Integrated microfluidic devices for in vitro diagnostics at point of care

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Abstract
Given the continuous and growing demand for point of care (POC) diagnostic tests, attention has been shifted toward integration and miniaturization of laboratory protocols into “sample-in-answer-out” devices. Microfluidic technologies have been considered an ideal solution to address the requirements of POC diagnostics since many laboratory functions can be miniaturized and incorporated onto a single integrated chip. In this review, we summarize the advances of integrated microfluidic devices for POC diagnostics in the last 3 years. Particularly, we summarize current materials used for microfluidic chip fabrication, discuss the innovation of versatile integrated microfluidic devices, especially the strategies for simplifying sample preparation in manual or self-driven systems, and new detection methods of microfluidic chips. In addition, we describe new integrated microfluidic devices for POC diagnostics of protein-targeted immunodiagnostics, nucleic acid molecular tests, and small molecule metabolites analysis. We also provide future perspectives and current challenges for clinical translation and commercialization of these microfluidic technologies.

KEYWORDS
in vitro diagnostics, microfluidic devices, point of care

1 | INTRODUCTION

In vitro diagnostic (IVD) is a vital component of clinical care, which contributes to the prevention, diagnosis, treatment, and management of diverse diseases by the analysis of biomolecules from body fluids, including proteins, nucleic acids, and small molecule metabolites.1–3 Conventional methods for IVD such as enzyme-linked immunosorbent assay (ELISA), polymerase chain reaction (PCR), and mass spectrometry (MS) are well-established and commonly accepted for disease diagnostics due to their high selectivity and sensitivity. However, these technologies are limited to laboratory analysis because they require careful preparation of infectious samples, large and sophisticated

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instruments, as well as considerable time, labor, and highly trained technicians.\[14\] Thus, there is a growing need for reliable point of care (POC) diagnostic techniques that are available at any time and in any place, especially when decentralized from clinical and laboratory settings. In recent years, increasing research attention has been focused on the integration and miniaturization of analytical devices for POC diagnostics, since advances in miniaturization have facilitated better portability and more advanced analytical devices, while integration enhances the simplicity of operation, resulting in “sample-in-answer-out” systems that can be operated in any setting by patients or technicians. The growing availability of technologies for a wide range of medical conditions, accompanied by these advances in miniaturization and integration, has thus fueled growing demand for POC diagnostics. In addition, the global severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic resulted in a high demand for POC diagnostic devices, which also drove numerous recent advances that can be readily applied to other diseases.

Microfluidic technologies have been considered as an ideal solution to address the requirements of POC diagnostics since many laboratory functions can be miniaturized and incorporated onto a single integrated chip. Using the microfluidic approach, very small volumes of fluids can be manipulated at the microscale in miniaturized total analysis systems or lab-on-a-chip devices.\[5–9\] Compared with conventional laboratory analytical techniques, microfluidic offers several obvious advantages. First, microfluidic systems can integrate multiple procedures such as sampling, chemical reaction, chromatographic separation, and detection into a small chip, thereby streamlining complex assays, enabling multiplexed analysis, and providing “sample-in-answer-out” capabilities. Second, microfluidic methods require only low volumes of reagents, where the mass and heat transfer are easily controlled, thus enabling high speed and low-cost analysis. Finally, the small size of integrated chips also facilitates the portability for POC devices, allowing applications of complex laboratory procedures. These properties and advantages have driven the development of integrated microfluidic devices for in vitro POC diagnostics.

Advances in material science have propelled the design of microfluidic devices. Synergistic innovation of materials and microfluidic platforms is essential to increase the sophistication of microfluidics-based technologies. The development of new materials that can be combined with existing materials has led to the design of microfluidic systems with new and diverse functions. Different materials can confer new properties and capabilities to microfluidic devices, or can be used for easier or more cost-effective device production while retaining the original device function. Currently, numerous materials such as glass, polydimethylsiloxane (PDMS), polymethyl methacrylate (PMMA), and paper, etc. are widely used for fabrication of POC diagnostic microfluidic devices. Microfluidic devices can combine various functions within a single chip that includes both sample preparation steps (such as mixing, reaction incubation, and product separation) and detection. Conventional laboratorial sample preparation requires multistep reactions with manual pipetting and repeated use of equipment. Microfluidic devices resolve these issues by integrating the fluidic handling, necessary consumables, and specimen processing into fewer steps with extremely low volumes. However, the functionality of microfluidic-based diagnostic platforms is often limited by the dependence on pump systems or accessory tools for liquids loading. Several approaches to simplify sample preparation have been developed using hand-operated or self-driven preparation systems. In addition, new and advanced medical technologies depend on sophisticated or expensive laboratory instruments (e.g., fluorescence plate readers, spectrophotometers, MSs, etc.). Therefore, novel and simpler detection strategies compatible with microfluidic chips are needed to foster the adoption of new technologies. As illustrated in Figure 1, numerous solutions to miniaturize and integrate various laboratory functions have been incorporated into microfluidic devices, with emphasis on simplified sample preparation through either manual or self-driven processing systems, and new detection strategies without the requirement of sophisticated instruments. A number of reviews have summarized the state of microfluidic technologies for biomedical analysis and POC diagnostics.\[10–14\] However, since the last major review, recent years have seen several innovations in the versatility and function of integrated microfluidic devices, especially in strategies for simplified sample preparation in manual or self-driven systems and new detection platforms for microfluidic chips, which have changed the field, but are not covered by other reviews of in vitro POC diagnostics. Moreover, several research groups have continued to build and improve on their established methodologies over the past 3 years, driving rapid development of microfluidic devices for new POC tests. This review thus specifically focuses on the most recent advances in integrated microfluidic devices for POC diagnostics.

