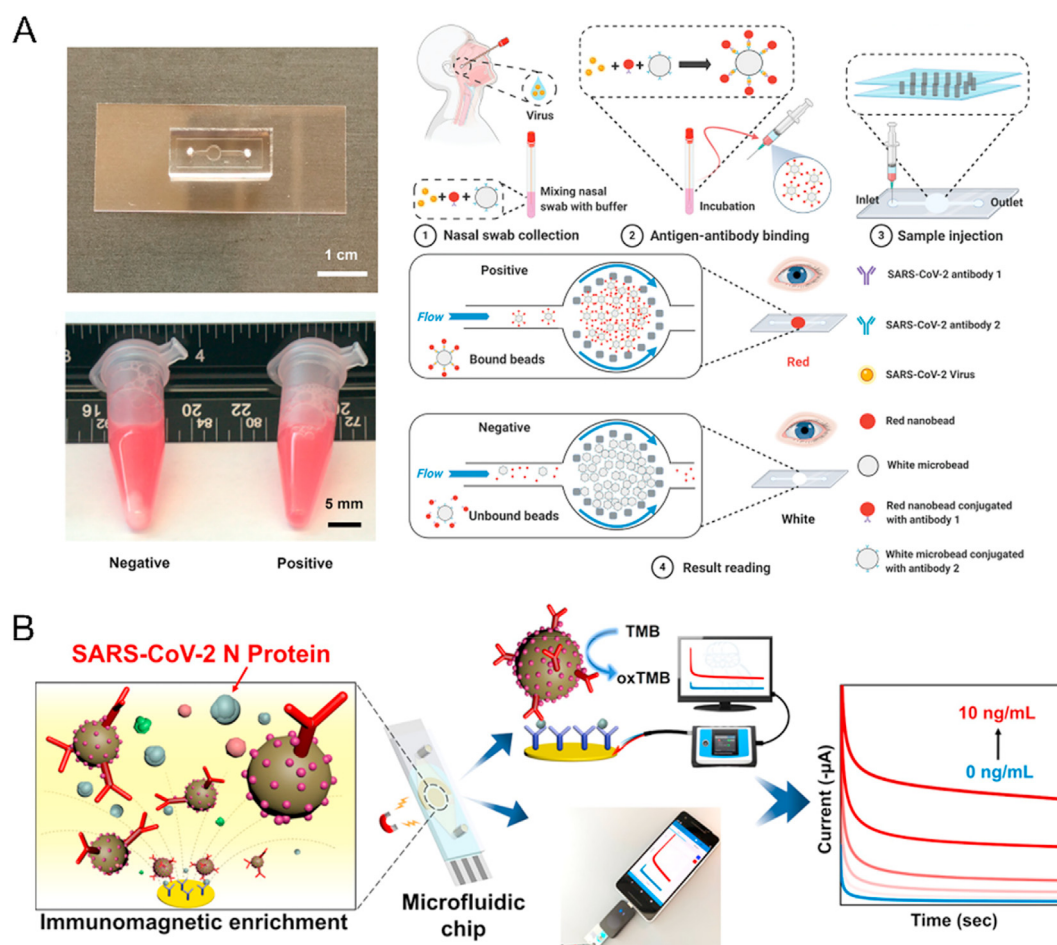


Figure 4 Example of microfluidic technology for SARS-CoV-2 antigens detection. (A) The handheld microfluidic filtration platform enables rapid, low-Cost, and robust self-testing of the SARS-CoV-2 virus. Reprinted with the permission from Ref. 86. Copyright © 2021 John Wiley and Sons. (B) Microfluidic magneto immunosensor for rapid, high sensitivity measurements of SARS-CoV-2 nucleocapsid protein in serum. Reprinted with the permission from Ref. 87. Copyright © 2021 American Chemical Society.

confirmed by rapid reconfiguration to detect SARS-CoV-2 antigens in nasal swab samples ($n = 62$) with 100% accuracy. As SARS-CoV-2 can spread through bioaerosol, Kim et al.⁹¹ proposed a handheld, rapid, low-cost, smartphone-based paper microfluidic assay capable of directly detecting SARS-CoV-2 in the droplets/aerosols from the air without the need for air samplers and long collecting time.

