Analyst

ROYAL SOCIETY OF CHEMISTRY

CRITICAL REVIEW

View Article Online
View Journal | View Issue

Cite this: *Analyst*, 2021, **146**, 5800

Application of centrifugal microfluidics in immunoassay, biochemical analysis and molecular diagnosis

Yuxing Shi,[a] Peng Ye,*[a] Kuojun Yang,[a] Jie Meng,[a] Jiuchuan Guo,[a] Zhixiang Pan,[a] Wenhao Zhao[a] and Jinhong Guo *[b]

Rapid diagnosis plays a vital role in daily life and is effective in reducing treatment costs and increasing curability, especially in remote areas with limited availability of resources. Among the various common methods of rapid diagnosis, centrifugal microfluidics has many unique advantages, such as less sample consumption, more precise valve control for sequential loading of samples, and accurately separated module design in a microfluidic network to minimize cross-contamination. Therefore, in recent years, centrifugal microfluidics has been extensively researched, and it has been found to play important roles in biology, chemistry, and medicine. Here, we review the latest developments in centrifugal microfluidic platforms in immunoassays, biochemical analyses, and molecular diagnosis, in recent years. In immunoassays, we focus on the application of enzyme-linked immunosorbent assay (ELISA); in biochemical analysis, we introduce the application of plasma and blood cell separation; and in molecular diagnosis, we highlight the application of nucleic acid amplification tests. Additionally, we discuss the characteristics of the methods under each platform as well as the enhancement of the corresponding performance parameters, such as the limit of detection, separation efficiency, *etc*. Finally, we discuss the limitations associated with the existing applications and potential breakthroughs that can be achieved in this field in the future.

Received 13th April 2021,
Accepted 20th July 2021

DOI: 10.1039/d1an00629k

rsc.li/analyst

1. Introduction

Recently, many researchers have focused on implementing experimental operations in "lab-on-a-chip" devices to address health monitoring, environmental testing, and food safety as these devices can achieve satisfactory results with low reagent consumption. Several microfluidic systems exist based on different driven forces, including centrifugal microfluidics, digital microfluidics, magnetic microfluidics, and capillary-driven microfluidics.[1]

At the end of the 1960s, with the development of centrifugal analyzers, the field of centrifugal microfluidics was born. N. Anderson first developed a chemical analyzer consisting of a rotating disk, a multi-cuvette assembly and an optical detector.[2] In 1998, the next generation centrifugal microfluidic diagnostic platform was developed.[3] At the beginning of the 21st century, the development of centrifugal microfluidic platforms ushered in an explosive advance.

A centrifugal microfluidic chip system integrates reagents, pretreatment, mixing, sequential loading of various liquids, valve control, and metering in an immunoassay along with other experiments on a lab-on-a-chip. The typical setup of centrifugal microfluidics generally includes a centrifugal drive source assembly, a liquid transmission channel, a liquid mixing chamber, a valve control assembly, a separation assembly, a reagent storage chamber, a reaction chamber, *etc*.

As shown in Fig. 1, when the centrifugal disk rotates, there will be centrifugal force in every corner of the disk. If the liquid exists in the capillary valve of the centrifugal disk, the centrifugal force will act on the liquid. By controlling the relationship between the capillary force and the centrifugal force, the valve control and the fluid pushing can be realized.[4] This system has the following advantages: first, it has a minimal risk of cross-contamination during automatic liquid mixing, sequential loading, valve control, and metering by strictly distinguishing and sealing each module; moreover, it is less influenced by the samples' characteristics, such as conductivity, hydrogen ion concentration (pH) and viscosity, which is an ideal choice for processing biological samples

[a]*School of Automation Engineering, University of Electronic Science and Technology of China, Chengdu 611731, China. E-mail: yepeng_uestc@163.com*
[b]*School of Information and Communication Engineering, University of Electronic Science and Technology of China, Chengdu 611731, China. E-mail: guojinhong@uestc.edu.cn*

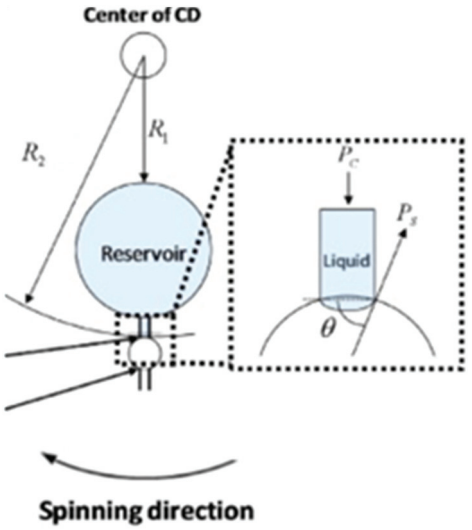

Fig. 1 Illustration of the principle of fluid propulsion and principle of the conventional capillary valve.[5] R_1: Radius 1; R_2: Radius 2; θ: the contact angle of the liquid with the channel surface; Pc: balance of pressure; Ps: surface tension.

such as blood; additionally, the inherent centrifugal force in this system exists in every corner of the disk, which makes liquid transmission simple and efficient, while effectively removing bubbles and residual liquid; furthermore, the radial distribution of the centrifugal force can simplify multiple parallel tests with only one single motor providing the source of the centrifugal force, which is a good news for system miniaturization; and finally, this system can play a significant role in cell or particle separation based on centrifugal density gradient separation.[3]

Immunoassays can be of different types, such as enzyme-linked immunosorbent assay (ELISA), fluorescence immunoassay, chemiluminescence immunoassay, and turbidimetric immunoassay;[6] molecular diagnosis mainly involves the extraction, purification, amplification, and detection of nucleic acids; and biochemical analysis includes quantitative analyses of creatine, trace elements, and glucose along with plasma and blood cell separation, particle isolation, and filtration.

To date, several reviews have focused on different applications of centrifugal microfluidic systems,[7] methods of particle processing on centrifugal platforms,[8] and molecular diagnostics on centrifugal platforms.[9] However, there is no critical review about the application of the system in immunoassay, biochemical analysis and molecular diagnosis. Thus, there is an urgent need to summarize these applications, especially for researchers who study medically related point-of-care testing technologies in resource-limited areas. Although Gorkin *et al.* have published two critical reviews about the centrifugal microfluidic platform,[3,8] research on this platform has been changing rapidly. For instance, Miyazaki *et al.* have developed a method that enables label-free, spatially multiplexed, surface plasmon resonance (SPR)-based detection of immunoassays on a highly integrated, centrifugal lab-on-a-disk platform,[10] Bhamla *et al.* have invented an ultra-low-cost paper centrifugal operating system that can be driven by the centrifugal force coming from hand power,[11] and Lin C-T *et al.* have performed a quantitative detection of antibodies on a manual, centrifugal, microfluidic disk based on magnetic chitosan beads.[12]

In this review, we comprehensively introduced the latest progress in centrifugal microfluidic platforms in the fields of immunoassays, biochemical analysis, and molecular diagnosis. While discussing immunoassays, we focus on ELISA; for biochemical analysis, we expound the applications of particle and cell separation technology; and in molecular diagnosis, we review nucleic acid amplification technology. Additionally, we discuss the methods adopted by each application, focusing on the structure, principle, performance, efficiency, and innovation of each platform. Finally, we present the limitations of the existing applications and potential breakthroughs that can be achieved in the future.

2. Application of centrifugal microfluidic systems in immunoassays

Since Low and Berson discovered radioactive insulin immunoassay in the 1950s, immunoassays have gradually become a research hotspot. Nowadays, immunoassays are used as one of the main methods for disease diagnosis, pathological research, and pharmacological analysis. An immunoassay is based on the principle of using labels, such as enzymes to identify a test substance, which will in turn be quantified by testing the quantity of the biomarkers in an immune complex.[6]

Although immunoassays have been widely used in medical laboratories, complicated operations, enormous experimental equipment, and long incubation periods limit the widespread application of immunoassays in resource-limited areas. At present, with the help of a micro-total analysis system (μ-TAS), an immunoassay can be combined with the centrifugal microfluidic system. The centrifugal microfluidic system has a unique advantage, wherein, the driving force provided by the rotation of the disk enables the system to conduct a series of reactions, including valve control and sequential loading, without any external pump equipment.[1,3,7] Recently, many researchers have combined immunoassays with a centrifugal microfluidic system and have used them in health monitoring,[13–16] disease diagnosis,[17–19] and drug screening experiments.[20,21] Compared with immunoassay analyzers in the laboratory, these automated centrifugal microfluidic immunoassay systems have shown an equivalent or even better limit of detection (LOD). Thus, this system is a promising candidate for resource-limited areas. Table 1 summarizes some typical centrifugal microfluidic immunoassay systems.