We first review the most commonly used fabrication materials for POC diagnostic microfluidic devices and their respective functional properties in devices. Next, we discuss recent progress in integrating various laboratory tasks into microfluidic devices, including reagent handling, sample processing, as well as the innovation in sensitive and quantitative visualized detection by naked eyes. We then describe the latest advances in integrated microfluidic devices for POC diagnostics for protein-targeted immunodiagnostics, nucleic acid-based molecular tests, and small molecule metabolite detection from minimally processed samples. Finally, we provide some perspectives on the future of integrated microfluidic chips and other miniaturized diagnostic devices, as well as the barriers for their clinical translation and commercialization.

2 | MATERIALS AND FABRICATION OF INTEGRATED MICROFLUIDIC DEVICES

Integrated microfluidic devices typically require a set of common components including inlet and outlet microchannels along with channel mixers, which can be assembled to combine sequential reaction steps in a single chip. In addition to recent techniques for micro-scale/micron-scale manufacturing, advances in materials science have also driven improvements in the design of microfluidic devices. Although a wide range of materials are available, surface properties (e.g., hydrophobicity or porosity) are paramount for fluid dynamics or reaction kinetics; thus device function should be carefully considered when selecting materials for fabrication. In general, these materials are categorized as inorganic, polymeric, or paper materials. In this review, we
highlight general materials such as glass, PDMS, PMMA, and specialized papers appropriate for microfluidic devices.

2.1 | Inorganic materials

Inorganic materials with relatively high surface stability, modifiable heat conductance, and solvent compatibility were adopted in micro-electro-mechanical systems, among which silicon or glass are widely used due to their versatility and wide availability. But silicon has become increasingly limited in microfluidics due to its opacity, impeding optical monitoring required by many applications. Glass has therefore served as a reliable and long-standing alternative because of its easily adjusted optical properties, long-established surface chemistry, and resistance to high pressure required for many biomedical applications. Glass can be etched through wet methods with chemical solutions or dry methods using plasma to imprint microstructures onto its surface, which is especially relevant for devices that require bonding with the glass itself or a PDMS layer to enclose the reaction system.[15–16]

Even prior to the introduction of microfluidics as a concept, glass capillary microchannels were used in gas chromatography.[17] Recently, Wen and coworkers applied a sensitive and self-driven glass capillary device to detect cocaine based on small changes in hydrogels.[18] Even small concentrations of the target compound can activate obvious changes in the internal hydrogel structure, which influenced the permeability and led to different flow velocities of sample solutions in glass capillary (Figure 2A). Also, glass is hydrophilic, readily available at relatively low-cost, and inert (impacting biocompatibility with biological samples), exhibits relatively low, nonspecific adsorption, and provides a uniform surface. These properties collectively render glass highly useful in biomedical devices. Qin’s group designed a series of low-cost glass microfluidic devices with distance-based readouts as volumetric bar-charts, in which the oxygen production was proportional to analyte concentrations for POC diagnostics.[19–24] Specific recognition molecule can also be covalently immobilized on the surface of epoxy-coated glass, with the roughness of the etched glass surface greatly increasing the coating efficiency of the recognition molecule. Since glass has large, composition-dependent elastic modulus, devices with active components such as valves and pumps commonly require the incorporation of other materials with glass. For example, Lunte and coworkers designed a PDMS/glass hybrid microchip using a carbon pyrolyzed photoresist film electrode to integrate microdialysis sampling, electrophoretic separation, and subsequent electrochemical detection for continuous monitoring of dopamine pathway metabolites.[25]

2.2 | Organic materials

In comparison with inorganic materials, organic polymers are cost-effective and easy for quick and simple fabrication,
FIGURE 2  Material science innovations applied in microfluidics. (A) A glass capillary device for cocaine detection through changes in hydrogel substrate. Reproduced with permission.[18] Copyright 2019, Springer Nature. (B) A microfluidic polydimethylsiloxane (PDMS)-based device that incorporates contour-mode acoustic streaming tweezers with a bead-based immunoassay. Reproduced with permission.[28] Copyright 2021, American Chemical Society. (C) A polymethyl methacrylate (PMMA) strip test for detection of ABO and Rh(D) antigen in red blood cells. Reproduced with permission.[32] Copyright 2018, Elsevier. (D) A hydrophobic valve for integrated liquid handling in paper-based microfluidic devices. Reproduced with permission.[37] Copyright 2019, American Chemical Society.

while retaining some of the robust and versatile properties of inorganic materials. The most common organic polymers used in microfluidic devices are PDMS, PMMA, polystyrene, and polycarbonate. Compared with other materials, PDMS has significantly higher gas permeability and higher biocompatibility. In addition, the fabrication of PDMS-based microfluidic devices from a master mold is a straightforward process that requires only mixing prepolymers with curing agents, followed by casting and heat curing. PDMS also confers useful properties such as lower cost and optical transparency, which make it particularly well-suited for microfluidic platforms designed for biomedical applications.[26,27] For example, Duan’s group recently proposed a PDMS POC device that employed contour-mode acoustic streaming tweezers in a bead-based microfluidic immunoassay.[28] Notably, the contour-mode resonator was fixed into the middle of a PDMS microfluidic channel fabricated by standard soft lithography (Figure 2B).