LFAs have also been widely used in SARS-CoV-2 antigens detection. In 2021, Wang et al.⁹² developed a magnetic quantum dot-based dual-mode LFIA biosensor for the high-sensitivity simultaneous detection of SARS-CoV-2 S and NP antigens, and LODs achieved 1 and 0.5 pg/mL, respectively. In 2022, Peng et al.⁹³ reported an immunoassay based on colloidal gold nanoparticles for rapid NP antigen detection. The sensitivity was improved through copper deposition-induced signal amplification, and three orders of magnitude promoted the LOD to 10 pg/mL after copper deposition signal amplification.

In conclusion, microfluidic technology has been broadly used for SARS-CoV-2 detection. Although several commercial products have appeared in the market, there is still a gap between scientific research and commercialization as their temporarily poor stability and high cost. Development of higher throughput, cheaper, more targets, and automated systems are common goals

to address the current COVID-19 pandemic. Furthermore, the detection sensitivity and speed need to be improved according to more novel designs and more straightforward processes, especially to omit the sample pretreatment procedure. Additionally, the generation of machine learning, mobile networks, and artificial networks would also help to boost the development of SARS-CoV-2 detection methods.

3. Microfluidic technology for COVID-19 drug discovery

3.1. Organ-on-a-chip for mimicking SARS-CoV-2 infection and drug screen

The current COVID-19 pandemic has harmed the whole world. It is significant to explore the mechanism of how SARS-CoV-2 injures the immune system and each organ and further develop more efficient drugs for COVID-19 treatment. Nowadays, cell lines (e.g., human airway epithelial cells and Vero E6 cells) and animal models (such as rhesus macaques and cynomolgus macaques) are mainly used to study the pathogenesis mechanism of viruses and drug testing^{94–97}. However, their biological structures and functions could not mimic the actual viral response in the human body,

thus not the same compared with human¹². Organoid technology has been a helpful tool and could facilitate COVID-19 drug screen. Organoids, induced by pluripotent stem cells, embryonic progenitors, and organ-specific adult stem cells, consist of multiple tissue-specific cell types and present similar organization of cells as well as crucial tissue-specific functions compared with their *in vivo* counterparts^{98,99}. Thus, organoids can provide the virus with a more natural environment and virus-host interactions *in vivo*, better to access drug responses in 3D microenvironment^{100,101}.

However, organoids still have some limitations, including incomplete tissue microenvironment, incomplete maturation, and presence of the often-used Matrigel, and lack of crosstalk between different organs¹⁰². Organoids incorporated with the microfluidic systems can optimize the organoid culture environment and improve the simulation. The organ-on-a-chip system combines the development of organoid culture, tissue engineering, microfluidic chip design, and micro-electromechanical system assistance, including single or multiple organoids¹³. Besides, flow in microchannels mimics the blood circulation *in vivo*, providing a dynamic culture condition instead of a traditional static state. Furthermore, high-throughput performance and low-volume reaction allow larger-scale drug testing incorporating drug gradient and dose studies on organ chips¹⁰³.

As the most susceptible organ to SARS-CoV-2, the lung and airway organ systems are common objectives for virus

pathological studies. A human alveolar chip cocultured human alveolar epithelium, microvascular endothelium, and circulating immune cells under fluidic flow to recapitulate lung injury and immune responses induced by SARS-CoV-2. They proved that viral infection caused immune cell recruitment, endothelium detachment, and increased inflammatory cytokines release (Fig. 5A) (Table 5)¹⁰⁴. As the only US Food and Drug Administration (FDA)-approved drug for treating COVID-19¹⁰⁵, remdesivir was proven to inhibit viral replication and alleviate barrier disruption on chips. On the other hand, Thacker et al.¹⁰⁶ used a vascularized lung-on-chip model and found that rapid endotheliitis and vascular damage characterized SARS-CoV-2 infection (Table 5). They validated that inhibition of IL-6 signaling with tocilizumab reduced but did not prevent loss of barrier integrity. Thus, SARS-CoV-2-mediated endothelial cell damage occurred independently of the cytokine storm. Another microfluidic bronchial-airway-on-a-chip lined by human bronchial-airway epithelium and pulmonary endothelium can model viral infection, strain-dependent virulence, cytokine production, and the recruitment of circulating immune cells. In the chips infected with SARS-CoV-2, clinically relevant doses of the antimalarial drug amodiaquine inhibited infection. However, clinical doses of hydroxychloroquine and other antiviral drugs did not inhibit the entry of SARS-CoV-2 in cell lines under static conditions (Table 5)¹⁰⁷. However, the above lung models lack a microvascular network to investigate viral infectivity and infection-induced thrombotic