2.1 ELISA

ELISA is a technique that is used to detect an antigen (or antibody) by using an enzyme-labeled antibody (or antigen)[26] and

Table 1 Summary of immunoassay applications using the centrifugal microfluidic platform

Application	Analyte	Performance	Characteristics	Ref.
Disease diagnosis	Non-structural protein 1 of dengue virus	Sample volume = 75 μL Detection time = 1 h	Large specific volume Short diffusion length	4
Disease diagnosis	HBV	LOD = 8.6 mIU mL^{-1} Detection time = 30 min	Based on LIFM	22
Disease diagnosis	CEA	Range = 0.5–27 ng mL^{-1}	No complex washing step	18
Health monitoring	CK-MB	LOD = 0.92–100 ng mL^{-1} Detection time = 22 min	Laser irradiated ferrowax microvalves are utilized Large silicon bead is utilized	23
Health monitoring	IgG	Sample volume = 3–10 μL	Stacked multi-layer, 10 reactors	5
Health monitoring	cTnI	LOD = 37 pg mL^{-1} Detection time = 22 min	Use of a TiO_2NF pad	13
Health monitoring	Human albumin	LOD = 0.516 ng mL^{-1} Detection time = 18 min	CLOCK-controlled	14
Health monitoring	Human albumin	LOD = 0.75 ng mL^{-1} Detection time = 12 min	Lab-in-a-bento box, CLOCK-controlled	24
Health monitoring	Periodontal markers	99% linear correlation Detection time = 9 min	Magnetic beating is utilized	25
Animal experiment	Goat-anti-human IgG	Reduce 75% reagents Detection time = 5–8 min	Reciprocating flow is utilized	20
Animal experiment	Goat-anti-mouse IgG	NR	Flying ball governor, spring plunger	21

Abbreviations: HBV, hepatitis B; CEA, carcinoembryonic antigen; CK-MB, creatine kinase-MB; IgG, immunoglobin G; cTnI, cardiac troponin I; LOD, limit of detection; LIFM, laser irradiated Ferrowax microvalve; CLOCK, control of liquid operation on centrifugal fluid kinetics; NR, not reported.

is based on colorimetry in the presence of a substrate. ELISA can be divided into direct methods (detecting antigen) and indirect methods (detecting antibody). Direct methods can be divided into sandwich format and competitive format, in which the sandwich method is generally used to detect large molecule antigen, while the competitive method is used to detect small molecule antigen. An important aspect of ELISA research is to find a convenient and accurate measurement of the colored products.

2.1.1 Improvement in step simplification, system stability, and reduction of reaction time. Centrifugal microfluidic ELISA has been researched and developed in the past decade in terms of step simplification, improvement of system stability, and reduction of reaction time.

The long analysis time required by the traditional methods resulted from an extended incubation time, long diffusion length of the well, large reagent quantity, and complicated operation steps. The extended incubation time is possibly responsible for the low transfer efficiency of the antigen/antibody from the solution to the surface of the well. However, a study[27] found that the immune response of antigen and antibody is a rapid process. Therefore, a new technology has been proposed to address this. A prominent example is performing Dengue non-structural protein 1 (NS1)-ELISA on a microfluidic platform "lab-on-a-disk".

As early as 1999, a centrifuged microchip was first proposed,[28] and the first centrifugal microfluidic immunoassay platform was published in 2004.[29] Since then, the research of immunoassay based on centrifugal microfluidics has entered a stage of rapid development. In 2009, Yusoff *et al.* proposed a lab-on-a-disk that exerts centrifugal force as a potential microfluidic platform.[4] The lab-on-a-disk uses centrifugal force and capillary force as passive valves to control the flow sequence of different solutions. The small structure of the CD increases the reaction efficiency because of its large specific volume and short diffusion length. The principle of this technology is using centrifugal force and capillary force to properly mix samples and reagents to control the liquid flow. Therefore, by controlling the speed of the CD, each step of ELISA can be carried out automatically.[29] This method shortens the detection time by effectively mixing and separating liquids for ELISA detection. Additionally, micro-volume detection of reagents and samples, which amount to approximately 75 μL combined, can be conducted on the CD. This remarkably saves reagent consumption when compared with a total volume of 760 μL that is typically required for a microwell.

As the microfluidic platform developed by Yusoff *et al.* led to rapid detection,[4] researchers then started focusing on improving the mixing efficiency in a centrifugal microfluidic system. In 2011, Lee *et al.* developed an innovative laser-irradiated ferrowax microvalve, the first real-time detection medical device, that could concurrently perform immunoassay and biochemical analysis (Fig. 2a);[23] here, whole blood could be added directly to a disposable lab-on-a-disk. In this immunoassay, the researchers did not use polystyrene particles, rather, used large silica beads, which were preloaded into the mixing chamber before the experiment. The large silica beads promoted the mixing performance of the system and were more tractable than the polystyrene particles. The final limit of detection was 0.92 ng mL^{-1}, which was compar-

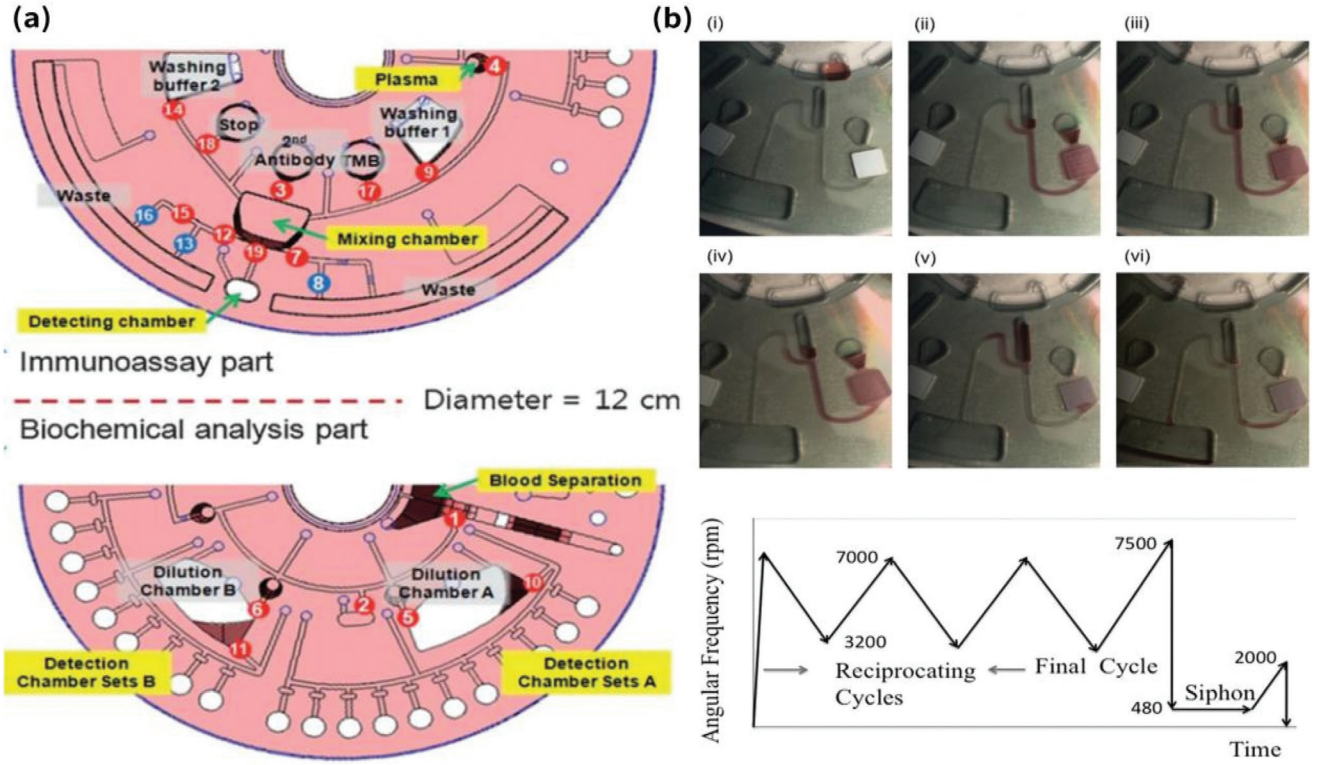

Fig. 2 (a) Complete layout of a centrifugal microfluidic system. The number[1–19] indicates how a laser irradiated ferrowax microvalve (LIFM) operates. TMB, tetramethyl benzidine.[23] (b) Time-lapse images of the system in operation and reciprocating cycle profile: angular frequency *vs.* time.[20]

able with that of ELISA measurement. Additionally, the experimental time was reduced to 22 min. Thus, this device paves the way for the subsequent research of multiple immunoassays in a micro-system.

However, sometimes improving mixing efficiency is accompanied by high reagent consumption. In 2011, Noroozi *et al.* developed a new μ-TAS based on centrifugal disks for low-cost, high-throughput, semi-automatic immunoassay processing.[20] A major innovation of this platform was that by reciprocating flow, fluid mixing could be enhanced on a disk of a relatively small available area (100 mm^2). Here, centrifugal force acted on the liquid and then generated air-pressure energy, which was stored. Consequently, the researchers reduced the rotation frequency and used the air-pressure energy to reverse the moving direction of the fluid to achieve the reciprocating flow (Fig. 2b). Consequently, this centrifugal microfluidic platform greatly reduced the consumption of reagents by 75% and operation time by 85%.

While optimization of mixing efficiency and reagent consumption are crucial, the stability of the system is also of significance. In 2016, Park *et al.* used a lab-on-a-disk composed of TiO_2 nanofibrous (NF) mats to perform ultra-sensitive detection of human albumin, C-reactive protein (CRP), and cardiac troponin I (cTnI).[13] Conventional TiO_2NF can only be prepared on conductive and thermally stable surfaces, which is difficult to be used in centrifugal microfluidic ELISA. However, in this device, TiO_2NF could be transferred to any substrate (including non-conductive and plastic materials) along with a thin polydimethylsiloxane (PDMS) adhesive layer. High adhesion of the PDMS enabled TiO_2NF to withstand pressure and remain stable during the washing step in ELISA. The final experimental results were: 30 min experimental time, high sensitivity, and large dynamic detection range, using a small amount of whole blood (the limit of detection for CRP is 0.8 pg mL^{-1}, and the limit of detection for cTnI is 37 pg mL^{-1}).