Formally, the PDMS surface from a template is flat at nanoscale. Although nanostructures can be potentially used as a template for molding PDMS, similar to work in microstructures, preserving the template morphology when peeling the PDMS from a nanostructure mold presents difficulties. To overcome this obstacle, Zheng’s group developed a new method to create a nanostructured PDMS surface using inexpensive ZnO nanorods as the template for a PDMS mold. A POC virus detection platform was described that combined nanostructure-integrated PDMS chips with colorimetric detection.[29] Instead of directly peeling the PDMS from the ZnO nanorod template, PDMS is released from the template by dissolving ZnO nanorod in diluted acid, which preserves the PDMS nanostructures. The whole fabrication process costs less than other methods and can be performed without a cleanroom.

Composed of polymerization of methyl methacrylate, PMMA is widely known under the commercial names of Plexiglass and Lucite. Patterns in PMMA can be printed through hot embossing or injection molding. Several bonding methods have also been developed to generate microfluidic networks in a PMMA housing. Moreover, PMMA has the merits of high biocompatibility, gas impermeability, and ease of micromachining at relatively low temperatures (∼105°C).[30,31] For example, Srikhirin’s group recently developed a PMMA strip test for highly precise and accurate detection of ABO (ABO blood types) and Rh(D) antigen on red blood cells. A POC device for deployment of this blood typing test incorporated visual A, B, and + patterns for a readout.[32] To generate this readout, ultraviolet (UV)-ozone was used to hydroxylate the PMMA, after which epoxy termination was introduced by reaction with epoxy-silane, which was then converted to carboxy methyl dextran (CMD) through a reaction with dextran (Figure 2C). Antibodies against A, B, or D red blood cell antigens were then immobilized on the surface of each CMD readout pattern. This design was particularly elegant, since the different combinations of A, B, and + conformations can be easily interpreted as A, B, AB, O, or Rh(D) blood types by users without advanced training.

Due to its hydrophobic surface, PMMA exhibits poor wettabiliy, which has limited its application in autonomous capillary microfluidic devices.[33] In order to resolve this issue, Li and coworkers developed a protocol for UV irradiation in a controlled gradient that alters the contact angle of target areas on the PMMA surface, resulting in selective wettabiliy of the PMMA. This innovation enables the incorporation of various functions into specific regions on the microfluidic device surface. Using this approach, they developed a fully integrated POC device driven entirely by capillary action for detection
TABLE 1 Summary of properties of various materials

| Material | Optical properties         | Surface properties                  | Gas permeability/ moisture evaporation | Cost | Scale production | Refs. |
|----------|----------------------------|------------------------------------|----------------------------------------|------|-----------------|-------|
| Glass    | Transparent/UV absorption | Hydrophilic/Easily modified         | None/Rarely                             | ~10–200 ¥/test | Unsuitable/hard to process | [15, 16] |
| PDMS     | Transparent/No UV absorption | Hydrophobic/Requires surface activation | High/Rapid | ~1–10 ¥/test | Challenging/variable between batches | [26, 27] |
| PMMA     | Transparent/Weak UV absorption | Hydrophobic/difficult to modify the surface | Low/Slow | ~0.5–10 ¥/test | Suitable for mass-production | [30, 31] |
| Paper    | Opaque/High background autofluorescence | Hydrophobic/difficult to modify the surface | High/Rapid | ~0.05–1 ¥/test | Suitable for mass-production | [35, 36] |

Abbreviations: PDMS, polydimethylsiloxane; PMMA, polymethyl methacrylate.

of cardiac Troponin I (cTnl), a gold standard biomarker for acute myocardial infarction.[34]

2.3 | Paper

Recent studies have proposed the use of paper in microfluidic devices due to several properties that outweigh other materials. For example, it is inexpensive and ubiquitous. Moreover, it can be easily functionalized or patterned by inkjet, solid wax, or other printing methods. The modifiable porosity of its structure makes it readily used for controlled flow, filtration, and material separation. Furthermore, paper is biologically compatible, and its typical white fiber color provides a high-contrast background for colorimetric assays. In particular, paper-based microfluidics use passive capillary to manipulate reagents and analytes through devices.[35, 36] For example, Lammertyn’s group developed a broadly applicable hydrophobic valve for complex, fully integrated, and self-contained liquid manipulation systems within paper-based microfluidic devices that require no external power source.[37] For this valve system, the hydrophobicity of filter paper fibers was increased by the treatment of fluoridated compounds (contact angle up to 155°), where the porosity for gas permeability was retained, while liquids were prevented from passing through the membrane (up to 9 kPa) (Figure 2D). Notably, fluidic control is essential for sensitive and multistep assays based on paper devices. Limited control over flowrate represents a major drawback of sucrose-based paper devices. Tu’s group developed a paper device using fructose-sucrose mixtures as localized dissolvable delay to effectively prolong flow time for enhanced sensitivity, while a horizontal valve was designed to automatically control the multistep process.[38]

The merits of materials suitable for different applications are also accompanied by inherent disadvantages. For instance, although glass is a historically versatile and design-friendly substrate, its rigidity and fragility limit the use in integrated microfluidic devices that require flexibility. Similarly, PDMS is relatively cost-prohibitive for mass production. Paper is strictly used for devices that rely on capillary action for fluid migration. Thus, when determining which material is suitable, these factors should be mainly considered: (1) the types of reactions and reagents used in the intended application; (2) the number and compatibility of different functions that will be integrated; and (3) specific requirements of its POC diagnostic applications. Table 1 summarizes the properties of various materials used in chip fabrication.