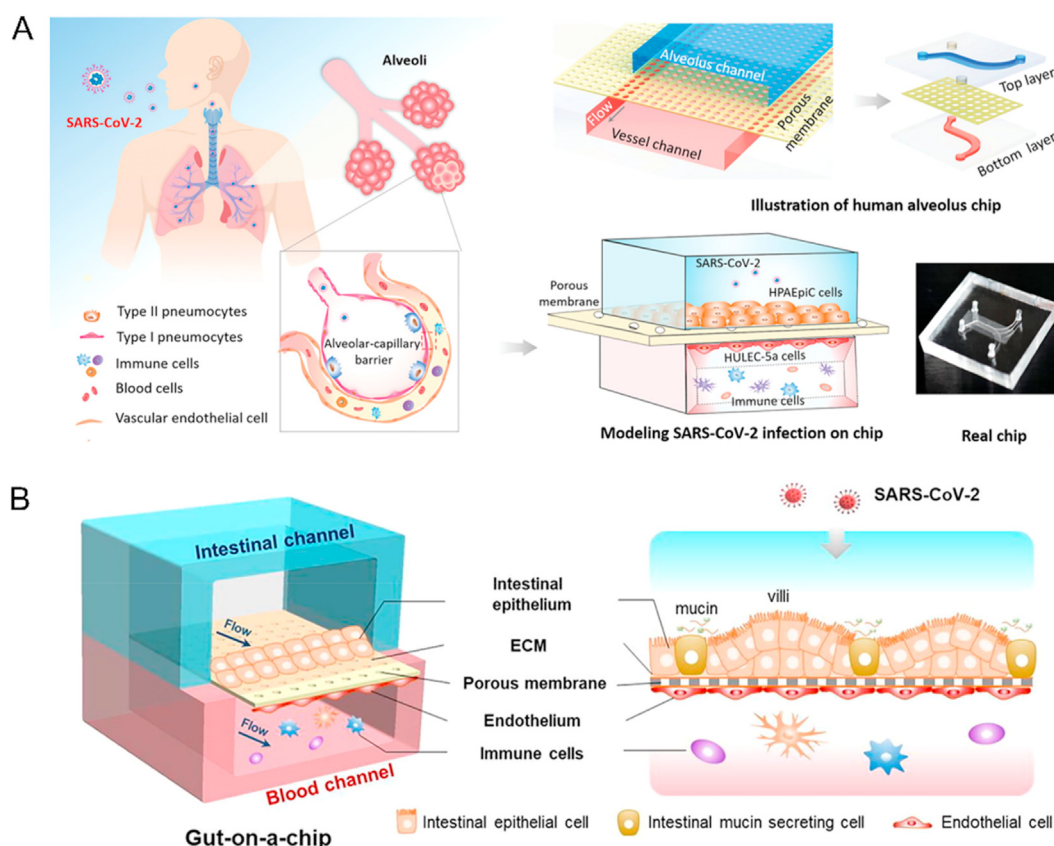


Figure 5 Example of organ-on-a-chip for SARS-CoV-2 drug screening. (A) The biomimetic human disease model of SARS-CoV-2-induced lung injury and immune responses on organ chip. Reprinted with the permission from Ref. 104. Copyright © 2020 John Wiley and Sons. (B) SARS-CoV-2 induced intestinal responses with a biomimetic human gut-on-chip. Reprinted with the permission from Ref. 110. Copyright © 2020 Elsevier.

Table 5 Summary on organ-on-a-chip for mimicking SARS-CoV-2 infection and drug screen.