Traditional ELISA detection not only takes a long time, but also requires trained personnel, which is inconvenient for point-of-care diagnosis. Among all the ELISA steps, the washing step is indispensable. If this step is omitted, all other experimental steps can be greatly simplified. Recently, Gao *et al.* developed a high-throughput centrifugal microfluidic device that does not require complicated operations to detect carcinoembryonic antigen (CEA).[18] This device is based on the principle of density gradient centrifugation. They used chitosan as the dense medium in the centrifugal microfluidic device. The CEA was separated as the disk rotated and researchers were able to detect the concentration of CEA using a semi-quantitative fluorescence immunoassay method. With the aid of a dense medium, routine washing steps can be omitted, thereby simplifying the system operation. Here, 34 clinical serum samples were measured. They obtained satisfactory results with a 9.22% average deviation and good repeatability ranging from 0.5 ng mL^{-1} to 27 ng mL^{-1}. Thus, this centrifugal microfluidic platform, based on the principle of

centrifugal density gradient equilibrium, can be useful for detecting CEA in resource-limited areas.

2.1.2 Improvement of system integration, automation, and miniaturization. The main purpose of a centrifugal microfluidic platform is to make point-of-care testing more convenient. Apart from step simplification, improvement of system stability and reduction of reaction time, system integration, automation and miniaturization also need to be considered.

Lee *et al.* have developed a portable, disk-based, fully-automated ELISA system to detect infectious agents, particularly for measuring the concentration of hepatitis B virus (HBV) antigen and antibody, in whole blood.[22] This was the first report of a fully automated immunoassay for whole blood testing (Fig. 3a). Separation of plasma and blood cells requires high speed; as the separation step is always the first step, none of the previously reported systems could perform analysis from whole blood. Based on the phase transition of ferrowax, Lee *et al.* introduced an innovative laser irradiated ferrowax microvalve (LIFM).[22] Since a high intensity laser beam alone could not melt the wax, they embedded iron oxide nanoparticles (10 nm in size) in the paraffin wax, thereby melting the paraffin wax. With the help of LIFM combined with pathogen-specific magnetic particles, pathogen-specific DNA extracted from whole blood could be fully integrated into the portable lab-on-a-disc device. The proposed assay had an improved LOD of 8.6 mIU mL^{-1} with a detection time of 30 min.

In addition, although integrating multiple functions on one disk has become the mainstream research direction, it is difficult to integrate many reactor units and various modules compatibly. To overcome this, Ukita *et al.* (2012) developed a centrifugal microfluidic immunoassay system (Fig. 3b) with multiple reactor units and integrated each module to detect immunoglobulin G (IgG).[5] Ukita *et al.* integrated up to 10 reactors on a disk with a diameter of 8 cm by stacking multiple layers. Next, they transmitted liquid in an original three-dimensional centrifugal microfluidic device to overcome bubble formation and sequential fluid loading. The novelty of the idea is to use multiple stacked layers (with integrated components, such as capillary bundle structures which interconnect the layers of the disks vertically) to realize various operations. Therefore, this system may be used as a reference to develop high-throughput, high-sensitivity, three-dimensional, centrifugal microfluidic systems.

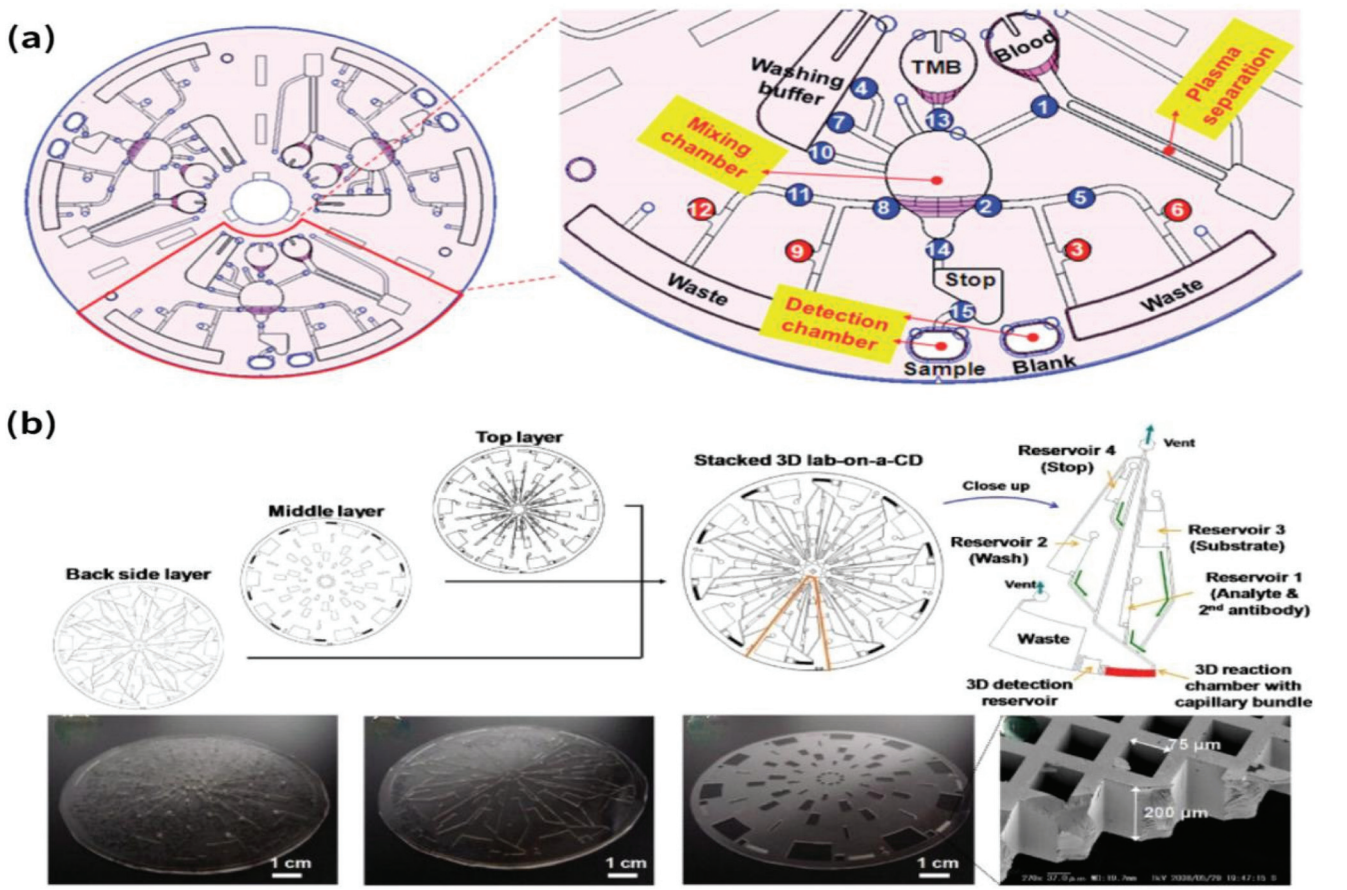

Fig. 3 (a) Detailed structure of a centrifugal microfluidic system.[22] TMB, tetramethyl benzidine. (b) Detailed structure of a three-dimensional centrifugal microfluidic device with multiple layers (top layer: for dispensing, sample loading and waste containing; middle layer: for mixing, detection and reaction; back side layer: for buffer containing and dispensing), photos of fabricated individual layers (the first picture to the third picture at the bottom left), SEM image of the capillary bundle structure (the first picture at the bottom right).[5]