3 | FUNCTIONS IN INTEGRATED MICROFLUIDIC DEVICES

Microfluidic devices can integrate various functions within a single chip to construct a “sample-in-answer-out” system for POC diagnostics. Primary functions include sample preparation steps (such as mixing, reaction incubation, and product separation) and signal detection. In this section, we detail recent innovation in the integration of different fluid handling functions in microfluidic devices, especially for the simplification of sample processing through hand-operated or self-driven preparation systems, and advanced detection strategies to enhance the sensitivity and reliability for quantitative detection using naked eyes or other instrument-free readout.

3.1 | Functions in microfluidic devices:
Simplified sample preparation by microfluidic handling

“Sample-in-answer-out” systems, which show the results of detection after sample injection without further processing steps, are considered as ideal POC devices. Among the procedures for POC diagnostics, sample preparation typically represents the biggest bottleneck in terms of processing time and thus should be carefully considered when developing ideal POC diagnostic devices. Conventional sample preparation in the laboratory requires multistep reactions with manual pipetting and repeated use of equipment such as centrifuges. By contrast, microfluidic POC devices resolve the issues by integrating the fluidic handling, reagents mixing, and specimen processing into several steps with extremely low volumes in a single chip. However, microfluidics-based diagnostic platforms are often limited by the need for pump systems or accessory tools for loading liquids, which increase the cost and device size. Sample processing is also simplified by circumventing the need for external pumps. In particular, centrifugal force has emerged as a robust means of controlling fluid migration through the chip. A number of reviews summarize these advances in centrifugal microfluidic devices in
3.1.1 Hand-operated sample preparation

**SlipChip technology**

The SlipChip technology, which enables the connection of separated flow channels by manually slipping two layers of glass plates, eliminates the need for microfluidic valve switches or pump accessories and simplifies liquid handling. Qin and coworkers developed a series of volumetric bar-chart diagnostic devices using SlipChip technology to allow precise control of fluids for POC diagnostics. Based on the conventional SlipChip technology, Li’s group recently built a platform for multiplexed ELISA platform, a composable microfluidic plate system where the flow of low volumes of fluids can be simply and accurately manipulated by the assembly of 3D-printed microscale channels and wells by superhydrophobic coating. Samples were loaded in a single step, the addition of reagents and washing steps were handled in a single-phase system, and each ELISA reaction was spatially isolated from others to prevent crosstalk between antibodies. This approach was self-contained and scalable as the original SlipChip concept except that the fluid flow was manipulated by the device.

**Finger actuation**

A major alternative strategy for hand-operated sample preparation is finger actuation, which supplies the initial pressure to the microchannel. For example, immunoassays microfluidic devices were developed where the flow of reagents was driven by the finger actuation of air pouches and sequentially delivered to microchannels. However, the pushbuttons needed for finger actuation have limited the application of this design in conventional microfluidic devices. Recent study described a modular finger-actuated pump that was more compatible with current microfluidic devices. In order to ensure the accuracy of micro-scaled reagent volumes, an indirect pressurization method was developed to reduce the variability introduced by operator errors, and the finger-actuated system was first applied in a blood crossmatching test. In this approach, a user can dispense several fluid reagents in the correct proportions by pressing and releasing a pressure chamber (Figure 3A), and results were obtained by cross-reacting plasma from donor blood with the whole blood from recipients, and vice versa.

**Hand shaking**

Another extremely simple but innovative method for driving flow in microfluidic devices is hand shaking. For example, Li’s group developed a portable multiplexed bar-chart SpinChip (MB-SpinChip), which incorporated nanoparticle-mediated magnetic asptasensors and was shaken by hand for liquid processing. The MB-SpinChip used a “T” phase exchange channel to deliver or change reagents within a sealed compartment, and the flow was manipulated by shaking the device. Recently, our group developed a microfluidic device that integrated a lateral flow (LF) design with recombinase polymerase amplification (RPA) for rapid and sensitive detection of SARS-CoV-2. The reverse transcription-RPA (RT-RPA) mixture was transferred to the LF strip by inverting and shaking the chip device without any valves. In this integrated and self-contained POC diagnostic chip, the RT-RPA reagents and samples were mixed with running buffer, then delivered to the LF detection strips (Figure 3B). The potential for exposure to aerosol contaminants was thus avoided by encapsulating the reactions within the chip and limiting the number of steps required for sample processing (i.e., incubation, reagents mixing, and LF dipstick detection).

External manual fluidic manipulation

Although several innovations for integrated fluid manipulation have been proposed, the use of simple, conventional equipment (such as syringes) remains the most effective means of pressurized fluid delivery to microchannels in some device designs. For example, some microfluidic devices used a port that could be connected to a syringe to apply positive or negative pressure for controlling the flow in microfluidic channels. To correct different flow rates resulted from users operators, Ni and colleagues proposed a novel syringe flow-stabilizer for hand-powered, continuous-flow microfluidic sample injection based on passive flow-resistance compensation for constant flow rates of sample or reagent delivery. The stabilizer was integrated with an inertial microfluidic cell concentrator for high-throughput continuous concentrating of trace blood cells from large-volume samples. More broadly, the flow-stabilizer could be used for manual sample loading with several POC testing devices that are widely used in resource-poor regions (Figure 3C).