Material	Fabrication method	Organ type	Key finding	Limitation	Ref.
PDMS	Soft lithographic	Lung	Treatment with remdesivir can inhibit viral replication and alleviate barrier disruption on chip	Use of only one cell type of alveolus epithelium and assess of only one type of antiviral candidates	104
PDMS	Soft lithographic	Lung	Rapid endotheliitis and vascular damage characterize SARS-CoV-2 infection	Lack of a complete recapitulation of resident innate immunity and the absence of an adaptive immune response and other cell types necessary for the complete recapitulation of vascular function	106
PDMS	Soft lithographic	Airway	Amodiaquine inhibited SARS-CoV-2 infection, but hydroxychloroquine did not.	Not use of native SARS-CoV-2 in BSL3 laboratories	107
Commercial device	Commercial device	Respiratory epithelial tissue	Models can recapitulate key, essential epithelial –capillary interactions specifically observed from <i>in vivo</i> pulmonology.	Timeline limit of three weeks	108
PDMS	Soft lithographic	Gut	In regard to the brain endothelium, the SARS-CoV-2 spike protein induced destabilization of the BBB, promoted a pro-inflammatory status but did not appear to alter cell viability acutely	Use of immortalized intestinal epithelial cell lines and lack of a comprehensive study on the complex responses of the immune cells involved in the host-virus interactions in this gut-on-a-chip system	110
PDMS	Soft lithographic	Blood–brain barrier	Introduction of spike proteins to <i>in vitro</i> models of the blood–brain barrier (BBB) showed significant changes to barrier properties	Ignorance on how permeability dynamics may change once these 3D microfluidic constructs are used with the whole SARS-CoV-2 virus	111
PDMS	Soft lithographic	Vasculature	Identification and inhibition of patient blood-specific coagulation in response to spike mutation or SARS-CoV-2	–	112
PDMS polystyrene	Soft lithographic Hot-embossing	Vasculature	Identification of angiotensin-1-derived peptide as a therapeutic for SARS-CoV-2-induced inflammation	Use of HUVEC to understand SARS-CoV-2 pathogenesis while some studies have shown that endothelial cells may not be the primary target of SARS-CoV-2 infection in all organs; the complex interplay between parenchymal tissue and endothelial cells in the context of infection was not demonstrated	113
PDMS	Soft lithographic	Lung endothelial barrier	SARS-CoV-2 disrupts respiratory vascular barriers by suppressing Claudin-5 expression	Lack immune cells, the mechanism is not fully understood	114

–, not applicable.

events. Jung's team¹⁰⁸ developed two novel and vascularized lower respiratory tract multi-chip models for the alveoli and the small airway based on a high throughput, 64-chip microfluidic plate-based platform (Table 5). They demonstrated the physiologically relevant cellular composition, architecture, and perfusion of the vascularized lung tissue models. The platforms will enable the development of respiratory viral infection and disease models for research investigation and drug discovery.

The intestine is also a high-risk organ for SARS-CoV-2 infection¹⁰⁹. A micro-engineered gut-on-chip reconstitutes the critical features of the intestinal epithelium-vascular endothelium barrier through the three-dimensional (3D) coculture of human mucin-secreting intestinal epithelial and vascular endothelial cells under physiological fluid flow. Transcriptional analysis revealed abnormal RNA and protein metabolism. It activated immune responses in both epithelial and endothelial cells after viral infection, which may contribute to the intestinal barrier injury associated with gastrointestinal symptoms. This human organ system can partially mirror intestinal barrier injury and the human response to viral infection, which is impossible in existing *in vitro* culture models (Fig. 5B) (Table 5)¹¹⁰. In addition, other organ chips are also used to study the potential mechanism of SARS-CoV-2. For example, the introduction of spike proteins to *in vitro* models of the blood-brain barrier (BBB) showed significant changes in barrier properties. Key to the authors' findings is that S1 promotes loss of barrier integrity in an advanced 3D microfluidic model of the human BBB. This platform resembles the physiological conditions at this central nervous system interface. Evidence suggests that the SARS-CoV-2 spike proteins trigger a pro-inflammatory response in brain endothelial cells that may contribute to an altered state of BBB function. These results are the first time to show the direct impact of SARS-CoV-2 spike protein on brain endothelial cells, thereby offering a plausible explanation for the neurological consequences seen in COVID-19 patients (Table 5)¹¹¹.