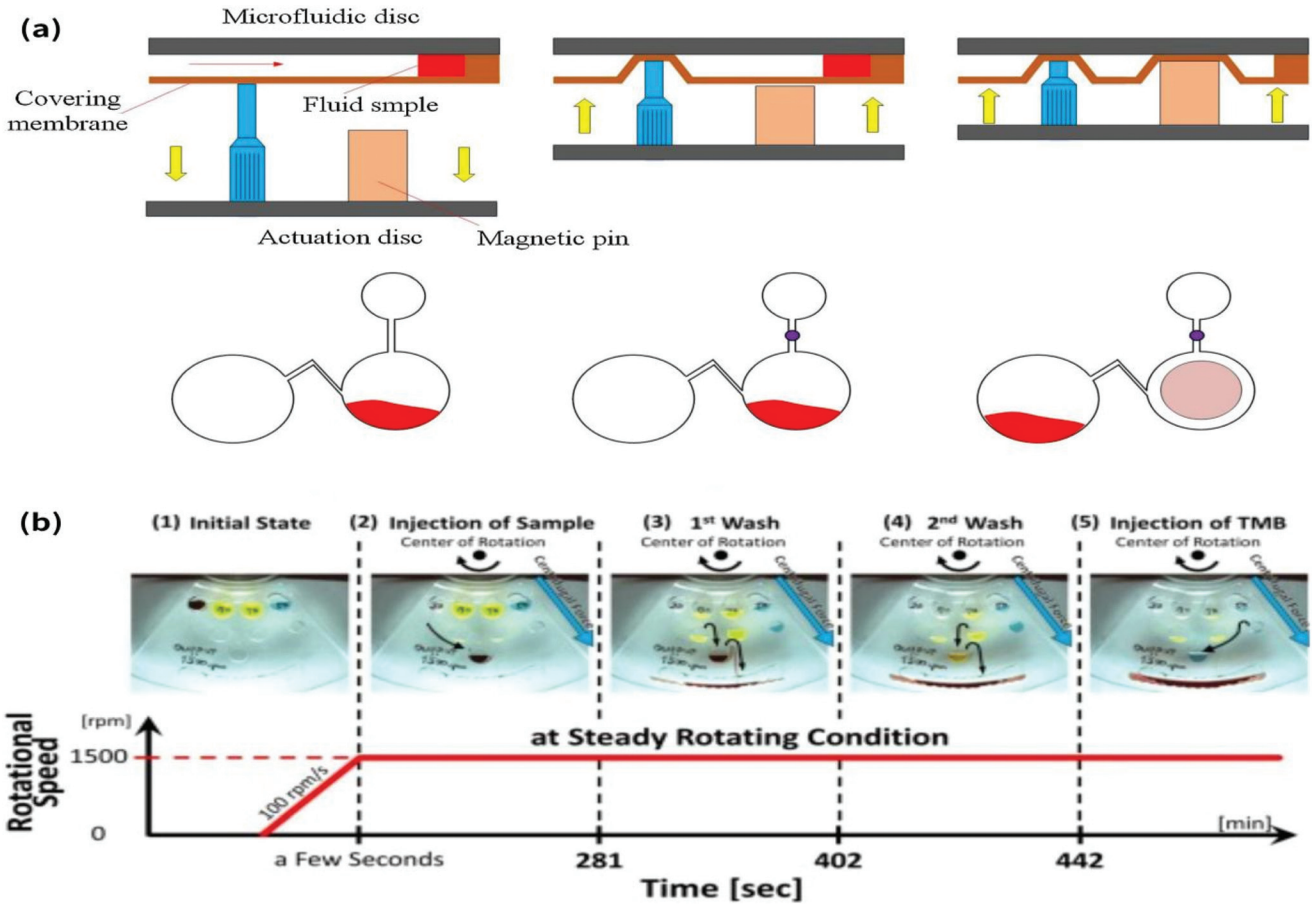

Fig. 4 (a) Principal demonstration of a mechanical valve used to control sample transport and waste liquid discharge.[21] (b) Illustration of the detailed operation step with the rotational speed set at a constant value.[14]

Similarly, for the miniaturization of the system, as shown in Fig. 4a, Wang *et al.* (2017) have designed and tested a CD-based laboratory immunoassay system.[21] The system consists of a flying ball governor and a set of spring plungers. Four sequence valves and an internal pump are integrated on a rotating platform. Wang *et al.* regulated the burst frequency of the valves by adjusting the preload of respective spring plungers and opened and closed the valves by mechanically compressing the cover film of the valve chamber. This technology is advantageous as the burst frequency of each valve becomes independent by adjusting the preload of the individual spring plunger. This fabrication facilitates miniaturization, and cross-contamination can be resolved as the system avoids complicated fabrication processes. The system was used to conduct experiments on mouse IgG, and a goat-anti-mouse IgG sample solution coupled with magnetic nanoparticles was used as the magnetic trapping mechanism. High-sensitivity detection results indicated that this method has the potential for developing micro-systems for use in biochemistry and medicine.

Both switching the rotation frequency[29–34] and mechanically controlling the opening and closing of the valve[35,36] to realize the sequential loading of the flow inevitably increase the cost and size of the micro-system. In 2018, Okamoto and Ukita developed an automatic, microfluidic ELISA based on Control of Liquid Operation on Centrifugal fluid kinetics (CLOCK)-controlled autonomous centrifugal microfluidics.[14] This device was made up of a single-layer, simply patterned polydimethylsiloxane (PDMS) chip which is driven by a steady rotational frequency. The water clock network and siphons are integrated into a single chip. In this way, the device maintains a constant injection timing for all reagents, without a rotational speed control program, which in turn greatly facilitates the miniaturization of the platform. Eventually, the device achieved a deviation of less than 5% in the execution interval time of each unit, such as reaction of the antigen and antibody and the washing step. An LOD of 0.516 ng mL^{-1} was achieved within 18 min of operation time, which was on a par with that of a traditional method (LOD = 0.707 ng mL^{-1}).

However, the aforementioned ELISA system with polydimethylsiloxane (PDMS) as a substrate is unsuitable for large-scale production; moreover, the water clock controller is expensive, bulky, and difficult to carry, making the aforementioned system an inconvenient point-of-care testing platform. Therefore, in 2020, Abe *et al.* developed a "lab-in-a-bento box", which is an autonomous centrifugal microfluidic system for ELISA based on CLOCK control.[24] An injection molding box

was used to detect human albumin, and with a bento box (*i.e.*, a shell), the entire assembly could be used for point-of-care testing. Based on the clock control principle, this device could automatically perform operations of each unit at a stable rotational frequency. The final LOD of human albumin was 0.750 ng mL^{-1}, indicating a good detection performance.

2.1.3 Others. Recent studies have focused on improving the performance of a centrifugal microfluidic platform for processing complex samples. A complex matrix of the saliva along with its non-Newtonian behavior and high viscosity makes the analysis of saliva challenging. Moreover, there are significant differences between stimulated and non-stimulated sample collection methods.[37,38] These characteristics may lead to inaccurate volume measurement in the process of sample processing.[39,40] Therefore, most of the concentration ranges for saliva biomarker detection are inferior to those for blood biomarker detection.[41] Johannsen *et al.* developed an automated pretreated system for saliva, by using magnet-beating, for point-of-care protein biomarker analysis.[25] After subjecting saliva to magnetic force-beating for 4 min, its viscosity decreased from 10.4 mPa s^{-1} to 2.3 mPa s^{-1}, and the processed sample could be used for subsequent protein detection. After magnetic beating, immunoassay results of three periodontal markers (MMP-8, MMP-9, and TIMP-1) were linearly correlated with results from the laboratory with a linear slope of 0.99. In addition, while the processing time of the standard method takes several hours, this method only takes 5 min. Thus, this demonstrates that magnetic beating may be suitable for pre-analyzing saliva.

2.2 Centrifugal microfluidic chemiluminescence immunoassay

Although ELISA is rapid, inexpensive, and portable, it is usually a diagnostic tool whose sensitivity needs to be improved. Compared with conventional ELISA, chemiluminescence immunoassay (one of the most advanced labeling immunoassay techniques), as a chemical method based on photon counting, has several orders of magnitude higher sensitivity. The coating plate, substrate and detection system of the two methods are different.

Chemiluminescence immunoassay utilizes a combination of a highly sensitive chemiluminescence assay and a highly specific immune response to analyze various antigens, haptens, antibodies, hormones, fatty acids, vitamins, and drugs. In fact, this is the latest immunoassay technology developed after radioimmunoassay, enzyme immunoassay, fluorescence immunoassay, and time-resolved fluoroimmunoassay.[42]

Czilwik *et al.* have introduced a magnetic chemiluminescent immunoassay (MCIA) for human CRP, on a centrifugal microfluidics platform.[43] After the initial loading of the sample at the inlet ports, the platform operates automatically as the centrifugal force propels the liquid without cross-contamination. The platform was made up of a set of stationary magnets, a microfluidic polymer disposable and a specific centrifugal protocol to transport magnetic capture micro particles between adjacent reaction compartments. As a result, sample-to-answer detection can be completed within 25 min, and an LOD of 1.5 ng mL^{-1} and a limit of quantification of 1.8 ng mL^{-1} can be achieved in this short time (the sample used was diluted human serum of 3–81 ng mL^{-1}). In 2016, Delgado *et al.* introduced a fully automated chemiluminescence detection system using an electrified lab-on-a-disk (eLoaD) platform, which was the first report of a complete integration of chemiluminescence onto a lab-on-a-disk platform.[44] The eLoaD enables measurements without any manual intervention, or even without stopping the disk during the measurement.

Many researchers have used the eLoaD platform to conduct chemiluminescence detection for human C-reactive protein (CRP)[44] and valve actuation.[45] For the first time, Delgado *et al.* elaborated the design of an open source hardware, which composed the core of the system. They discussed the current functions of the platform and the new functions that may be developed in the future, and characterized the performance of the platform.[46] They found that the eLoaD platform could produce wireless power by spinning and the eLoaD platform could make the centrifugal microfluidics analyzer controllable. Moreover, they also demonstrated the sensing, driving, and interface capabilities of the platform.

2.3 Other centrifugal microfluidic immunoassay systems

Recently, there has been a focus on other detection methods as well, such as electrochemical immunoassay, SPR, and turbidimetric immunoassay.

Turbidimetric immunoassay is based on the reaction of an antigen and antibody under specific conditions to form an immune complex, wherein the analyte is quantitatively analyzed by detecting the antigen–antibody immune complex particles suspended in the buffer. The amount of analyte can be calculated by comparing the turbidity of the reaction solution with a series of standard substances. Both transmission and scattering (two commonly used methods in immunoturbidimetry) need to apply light to pass through the solution, which is either absorbed by the immune complex or refracted by the immune complex. This method is simpler and cheaper than enzyme-linked immunosorbent assay and chemiluminescence assay because turbidimetric has no need for separation by washing.