Magnets are also used as an external means of manual fluid manipulation in bead-based sequential reactions. For example, our group developed an ELISA-chip with distance-based readout by incorporating multistep sample processing for ELISA detection into a microfluidic device. This chip consisted of circular chambers for aqueous phase components that were separated by elliptical regions containing oil. This microfluidic ELISA assay provided highly sensitive “sample-in-answer-out” detection of disease biomarkers by simple manipulation of an incorporated magnet (Figure 3D). In addition to ELISA, magnets have also been applied to microfluidic devices for multiplex nucleic acid testing. For instance, Mu’s group developed a multiplex digital RPA chip that could simultaneously detect three types of pathogens by “sample-in-multiplex-digital-answer-out” testing. This chip consisted of a nucleic acid extraction component and a digital amplification component. Nucleic acid extraction was completed in 15 min by manual manipulation of magnetic beads to lyse cells, wash the precipitated DNA (DeoxyriboNucleic Acid), and elute the final sample on chips.

3.1.2 Self-driven sample preparation

**Capillary force**

Capillary microfluidics are commonly described as “passive” systems because fluids migrate autonomously through the microchannel by capillary action without external force. In a microchannel, the properties of capillary action (such as flow rates) are directly related to the surface tension of liquids, the geometry of channels, and the solid contact between liquids and channel surfaces. Currently, capillary-based POC devices with improved functionality have been proposed.
For instance, Delamarche’s group adopted capillary action as an approach to control sample flow within a microfluidic chip that tested G6PD deficiency.\textsuperscript{[56,57]} The power of capillary action-driven platforms lies in small sample volumes (i.e., only a few microliters) and independence of active pumping for control of liquids. Multiple flow paths were designed to accommodate different steps required for hemoglobin measurement and enzymatic assays within a single chip (Figure 4A).

Alternatively, paper substrates can be used in place of capillary pumps to incorporate capillary liquid flow into a chip. This design relies on the fibrous and porous paper structure to mediate liquid transport via capillarity, without an external pump to mobilize the liquid reagents and samples. Similarly, Zhuang et al. recently presented a pump-free microfluidic chip in which flow was self-initialized by capillary action and continued by absorption of a filter paper.\textsuperscript{[58]} Teardrop-shaped chambers where reactions occurred were designed to ensure enough time for the binding between antigen and antibody. By spotting different antibodies onto the reaction area, four types of biomarkers could be measured simultaneously in one microfluidic chip (Figure 4B).

With intrinsic pores and capillary channels, thread and cloth have also been used as substrates in microfluidic devices. Notable examples such as thread-paper-based microfluidic devices (\(\mu\)TPADs) incorporated fluid transportation through thread with a colorimetric readout on paper substrate. Similarly, microfluidic bioassays have been designed by integrating a “sieving” or filtration step to prevent capillary blockage with colorimetric quantification of analytes using three-dimensional hollow fiber membrane (3D-HFM) technology.\textsuperscript{[59]} HFM-based microfluidic devices integrated cell separation, enzymatic reactions, and colorimetric readout by engineering a stereoscopic three-dimensional pore gradient based on capillary action (Figure 4C).

Vacuum-driven pressure

In addition to capillary systems, vacuum pressure has been applied in microfluidic chips for controlling fluid dynamics and migration. Notably, PDMS-based devices are often degassed in a vacuum chamber before use because PDMS is permeable to gases. After degassing, negative pressure is generated in the microchannel; fluids are driven from reaction areas to detection areas. Using this principle, Mu’s group developed an integrated digital RPA PDMS chip for DNA extraction and multiplex digital RPA, enabling “sample-in-multiplex-digital-answer-out” diagnostic testing.\textsuperscript{[60]} By coordinating screw valves with vacuum-based liquid pumping, the nucleic acids are extracted, then self-primed and compartmentalized into 12,800 microchambers for successive digital RPA. These microchamber-based digitally integrated devices have exhibited good performance in target detection, and moreover, the nucleic acid extraction and amplification steps can be completed on chip without any additional equipment (Figure 4D).

### 3.2 Functions in microfluidic devices: Signal detection and readout

Despite the advances of simplified sample preparation for microfluidic devices, the signal detection and readout still depend on the use of sophisticated or expensive laboratory instruments (e.g., fluorescence plate readers, spectrophotometers, MSs, etc.), which prevented the adoption of new microfluidics testing devices in field or clinical applications.
FIGURE 4  Self-driven sample preparation. (A) The capillary-driven microfluidic chip for G6PD assay and determining hemoglobin concentration. Reproduced with permission. Copyright 2021, the Royal Society of Chemistry. (B) The capillary self-starting microfluidic chip for detecting multiple biomarkers. Reproduced with permission. Copyright 2021, the Royal Society of Chemistry. (C) The mechanism of hollow fiber membrane (HFM)-based microfluidic device. Reproduced with permission. Copyright 2020, American Chemical Society. (D) The vacuum-based liquid pumping and sampling process. Reproduced with permission. Copyright 2020, the Royal Society of Chemistry

Thus, new and simpler detection strategies are needed to advance the adoption of new testing technologies. In this section, we discussed recent developments in signal detection and readout formats that can be potentially applied or have already been employed in integrated microfluidic devices.

3.2.1 Visual signal output

Colorimetric intensity

Considerable research focus has been committed to developing POC diagnostics that incorporate visual readouts as results. Most conventional bioassays use colorimetric intensity-based methods that rely on the analysis of different intensity of colored bands in the detection component of chips. The concentration of target analytes is determined by comparing the intensity of the sample band with those of standard targets. Recent innovations in colorimetric readout approach are CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats)/Cas9-mediated triple-line paper microfluidic devices developed by Zhou’s group, which integrated multiplex RT-RPA detection of two SARS-CoV-2 genes into one probe-based strip test. In this design, Cas9/sgRNA (single guide RNA) was used to recognize the target DNA recognition and served as a bridge for gold nanoparticle (AuNP)-DNA probes via recruitment to a binding site in the sgRNA scaffold (Figure 5A).