Evidence shows that vascular pathology could substantially affect COVID-19 disease outcomes, as endothelial cells are susceptible to SARS-CoV-2 infection. Besides, cases of thrombotic events are reported following vaccination. Satta et al.¹¹² recapitulated the human microcirculation environment using a novel microfluidic platform to elucidate Spike proteins underlying patient-specific vascular thrombosis (Table 5). It was coated with human endothelial cells and exposed to patient-specific whole blood. The blood coagulation effect is tested after exposure to Spike protein in nanoparticles and Spike variant D614G in viral vectors, and the results are corroborated using live SARS-CoV-2. Finally, the authors proved that nanoliposome-hACE2 and anti-Interleukin (IL) 6 antibodies can reduce blood clot formation. To further study the immune reaction of vascular after SARS-CoV-2 infection, Lu et al.¹¹³ also developed a vasculature-on-a-chip to model the interaction of the virus with the endothelial and virus-exposed-endothelial cells with peripheral blood mononuclear cells (PBMCs) (Table 5). They demonstrated the compounding effects of inter-cellular crosstalk between endothelial cells and monocytes in facilitating the hyperinflammatory state. They identified angiopoietin-1 derived peptide QHREDGS as a potential therapeutic capable of profoundly attenuating the inflammatory state of the cells consistent with the levels in non-infected controls, thereby improving the barrier function and endothelial cell survival against SARS-CoV-2 infection in the presence of PBMC. Moreover, Hashimoto et al.¹¹⁴ investigated the effect of SARS-CoV-2 on the endothelial barrier using an airway-

on-a-chip and found that SARS-CoV-2 disrupts the respiratory endothelial barrier by decreasing CLDN5 expression and disorganizing the VE-cadherin-mediated junction (Table 5). They proved that CLDN5 over-expression and Fluvastatin treatment could suppress the respiratory endothelial barrier disruption.

However, the application of organs on chips for studying virus infection still needs to be improved in research for unsatisfying reproducibility, stability, user-friendliness, and incomplete biological functions of engineering chips. In the future, many aspects need to improve, including a universal medium or a management system for different media, upscaled devices to accommodate growing organoids with higher resolution, strengthening the crosstalk between different organoids, integrating with different biosensors to monitor cells in real-time and machine learning for computational drug screen^{13,115}.

3.2. Microfluidic technology for COVID-19-associated nanoparticle engineering

Nowadays, the delivery of messenger RNA (mRNA) *in vivo* has been used to prevent and treat many diseases, including SARS-CoV-2^{116–119}. The carriers for the delivery have a significant effect on such strategies, including clustered regularly interspaced short palindromic repeats (CRISPR) antisense oligonucleotides (ASO), short interfering RNA (siRNA), microRNA (miRNA), and mRNA¹²⁰. Gene delivery based on engineered nanoparticles has the feasibility and tenability, proved to reduce the off-target related risks¹²¹. Nanoparticles enable the effective delivery of drugs, improving circulation time, targeting efficiency, and reducing potential adverse side effects. Nanoscale materials have features of a smaller size, high surface-to-volume ratio, tunable surface chemistry, and the capacity to load drugs¹²². Nanocarriers can also be used to deliver antiviral therapeutics into cells to intervene with the viral-replication cycle at the molecular level or to deliver plasmid DNA¹²³ and mRNA¹²⁴ for vaccination. Several mRNA nanoparticle-based vaccines have been used for COVID-19 prevention, even for clinical development¹²⁵. LNPs with properties of proper sizes, stable storage, efficient payload capture, and release rates will meet the requirements for nucleic acid delivery¹²⁶. However, they still lack high encapsulation efficiency and excellent scalability.

Microfluidic technology has significant benefits in preparing LNPs. By being highly versatile with a wide range of channel dimensions, multiple fabrication materials (*e.g.*, polymers or glass), and lipid formulations, microfluidic devices can produce homogenous-sized LNPs¹²⁷. A simple microfluidic-reactor-platform designed with continuous circular serpentine was reported for *in-situ* metal-nanoparticle synthesis¹²⁸. This novel microfluidic platform has the advantages of *in-situ* synthesis, flow parameter control, and reduced agglomeration of nanoparticles over the bulk synthesis due to the segregation of nucleation and growth stages inside a microchannel. Importantly, nanoparticle production does not need complex instrumentation. The fluid behavior in microfluidic chips is not controlled by the inertial ones but by the viscous forces¹²⁹. The continuous flows ensure that obtained nanoparticles have the same quality over time, preventing the issues of batch-to-batch variability. By utilizing the different geometries of microfluidic channels designed, the formation of different types of fast mixing patterns and controlled nanoparticle production becomes feasible^{130–132}.