In 2018, Arjmand *et al.* used a turbidimetric immunoassay to design a centrifugal microfluidic platform with a septum valve for detecting hemoglobin A1c (HbA1c) in human whole blood (Fig. 5b).[16] In the centrifugal microfluidic system, to ensure that an ultra-high rotation speed can be achieved in the mixing steps and to ensure that the flow of the reagents is accurately controlled, this study adopted a newly designed passive valve, namely, the septum valve. After testing 14 blood samples, HbA1c test results, with a standard deviation of ± 0.36%, were obtained within 8 min. In 2019, Yang *et al.* have introduced an immunoturbidimetric assay for specific protein identification from whole blood, based on a multi-layered centrifugal microfluidic chip.[15] As most systems do not allow both

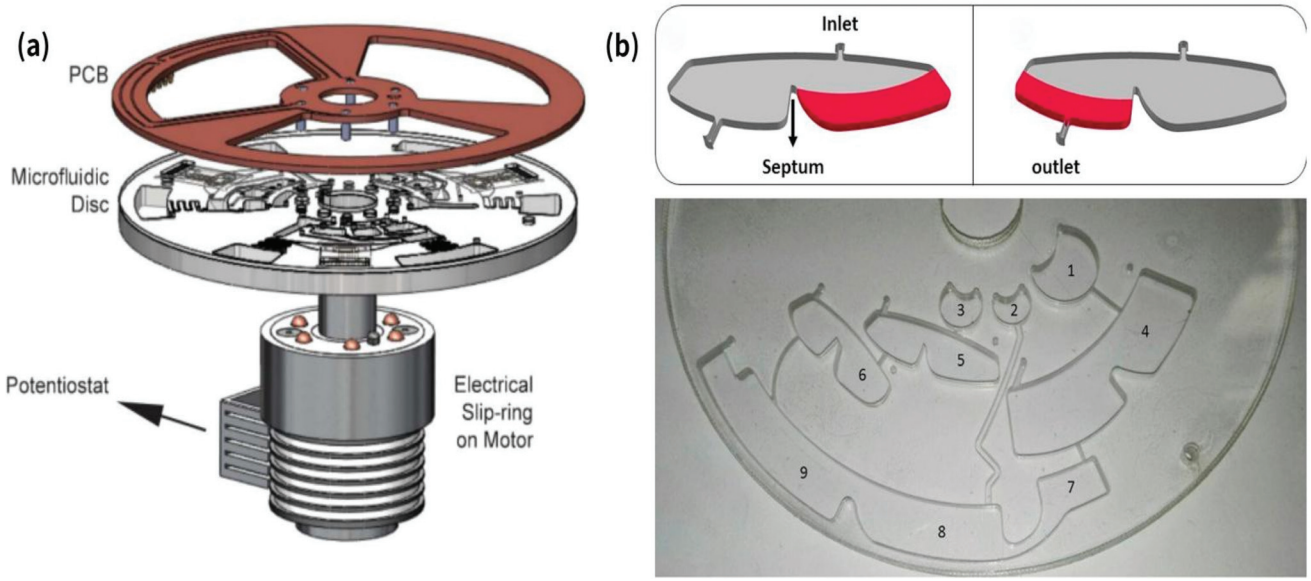

Fig. 5 (a) Illustration of electrochemical measurements with an electrical slip-ring, a stationary potentiostat, and a Printed Circuit Board (PCB) component.[17] (b) Illustration of the principle of the septum valve and layout of the whole disk.[16] 1: R1 inlet; 2: blood sample and R3 inlet; 3: R2 inlet; 4: septum valve; 5: septum valve; 6: septum valve; 7: lower mixing chamber; 8: upper mixing chamber; 9: detection chamber; R1: suspended latex particles and a stabilizer; R2: mouse anti-human glycosylated hemoglobin monoclonal antibody, goat anti-mouse IgG polyclonal antibody and a stabilizer; R3: hemolysis buffer.

sample pretreatment and detection on a single platform, this study proposed a new, portable, turbidimetric immunoassay system that integrated whole blood sample pretreatment, real-time generation of standard curves, and portable absorbance detection in one system. This system fully utilized the characteristics of centrifugal microfluidics, used centrifugal force to sediment blood cells on the chip, and used a siphon valve to quantitatively extract purified plasma so that quantitative detection of specific proteins can be achieved within 35 min.

Apart from this, there are additional detection methods as well. In 2013, Kim *et al.* proposed a flow-enhanced electrochemical immunosensor on a centrifugal microfluidic platform (Fig. 5a).[17] This is an entirely automated centrifugal microfluidic platform, wherein the flow can enhance the electrochemical detection to achieve a low LOD of an analyte. Here, an LOD of 4.9 pg mL^{-1} was obtained for CRP biomarkers, which was five times better than that obtained by stagnant electrochemical measurement. In 2015, Nwankire *et al.* proposed a label-free detection of cancer cells from whole blood on an integrated centrifugal microfluidic platform.[47] This system utilizes siphoning, capillary, and centrifugo-pneumatic dissolvable-film valves (by rotational actuation) to control the flow. The system successfully extracts cancer cells from whole blood with specific capture and sensitive detection with the help of label-free electrochemical impedance. The system yielded a minimum capture of 214 mm^{-2} and a capture efficiency of 87%. Miyazaki *et al.* developed a label-free, spatially multiplexed, immunoassay process with SPR-based detection, on a highly integrated centrifugal lab-on-a-disk platform (Fig. 6).[10] This innovative method involved a 5-fold multiplexed SPR-detection of IgG in whole blood. From extracting plasma from the ordinary chamber to the final connection with a portable smartphone, this platform achieved an LOD of 19.8 μg mL^{-1}, and may thus, be considered for future development of point-of-care detection kits.

3. Application of centrifugal microfluidic systems in biochemical analysis

Biochemical analysis usually refers to the detection and quantification of such as glucose, electrolytes, creatinine, and biomarkers, which also includes plasma/blood cell separation along with the separation and extraction of other biological particles. In recent years, the research on the use of a centrifugal microfluidic platform for biochemical analysis has become a hot spot. Biochemical analysis can be classified into three categories based on the centrifugal microfluidic platform used: plasma/blood cell separation; separation, extraction, and filtration of biological particles; and qualitative and quantitative detection of other biochemicals. Table 2 summarizes biochemical analysis applications based on the centrifugal microfluidic platform.

3.1 Separation of plasma and blood cells

The separation of plasma and blood cells is of great significance in biochemical analysis and immunodiagnosis because the presence of blood cells may interfere with the detection

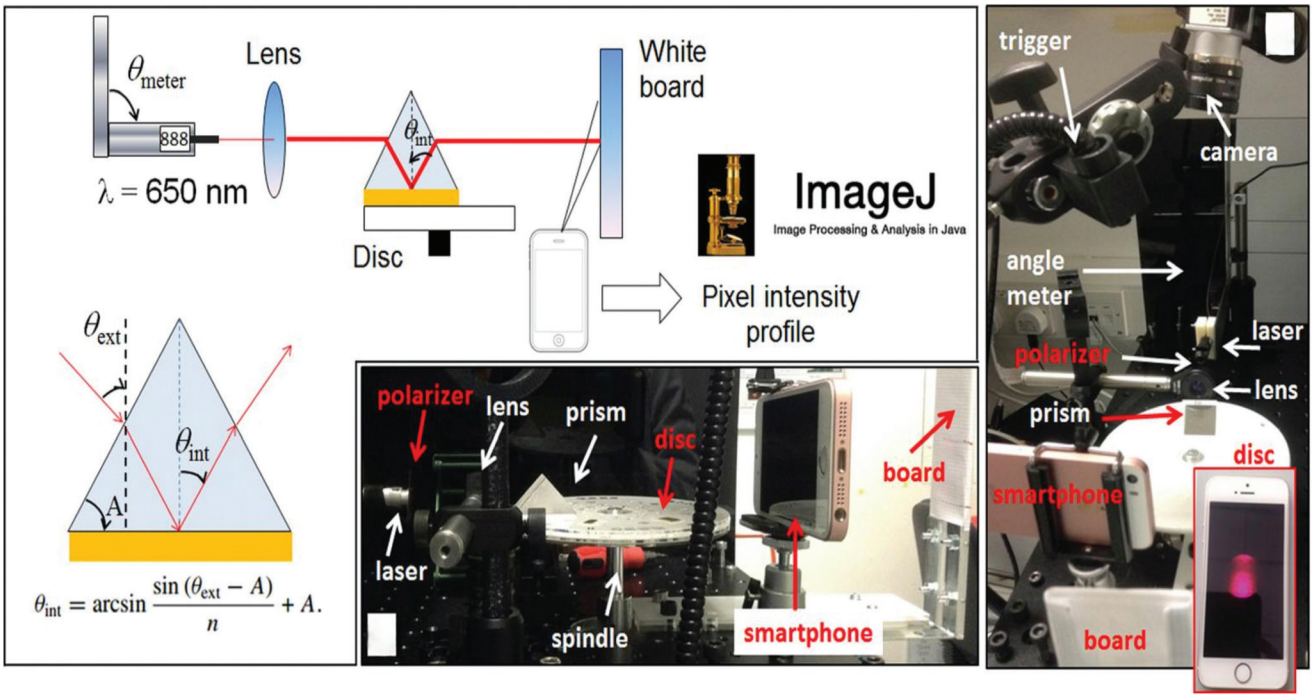

Fig. 6 Frontal and lateral views and illustration of the structure of the surface plasmon resonance (SPR) measurement disk with internal angle θ_{int}, externally measured angle θ_{ext} and the angle of light source θ_{meter}.[10]

results of certain biochemicals. Usually, the separated plasma and blood cells are stratified; however, they are all in the same chamber. Zhang *et al.* designed a microfluidic platform that enabled blood cells and plasma to flow into two microchannel branches at the same time.[48] The platform included a microchannel network composed of a straight main microchannel, a curved microchannel, and a branched microchannel. If the diluted blood was 6% hematocrit, then red blood cell separation efficiency could reach 99%. Similarly, Kuo and Chen designed a device wherein the plasma was first removed from human whole blood, divided into two samples of equal volume, and subsequently poured into a detection room for analysis.[49] The results showed that plasma volume discharged into the detection room could be accurately controlled by regulating the rotation speed of the disk, and for a dilute blood sample with a 6% hematocrit, a plasma separation efficiency of 96% could be achieved in approximately 5 min.