Multicolor

However, readouts in paper-based microfluidic devices that relied on the differences in intensity had limited precision, because subtle but meaningful differences could not be observed without the help of other detection instruments. Since the human eye is extremely sensitive to different colors but less sensitive to the differences in intensity, several groups have developed POC devices with multiple colors for visual readouts. Our group built a microfluidic chip that combined a multi-colorimetric gold nanorod (AuNR)-based immunosensor with a multistep horseradish peroxidase (HRP)-linked immunoassay for semiquantitative detection of HIV-1 p24 with relatively high sensitivity.
In this system, HRP catalyzed the oxidation of 3,3′,5,5′-tetramethylbenzidine (TMB) to etch the surface of AuNRs, resulting in various color changes dependent on the target concentration. This system integrated sample preparation and detection by antigen–antibody recognition in a simple “sample-in-answer-out” application with vivid colors for direct and semiquantitative visualization (Figure 5B).

Distance
It should be noted that variations in user interpretation of the signal output can influence the accuracy of colorimetric tests, particularly for color-blind or vision-impaired users. To address this disadvantage, distance-based readouts represent an attractive alternative for microfluidic devices by offering a readable scale based on the length output that is corresponding to target concentrations. The translation of molecular signals into visible signals (such as bands) distinguished by spatial separation represents a critical step in the development of distance-based assay readouts. This challenge was addressed by using paper-based devices that relied on capillary action or diffusion, where the length of bands was determined by precipitation or polymerization reactions. Nanoparticles or metalloenzymes (e.g., oxidoreductases or hydrolases) were frequently used for the transduction of molecular signals to distance readouts. [67] Recently, Yang and coworkers proposed a simple paper/PMMA hybrid ELISA-Chip with a distance-based readout for quantitative detection of clinical biomarkers for POC testing. [68] This integrated system utilized an alkaline phosphatase-conjugated antibody in the detection chamber to cleave glucose-1-phosphate into glucose, which was mobilized to a µPAD carrying glucose oxidase, HRP, and 3,3-diaminobenzidine (DAB). After a series of cascaded reactions, brown poly-DAB bands were produced, the band length could be observed by naked eyes to indicate target concentrations.

Alternatively, distance-based visual readouts can be integrated into a miniaturized chip device using ink bars that volumetrically expand due to gas-generating reactions. Volumetric barchart chips represent one class of innovative microfluidic devices with distance readouts. In a recent work, CRISPR-Cas12a was introduced to a volumetric barchart chip with a platinum nano-reporter for quantification of genetic polymorphisms at the single nucleotide level. [69] This was accomplished by tethering platinum nanoparticles to magnetic beads via single-stranded DNA fragments that could be cleaved by CRISPR-Cas12a, allowing quantitative detection of a panel of cancer-associated mutations in serum or isolated DNA (Figure 5C).

Volumetric barchart chips were based on gas generation driven by chemical or enzymatic reactions. Although innovative, this approach required optimization, since the reactions may generate gas inconsistently (i.e., lack controllability), resulting in inaccurate readout. In addition, the devices had a relatively short shelf-life (storage time) due to enzyme denaturation. One approach to resolve these drawbacks is the integration of photo-activated nanomaterials. For instance, a photothermal bar-chart microfluidic chip (PT-chip) was developed for quantitative detection of biochemical targets. [70]
In this application, the antibodies against the target (e.g., prostate specific antigen or PSA) were immobilized to reaction chambers, which also bound with Fe$_3$O$_4$ NP-labeled antibody proportional to the concentration of target antigen, resulting in a typical antibody sandwich structure that was strongly activated by near infrared light. The photothermal heat production increased vapor pressure that pushed dyed liquids, providing signals that were proportional to the concentration of PSA in samples. (Figure 5D).

In addition to the approaches described above, counting-, time-, and text-based strategies are also reportedly effective as equipment-free methods for signal readout. In quantitative counting-based readouts, a color change in separate zones indicates positive target detection, and target concentration is quantified by counting the positive signal zones. In time-based strategies, the time elapsed between the appearances of color in different detection zones and the duration required for a tracing agent to mobilize from the start to end points can both be used for quantitative assessment of target concentration. Text-based quantitative readouts provide the detection values for the target analyte based on the reading of displayed text. These strategies have been demonstrated to show good performance in the detection of a variety of analytes, suggesting their vast potential for POC testing in resource-limited settings (comprehensively discussed in a recent review by Li and colleagues).[71]

3.2.2 Handheld instrument-assisted output

Various handheld devices are commercially available for public use, including pressure meters, thermometers, and pH meters and smartphone. Several microfluidic devices have been produced for highly sensitive detection of disease markers by incorporating standard handheld devices into their design. Here, we summarize recent innovations in handheld instrument-assisted microfluidic technologies.