Nanomaterials also play roles in virus treatment to provide cofactors such as Zn²⁺, inhibiting RNA polymerase and

transcription¹³³. Zn²⁺-based nanomaterials that bind RNA polymerase could be designed and administered to inhibit virus replication. In contrast to dendrimers or polymers, DNA nanostructures' valency, and spatial structures can be efficiently designed. In addition, DNA nanomaterials are stable and non-toxic. Therefore, DNA nanostructures with particular shapes, modified with virus-targeting aptamers, can be applied for virus detection and inhibition¹³⁴. Owing to their customized spatial structure, such DNA nanostructures precisely match the viral surface, enabling efficient virus capture⁵. The proposed *in-situ* synthesized nanoparticle can also be utilized as a SARS-CoV-2 vaccine.

Additionally, the technology integrated with high-throughput microfluidic droplets, mammalian cell engineering, and molecular genomics were reported to capture diverse mammalian antibody repertoires and generate recombinant hyperimmune globulins, which were proven high specificity and activity against therapeutic targets, including SARS-CoV-2¹³⁵. When primary B cells ran through the microfluidic channels, antibody repertoires were created by fusing heavy and light chain immunoglobulin nucleic acid sequences on a single-cell level. The authors applied the technology to develop 10³ to 10⁴ diverse recombinant hyperimmune globulin drug candidates to address unmet clinical needs for the COVID-19 pandemic and validated them *in vivo*. In addition, there are other nanomedicines for COVID-19-associated symptom control. Achieving protection from the virus in the whole respiratory tract, avoiding blood dissemination, and calming the subsequent cytokine storm remain significant challenges. Wang et al.¹³⁶ developed an inhaled microfluidic microsphere based on droplet using dual camouflaged methacrylate hyaluronic acid hydrogel microspheres with genetically engineered membranes from angiotensin-converting enzyme II (ACE2) receptor-overexpressing cells and macrophages. By timely competing with the virus for ACE2 binding, the inhaled microspheres significantly reduced SARS-CoV-2 infective effectiveness over the whole course of the respiratory system *in vitro* and *in vivo*. Moreover, the inhaled microspheres efficiently neutralized pro-inflammatory cytokines, caused an alternative landscape of lung-infiltrated immune cells, and alleviated lymph nodes and spleen hyperinflammation.

LNP production based on microfluidics proved scalable, cost-effective, and versatile⁷. Besides, microfluidic systems can be integrated with multiple micromixers to achieve parallel reactions¹³⁷. However, the multifunctionality and biodegradability of lipids should be considered in practice to boost the payload's efficacy, minimizing long-term genome integration and immunogenicity¹²⁷.

4. Conclusions

As microfluidic technology entered the field of biomedicine in the early 2000s, it has dramatically affected many aspects. Like a small-sized laboratory, a microfluidic chip provides a platform to accomplish all the operations of traditional detection methods. On the other hand, organ-on-a-chip creates biomimetic and mini-organs to simulate the reactions and biological processes *in vivo*. When a viral disease outbreak occurs, it is essential to rapid response to get the basic information and continuously study antiviral strategies. Microfluidic technology plays a crucial role in anti-SARS-CoV-2 research, including constructing point-of-care devices to identify infected patients, establishing organ chips to

study viral pathogenic mechanisms, and screening drugs for efficient antiviral therapies, and the design of nanoparticle synthesis methods to deliver mRNA as vaccination.

To prepare for new viral outbreaks and to accelerate and coordinate our responses to COVID-19, we still need to overcome many challenges. Firstly, portable and inexpensive devices for separating and purifying viruses from saliva and whole blood samples are required to obtain viral knowledge rapidly. Secondly, microfluidic chips need to integrate with higher resolution microscopy and more advanced materials-enabled technologies to enable the evaluation of the real-time dynamics of viruses in cells or tissues and further the observation of the interaction between drugs and viruses. Thirdly, the cost of current diagnosis kits should be lowered when maintaining accuracy and sensitivity, considering resource-poor people who live in medical conditions limited regions. Finally, using the vectors based on biocompatible materials would benefit the delivery of antiviral drugs and vaccines, reduce systemic toxicity, improve circulation time, co-deliver multiple components, increase drug or vaccine stability, and target specific cells or tissues.

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Author contributions

Zhun Lin, Zhengyu Zou, and Zhe Pu drafted the manuscript. Minhao Wu and Yuanqing Zhang revised the manuscript.

Conflicts of interest

The authors have no conflicts of interest to declare.

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