Kinahan *et al.* defined the curvature of the sedimentation chamber by spiral mirabilis (equiangular spiral).[50,62] The results revealed that the stratification velocity of blood in the spiraled chamber was 39% higher compared with that of non-inclined linear chambers and 22% higher than that in the equivalently inclined linear chamber. Additionally, in order to solve the problem that the volume of liquid is very small in the application of the microfluidic system while the biological sample is usually expressed in milliliter, Amasia developed and verified a large-capacity plasma separation equipment based on a centrifugal microfluidic system that could process up to 2 mL of undiluted blood samples.[3] On adding three finger-like structures over an area of approximately 0.255 × 0.074 inches in the large chamber, the surface area between the disk layers increased (Fig. 7a). As a result, within 2.5 min, they obtained plasma samples of equal or even greater purity compared with traditional methods. Generally, after the sedimentation step, the next step is the extraction of the plasma supernatant, which is usually completed *via* the siphon channel.[63,64] However, Kuo and Chen effectively mixed reagents based on plasma separation.[52] They proposed a seamlessly connected centrifugal microfluidic platform that could separate plasma and mix it with appropriate reagents, thus, omitting the plasma supernatant extraction step, and acquiring a satisfactory result with a mixing efficiency of >97% within 5 s.

Many passive valves perform poorly with respect to plasma separation efficiency under airtight conditions. Hence, studies have focused on valves made of phase change materials, although these may cause cross-contamination. Cai *et al.* created a new type of a "pinch-valve" composed of a spring plunger and a flying ball governor (Fig. 7b).[53] Adjusting the height of the spring plunger and controlling the rotation speed can regulate the opening and closing of the valve to obtain sequential loading, which is indispensable for many biological and chemical applications. This system provides a residual cell concentration of <0.5% during plasma and blood cell separation.

Recently, Shamloo *et al.* proposed two models for separating target cells by using magnetic beads.[54] They separated blood cells using tortuous channels with an initial container and three output containers and obtained 100% separation of neutrophils. Interestingly, the centrifugal microfluidic plat-

Table 2 Summary of biochemical analysis applications using the centrifugal microfluidic platform

Application	Analyte	Performance	Structure and characteristics of the chip	Ref.
Plasma separation	Whole blood	SE = 99%	The microchannel network consists of a straight main microchannel, a curved microchannel and a branching microchannel	48
Plasma separation	Whole blood creatinine	SE = 96% ST = 5 min	The disc has a Y-shaped splitter network	49
Plasma separation	Whole blood	SS = 39% increase	Logarithmic spiral channel is utilized	50
Plasma separation	Whole blood	SE ≈ traditional method ST = 2 min	A finger-like structure is added to the large chamber	51
Plasma separation	Whole blood prothrombin	Mixing time = 5 s Mixing efficiency = 97%	Seamless connection of reagent mixing and plasma separation	52
Plasma separation	Whole blood	SE = 99.5%	Pinch-valve is utilized	53
Plasma separation	Whole blood	SE = 100%	Separation by magnetic beads	54
Plasma separation	Whole blood p24 capsid protein	SE = 98% ST = 4–7 min	Fidget-spinner is utilized	55
Particle separation	Yeast cell	MFR = 35 μL min^{-1} ST = 1–16 min	Carbon-DEP is utilized	56
Particle separation	Colorectal cancer cell line LIM1863	IAC-Exos is two times better than other methods (SE)	Ultracentrifugation, density-based separation and immunoaffinity capture are compared	57
Particle separation	Leukocytes	Extraction efficiency = 34%	CPSV is utilized	58
Particle separation	Nanoscale extracellular vesicles	Extraction time = 30 min	Two nanofilters are used	59
Particle separation	Solid phase extraction (SPE) of oil in water	PVA is six times better than other methods (EE)	Based on solid phase extraction Mechanical pinch valve is used	60
Quantitative detection	CK-MB	Detection time = 22 min	Laser irradiated ferrowax microvalve	23
Quantitative detection	Creatinine	LOD = 4.42×10^{-3} μmol mL^{-1} Detection time = 2 min	SERS substrate of nano Au/Ag film is utilized	61

Abbreviations: NR, not reported; ST, separation time; SE, separation efficiency; SS, separation speed; MFR, maximum flow rate; EE, enrichment efficiency; DEP, dielectrophoresis; PVA, polyvinyl alcohol; CPSV, centrifugo-pneumatic siphon valving; SERS, surface-enhanced Raman scattering; LOD, limit of detection.CK-MB, creatine kinase-MB; IAC-Exos, immunoaffinity capture using anti-epithelial cell adhesion molecule coated magnetic beads.

form has also been used for separating plasma and blood cells. In the study of Liu *et al.*, centrifugal force was provided by a "fidget-spinner", which comprised a ball bearing in the core of a multilobed flat structure made of either metal or plastic, wherein the spinner could rotate around the bearing (Fig. 7c), allowing easy separation of plasma and blood cells.[55] Compared with the standard method, the final results here revealed a recovery rate of 98%. Combining this affordable and low-power separation platform with a detection technology should benefit people in resource-limited areas.

3.2 Separation, extraction, and filtration of biological particles

Martinez-Duarte *et al.* combined a carbon-electrode dielectrophoresis (carbon-DEP) auxiliary filter with a centrifugal microfluidic platform by utilizing Carbon-Micro-Electro-Mechanical System (C-MEMS) technology to make a three-dimensional carbon electrode to capture particles (Fig. 8a).[56] 3D carbon electrodes are able to obtain superior filtering efficiency compared with traditional electrodes because the advantages of both metal-based and insulator-based DEP are reflected in Carbon-DEP. This platform achieved a high separation efficiency in capturing yeast cells from a mixture of emulsion and yeast cells, with a maximum flow rate of 35 μL min^{-1}, which is a significant consideration during the construction of an automated biomolecule separation platform. To determine the ideal approach for isolating human colon cancer cell line LIM1863-derived exosomes,[57] Tauro *et al.* compared the separation efficiency of purified exosomes by using three methods: ultracentrifugation (UC-ExOS), Optiprep™ density gradient centrifugation, and immunomagnetic bead method (IAC-ExOS). LIM1863 colorectal cancer cell culture medium was used, and IAC-Exos was determined as the best method. Proteins endosomal sorting complex required for transport (ESCRT-III) component charged multivesicular body (MVB) protein 4C (CHMP4C) and soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor (SNARE) synaptobrevin 2 (VAMP2) were also qualitatively detected for the first time *via* this method.

Kinahan *et al.* demonstrated a density-gradient-mediated band extraction of leukocytes from whole blood by using centrifugo-pneumatic siphon valving (CPSV) on centrifugal microfluidic disks, which eliminated the need for surface hydrophilicity treatment.[58] CPSV comprises a low-pass valve which holds a fluid at high rotation speed and releases it at low rotation speed. Kinahan *et al.* performed related operations on

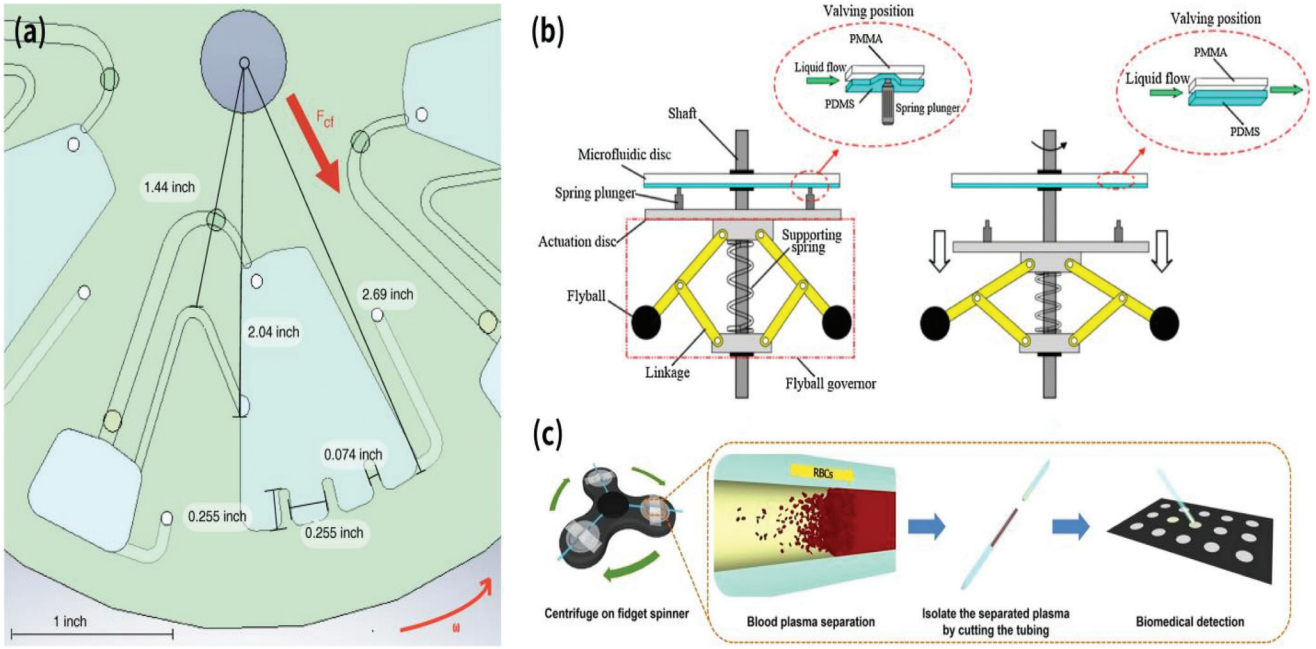

Fig. 7 (a) Layout of three finger-like structures, approximately 0.255 × 0.074 inches.[51] (b) A "pinch-valve" used to control fluid flow and its working principle.[53] (c) Schematic illustration of a fidget-spinner on a centrifugal microfluidic system,[55] RBC, red blood cell; PDMS, polydimethylsiloxane; PMMA, polymethyl methacrylate.