Smartphones have been proven highly suitable for incorporation with miniaturized detection technologies to produce portable microfluidic POC diagnostic and monitoring systems. When integrated with sensors, other hardware, and different detection methods, smartphones enable high-accuracy diagnoses and meet numerous clinical needs, especially in resource-limited regions. Smartphone apps and accompanying detection hardware have undergone continuous development in the past few years, with recent trends focusing on further miniaturization and software integration to improve detection accuracy (reviewed at length in other recent publications). [72–74]

Pressure meters have been integrated into miniaturized microfluidic devices as a readout that relies on pressure increases within a sealed environment caused by gas-generating reactions. Yang’s group initially used the handheld pressure-meter for highly sensitive bioanalytical applications.[75–80] To improve the sensitivity and expand the application of LF assays, they recently demonstrated a paper device with handheld pressure meter readout for rapid detection of disease-related protein.[81] The LF assay was used for sample processing, while the pressure meter was used for signal readout. In this paper device, the PtNPs formed a gray band by accumulating at the readout line through protein recognition and binding, the gray band part was then cut and added to the H$_2$O$_2$ solution in a gas-tight container and finally converted recognition signal into highly sensitive pressure readout for quantitative analysis (Figure 6A). Pressure-based assay using PtNPs can provide quantitative results using a portable pressure meter, but the instability of PtNPs poses an obstacle for their broad adoption. To resolve this problem, a Pt-staining method was developed based on test strips to create platinum nanoshells on the surface of colloidal gold for pressure-based quantitative detection with simple pressure meter.[82] After Pt-staining and catalytic gas generation, myoglobin in serum samples was readily detected by the portable pressure meter with an limit of detection (LOD) of 5.47 ng/mL, thereby satisfying the requirements for clinical monitoring of acute myocardial infarction.

In addition to pressure meters, digital thermometers represent one of the most widely used analytical tools for personal healthcare. Digital thermometers are easy to obtain, simple to operate, and inexpensive. As a signal readout, the thermometer was firstly developed by Li’s group through nanoparticle-mediated photothermal biosensing.[83–85] Given the merits of paper-based microfluidic devices, they developed a paper hybrid microfluidic photothermal biosensor for Mycobacterium tuberculosis where DNA capture probes were immobilized on the paper surface.[86] The probes formed a sandwich structure with target DNA and AuNPs surface functionalized with oligonucleotides to drive the oxidation of TMB, which had strongly photothermal signals in its oxidized state and thus generated heat under 808-nm laser irradiation in the presence of M. tuberculosis DNA. Finally, a handheld thermometer was used to record increases in temperature proportional to target DNA concentration (Figure 6B).

4 INTEGRATED MICROFLUIDIC DEVICES FOR POC DIAGNOSTICS

In light of these advances in detection strategies and chip design, we now discuss the application of these integrated microfluidic devices for POC diagnostic devices that target proteins, nucleic acids, and clinically relevant metabolites. Since this review cannot accommodate the vast quantity of publications and diversity of research topics, we provide selected examples of advanced integrated microfluidic devices for POC detection of different targets in the past 3 years.

4.1 Integrated microfluidic analysis for immunoassays

Two major advantages of microfluidics immunoassays in clinic are (1) reduced steps that minimize the required sample volume and the introduction of errors during multiple steps, and (2) the user-friendliness of these devices without extensive training. The recent corona virus disease 2019 (COVID-19) pandemic highlighted the value and urgency of developing COVID-19 tests for fast and reliable screening. For this purpose, Lillehoj and coworkers developed a device for the identification of SARS-CoV-2 nucleocapsid protein in serum samples, which used HRP-coated, dual-labeled magnetic nanobeads for enrichment of both target proteins and signal amplification.[87] DMB-antigen immunocomplexes were
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FIGURE 6 Signal detection via handheld instruments. (A) Detection of disease-related proteins using an external pressure meter with a paper-based device. Reproduced with permission.[81] Copyright 2019, the Royal Society of Chemistry. (B) Handheld digital thermometer for quantitative photothermal detection of bacterial DNA. Reproduced with permission.[86] Copyright 2020, American Chemical Society

mobilized on the sensor surface and bound to capture antibodies on a working electrode, where they were activated by bias potential to catalyze TMB reduction, thereby producing amperometric currents proportional to antigen concentrations. This device had a low limit of detection of SARS-CoV-2 nucleocapsid protein 50 pg/mL in whole serum, 10 pg/mL in 5× diluted serum, and 230 pg/mL in whole serum in a smartphone-adapted version of the device.

Integrated microfluidic chips have also been used in automated immunoassay systems for early-stage disease diagnostics. In one system, a membrane-based filter unit was combined with magnetic bead-based immunoassays for automated blood treatment in addition to circulating extracellular vesicle (EV) enrichment and quantification in whole blood samples.[88] For this purpose, three functional modules were incorporated into a serial separation, enrichment, and quantification process: the filtration module with an integrated stirring mechanism to separate plasma from blood cells and 94% of vesicles in 500-µL samples within 8 min. The magnetic bead module captured 45% of circulating EVs from plasma. The third module used on-chip ELISA for quantification of EVs in plasma. Since this device could be fully automated, it represented a promising research tool for clinical or fundamental applications requiring characterization of circulating EVs.

Lima’s group combined a multidimensional sensor with machine learning-based decision-making tasks for rapid, accurate, sample-to-answer quantification of EVs with simultaneous determination of their cargo proteins.[89] One innovation of this device was the integration of pencil cores as double-layer electric capacitors. Moreover, supervised models allowed relatively high accuracy in predicting cargos even using a small training set, and outputs were processed by smartphones. Up to now, this new platform showed the highest throughput for EV biomarker quantification among reported strategies.

4.2 Integrated microfluidic analysis of nucleic acids

In standard nucleic acid-based diagnostic detection systems, contamination represents a major challenge, which can be addressed by multistep integrated microfluidic systems. Thus, “sample-in-answer-out” microfluidic devices have advantages in nucleic acid detection of genetic diseases, cancers, infectious diseases, and other diagnostic applications. Fully integrated microfluidic devices allow nucleic acid extraction, nucleic acid amplification, and signal readout through a variety of approaches. For instance, Liu’s group described a simple but fully integrated paper chip comprised of various modified types of paper for detection of specific gene mutations.[90] Pipetting and sample manipulation were simplified by incorporating all the required DNA handling steps into a single chip driven by LF; gene mutation indicators in the detection strip were observed by naked eyes. In an alternative approach, Xing’s group reported a centrifugation-based chip for bacterial pathogen detection that combined DNA extraction, RPA, and fluorescence detection steps. One major innovation of this device was to seal the RPA reaction within the chip, significantly reducing the risk of sample contamination.[91] More recently, Sun and coworkers also built an automated centrifugal microfluidic system for viral nucleic acid detection through an internal loop-mediated isothermal amplification (LAMP) reaction to minimize viral contamination in aerosols.[92]

Integrated chip devices have been developed for multiplex nucleic acid detection. A slidable and paper/plastic hybrid chip was designed for simultaneous analysis of three common pathogens.[93] In this chip, six Whatman FTA cards were embedded in parallel for successive DNA extraction from bacteria, LAMP reaction, and colorimetric signal readout. Doyle’s group also developed a microfluidic device for multiplex detection of a small panel of miRNAs through hydrogel-based colorimetric assays.[94] A spatial encoding scheme was incorporated by a process of exchanging monomer solutions and polymerizing posts inside the device.

These integrated chips have been used for automated diagnostics of nucleic acids. An automated, self-driven microfluidic chip developed by Lee et al. facilitated rapid molecular diagnosis of pathogens using a colorimetric LAMP-based approach.[95] The liquid samples were loaded into the microfluidic chip, and the liquid flow and mixing were tightly manipulated to achieve successive analytical steps for pathogen detection. The 40-min process can be remotely controlled by smartphones and carried out with
little prior training, making it amenable for POC testing especially in remote sites.

### 4.3 Integrated microfluidic analysis of small molecule metabolites

Small molecule metabolites are widely used as markers in clinical diagnosis. For example, scurvy is a clinical syndrome caused by vitamin C deficiency. A fully integrated, pump-free, nano-scale microfluidic system that relied on bendable biofuel cell was developed for POC scurvy testing using 0.20-μL droplets of raw serum. This system bypassed the need to pretreat serum samples and provided a rapid analysis to avoid vitamin C degradation in the samples.

The detection of clinically valuable biomarkers (glucose, lactic acid, metabolites, etc.) in sweat samples poses an attractive strategy for many POC tests due to the relatively simple, noninvasive, and safe sample collection. Given the contributing factors of sweat secretion, it represents a relatively untapped source of metabolic information that could be interrogated using a well-adapted, multiplex microfluidic sensing system. Zhang demonstrated the use of a flexible band that attached to skin, which integrated superhydrophobic-superhydrophilic microarrays with nanodendritic colorimetric readouts for in situ sampling and analysis of sweat. In this device, the sweat was diverted by a superhydrophobic silica coating for collection specifically in a superhydrophilic micropatterns. A smartphone app was then used for analysis and readouts of the signal from sensors. Although it needed further optimization, this innovative approach could be a versatile and adaptable means of POC diagnosis for numerous markers in sweat.

### 5 CONCLUSION AND FUTURE PERSPECTIVES

This review summarizes recent state-of-the-art developments in integrated microfluidic devices, especially for POC testing and automated clinical diagnostics reported over the past 3 years. A wide range of materials, such as glass, PDMS, PMMA, and paper, are commonly used for fabrication of POC diagnostic microfluidic devices, depending on the strategy for fluid control and the required internal reactions. In particular, we highlight recent progress toward integrating greater functionality into microfluidic devices, including strategies for simplifying sample preparation through manual or self-driven sample processing systems, as well as innovations in detection strategies suitable for microfluidic chips. Moreover, we discuss innovations in protein-based immuno-diagnostics, nucleic acid-based molecular tests, and small molecule-based clinical diagnosis.

Microfluidic devices provide several unique advantages that make them powerful tools for POC diagnostics, most notably, their potential for integrating multiple, complex sample processing, and analytical steps. Despite considerable progress, several major challenges still need to be addressed to promote the adoption of these devices in clinical POC applications. Although fluid handling, consumables, and specimen processing can be combined in a single chip, expertise and training are still required for accurate loading of multiple reagents. A preloading system or single-step method for reagent loading could substantially reduce the necessary for user training. Such user-friendly microfluidic devices-integrated sample preparation tools are already in development for urgently needed POC devices. Additionally, new and simpler strategies for signal amplification and readout are highly desired. Handheld instruments such as pressure meters, thermometers, and phone cameras have already been integrated into microfluidic chips for signal readout in POC diagnostics. However, protocols for transforming signals from microfluidic chips to handheld instruments still need further innovation to ensure standardized and reliable results between different users. Improved production and surface modification techniques for microfluidic chips, along with automated sample preparation and detection, are also needed to enhance consistency and accuracy, especially for producing devices at scale. Although the commercialization of microfluidic devices for POC testing faces many challenges, future development of user-friendly and fully integrated “sample-in-answer-out” microfluidic systems will open an exciting, emerging area that can greatly streamline healthcare and improve clinical outcomes, especially for patients in resource-limited regions.

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Dan Liu performed the literature review and wrote the paper. Ying Wang and Mengmeng Li helped with figure preparation. Xingrui Li, Yanling Song, and Zhi Zhu revised the manuscript. Qiaoyi Wu and Chaoyong Yang provided expertise and critical revision of the manuscript.

### CONFLICT OF INTEREST

The authors declare no conflict of interest.

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