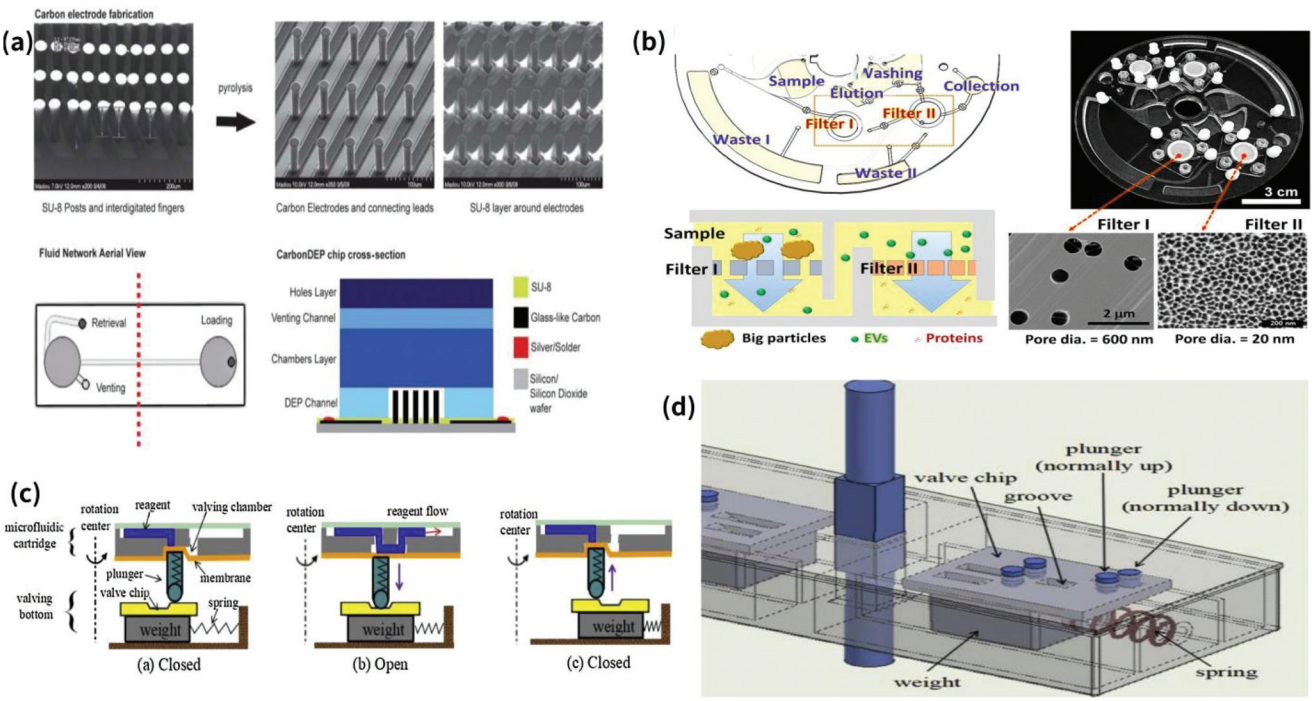

Fig. 8 (a) Illustration of a carbon-DEP chip structure.[56] (b) Illustration of the microfluidic device with its chamber distribution (left upper corner), principle of filters (left lower corner), photograph of exodisc (right upper corner) and SEM images of the two filters (right lower corner).[59] (c) Layout of the "mechanically programed" valve. Off state with low frequency(left), open state with increasing frequency(middle), and off state with further increase of frequency.[60] (d) Illustration of the operation of "mechanically programmed" valves (closing at a low frequency, opening with frequency increase, and closing again at a very high frequency).[60] DEP, dielectrophoresis; EV, extracellular vesicle; SU-8, a UV epoxy-based negative photoresist; SEM, scanning electron microscope.

the pneumatic chamber of the "split" valve to stabilize the density-gradient-medium layer during blood stratification. The extraction efficiency was also improved.

Isolation and analysis of nanoscale extracellular vesicles (EVs) from biological samples usually require ultracentrifugation; however, this is a time-consuming and low-purity process. To optimize this process, Woo *et al.* developed a centrifugal microfluidic platform for the rapid separation and quantitative analysis of EVs, where two nanofilters were important components (Fig. 8b).[59] Fully automatic enrichment of EVs in the range of 20–600 nm was achieved within 30 min. Recently, Zhang *et al.* developed a new centrifugal microfluidic platform based on centrifugal-driven solid-phase extraction technology for the enrichment of oil in water and the detection of trace oil pollution in water.[60] A mechanical pinch-valve was used in the platform to control the reagent flow (Fig. 8c and d). Precise fabrication of some suspending structures such as the valving chambers was achieved with the help of water-soluble polyvinyl alcohol. A 10 ppm oil–water sample was tested, and on comparing with the C18 column method, activated carbon, and three-dimensional printing porous polymer as the fixed adsorbent, they found that the enrichment efficiency of the three-dimensional printing was approximately six times that of the C18 column method and activated carbon method.

3.3 Qualitative and quantitative detection of other biochemical substances

Qualitative and quantitative detection of other biochemicals by a centrifugal microfluidic platform has also been studied recently. By using a unique active valve—an LIFM based on phase change—Lee *et al.* have developed a fully integrated lab-on-a-disk for performing simultaneous biochemical analysis and immunoassay of whole blood.[23] Manual operation of this platform requires the addition of 350 μL of whole blood to the platform, and the concentration of the analytes can be obtained within 22 min. Surface-enhanced Raman scattering (SERS) is advantageous because of its high sensitivity. Thus, the centrifugal microfluidic platform can be promisingly combined with SERS technology to perform rapid detection of biochemicals in the whole blood.[65–67] Su *et al.* developed a centrifugal microfluidic platform that integrated blood separation and *in situ* SERS detection and improved the detection sensitivity by fabricating a SERS substrate with a nano-Au/Ag membrane.[61] Ultimately, the LOD of creatine was determined to be 4.42×10^{-3} μmol mL^{-1}, and the lowest detected concentration in serum was 8.0×10^{2} μmol mL^{-1}.

4. Application of centrifugal microfluidic systems in molecular diagnostics

Molecular diagnostics uses molecular biology to determine sequence changes at the genetic level to aid in precise diagnosis. Change in DNA or RNA sequences is the key principle behind molecular diagnosis.[68] Molecular diagnosis plays an indispensable role in the prevention, detection, and treatment of diseases, environmental monitoring, and drug screening. Recently, there have been many studies on molecular diagnostics based on centrifugal microfluidic platforms.[69–73] Some typical centrifugal microfluidic platforms along with their advantages are summarized in Table 3. Based on different methods of nucleic acid amplification, we have categorized various detection platforms into three categories: those based on recombinase-based polymerase amplification (RPA), those based on polymerase chain reaction (PCR), and those based on loop-mediated isothermal amplification (LAMP).

4.1 Detection platform based on PCR or reverse transcription (RT)-PCR

In PCR, DNA is initially denatured into single strands at a high temperature and then annealed with primer pairs at a lower temperature to achieve complementary strand amplification. Highly sensitive detection and identification of pathogens based on unique DNA and RNA sequences is useful for the rapid detection of microorganisms, such as viruses and bacteria.[88,89]

Pre-amplification, which is the basis of many PCR protocols, is associated with a high risk of contamination which may be introduced during the processing of high-copy DNA samples. Therefore, Focke *et al.* invented a centrifugal microfluidic system for primary amplification and secondary real-time PCR (Fig. 9a).[69] This is a self-contained centrifugal system that includes pre-stored reagents, wherein DNA pre-amplification can be conducted in an improved commercial thermal cycler, followed by automated aliquoting and real-time PCR to achieve an amplification efficiency of 85%. In the same year, Focke *et al.* developed a micro-structured polymer film for enabling sensitive genotyping *via* real-time PCR on a centrifugal microfluidic platform (Fig. 9b).[70] This new technological process can enable molding of microfluidic ink cartridges (made of polymer films) and enable parallel processing of four independent samples and eight different genes with an LOD of less than 10 DNA copies, which is suitable for clinical diagnosis. Jung *et al.* proposed a new ultra-fast PCR system called a "rotary PCR genetic analyzer", which integrates a thermal block for thermal cycle control, resistance temperature detection, a disposable PCR chip, and an ultra-high speed rotating PCR system with a stepper motor to identify H3N2, H5N1, and H1N1 influenza virus RNA (Fig. 9c).[71] This system overcame the shortcomings of the requirement for an external pumping instrumentation and complicated manufacturing process because of the rapid transition of the PCR sample between the adjacent blocks, and amplified all three RNA types within 25 min. With a separation time of 5 min on a micro-capillary electrophoresis chip, subtype classification could be completed within 30 min. In 2014, Strohmeier *et al.* developed a real-time PCR based detection system for a panel of food-borne pathogens on a centrifugal microfluidic "LabDisk" with on-disk quality controls and standards for

Table 3 Summary of nucleic acid amplification and detection applications based on a centrifugal microfluidic platform

Principle	Analyte	Performance	Characteristics	Ref.
PCR	*Exfoliatin A* gene from MRSA	Amplification efficiency = 85% Detection time <20 min	Conducted in improved commercial thermal circulator	69
PCR	*Methicilin-resistant Staphylococcus aureus*	LOD = 10 copies Detection time = 110 min	Made of polymer film	70
RT-PCR	RNA of H3N2, H5N1, and H1N1	Detection time = 30 min	Polymer films is utilized	71
PCR	*Staphylococcus warneri* and *Streptococcus agalactiae*	LOD = 189 copies/141 copies Detection time = 1 h	Positive controls (PCs), no-template controls (NTCs), and standards (STDs) were integrated into a centrifugal microfluidic PCR cartridge	72
PCR	*1. Staphylococcus warneri* *2. Streptococcus agalactiae* *3. Escherichia coli* *4. Haemophilus influenzae*	LOD_1 = 3 cfu per 200 μL LOD_2 = 200 cfu per 200 μL LOD_3 = 5 cfu per 200 μL LOD_4 = 2 cfu per 200 μL Detection time = 45 min	Nested PCR Novel and easy to use MDS "sample-to-answer" system	74
PCR	HBV DNA	LOD = 10^2 copies Detection time = 32 min	Double-shaft disc is utilized	75
PCR	HBV DNA	LOD = 10^2 copies Detection time = 15 min	Integration of separations step, purification step and amplification step	76
PCR	*Bacillus atrophaeus* subspecies *globigii* spores	Detection time = 100 min	All-thermoplastic integrated sample-to-answer centrifugal microfluidic lab-on-disk system	77
RT-PCR	H3N2 virus	LOD = 2.39 × 104 copies	A fully automated sample-to-answer detection of influenza A H3N2 virus in a disc with pre storage of reagent	78
RT-PCR	Coronavirus	LOD = 200 copies Detection time = 90 min	Automatic multiple detection	79
LAMP	H1N1 Virus	LOD = 10 copies Detection time = 47 min	Micro optical detector is utilized	80
LAMP	Food-borne pathogens	LOD = 3×10^{-5} ng μL^{-1} Detection time = 60 min	Interfaced with smartphone	81
LAMP	1. *Staphylococcus aureus* 2. *Salmonella typhimurium* 3. *S. typhimurium*	LOD_1 = 0.1 cfu μL^{-1} LOD_2 = 1 cfu μL^{-1} LOD_3 = 1 cfu μL^{-1} Detection time = 70 min	Magnetic beads are used for cell lysis Final test results observed with naked eye	82
LAMP	HPAIV	Detection time = 70 min	Membrane resistance valve is used	83
LAMP	Six pathogens	LOD = 2×10^2 cells per μL	Hand-powered platform inspired by the spinning top (a kind of old toy)	84
LAMP	Subtypes of influenza A virus (H1, H3, H5, H7, and H9) and influenza B virus	Detection time = 45 min $LOD_{H1,H5,influenza\ B}$ = 50 copies $LOD_{H3,H7}$ = 20–50 copies LOD_{H9} = 50–100 copies Detection time = 45 min	SYBR Green I and calcein are two fluorescent dyes, which can be used for fluorescent detection or visual inspection	9
RPA	Antibiotic resistance gene *mecA* of *Staphylococcus aureus*	LOD = 20 copies Detection time = 20 min	Foil-based centrifugal microfluidic cartridge is utilized	85
RPA	Salmonella	LOD = 10 cfu mL^{-1} in phosphate buffered saline LOD = 102 cfu mL^{-1} in milk Detection time = 30 min	Laser diode is utilized DNA extraction, isothermal recombinase polymerase amplification (RPA) and detection are integrated on a disc	86
SEA	*Vibrio parahemolyticus*	LOD = 10^3 cfu g^{-1} (no enrichment) LOD = 10 cfu g^{-1} (with enrichment) Detection time = 55 min	Sample preparation, strand exchange amplification (SEA) and visual fluorescence detection are integrated on a disc	87

Abbreviations: PCR, polymerase chain reaction; RPA, recombinase-based polymerase amplification; LAMP, loop-mediated isothermal amplification; cfu, colony forming unit; LOD, limit of detection; HBV, hepatitis B virus; PC, positive control; NTC, no-template control; STD, standard; HPAIV, highly pathogenic avian influenza virus; SEA, strand exchange amplification; MRSA, methicillin-resistant *Staphylococcus aureus*; MDS, molecular diagnostic based.

quantification.[72] On this platform, positive controls, no-template controls (NTC), and standards were integrated into a centrifugal microfluidic PCR cartridge. They found that the LODs of *Listeria monocytogenes* and *Salmonella typhimurium* were 189 DNA copies and 141 DNA copies, respectively. To increase the sensitivity of detection after PCR amplification, Czilwik *et al.* developed a centrifugal microfluidic "LabDisk" system, which could perform high-sensitivity detection of multiple pathogens, based on nested PCR.[74] DNA extraction, multiplex PCR pre-amplification, and multiple species-specific real-time PCR

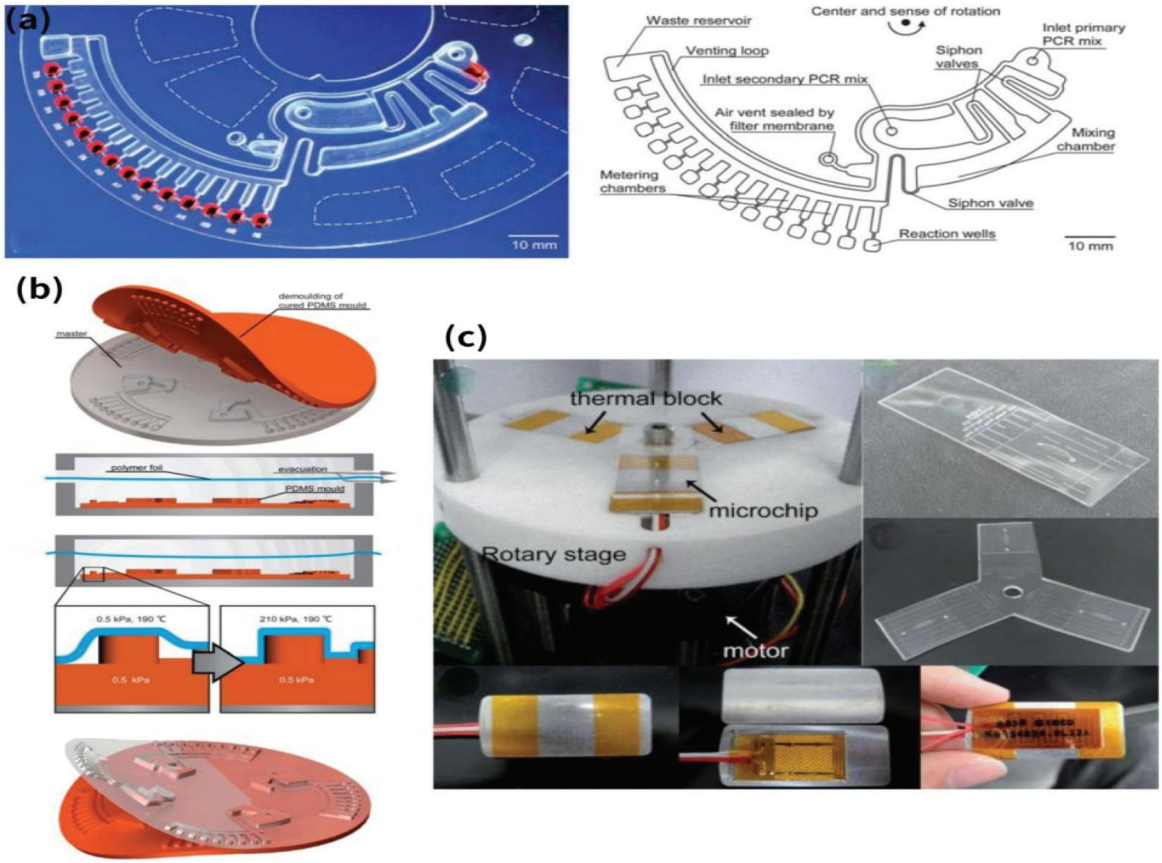

Fig. 9 (a) Design of a disk with one chamber for primary PCR and reaction wells for secondary PCR.[69] (b) Production flow chart of COP foil, from top to bottom: 1. PDMS mould fabrication (the first picture of Fig. 9(b)) 2. Use a modified hot embossing machine to realize assembly and evacuation (the second picture of Fig. 9(b)) 3. Clamp the foil (the third picture of Fig. 9(b)) 4. Use pressure difference to form the mould of the foil (the forth picture of Fig. 9(b)) 5. Venting and demoulding (the fifth picture of Fig. 9(b)).[70] (c) Layout of the whole centrifugal microfluidic PCR platform along with the illustration of a PCR microchip, thermal block, resistance temperature detection, and film heater.[71] PCR, polymerase chain reaction; PDMS, polydimethylsiloxane.

monitoring were integrated in this system (Fig. 10). Here, in a 200 μL serum sample, as few as 3 cfu of *Staphylococcus warneri*, 200 cfu of *Streptococcus agalactiae*, 5 cfu of *Escherichia coli* and 2 cfu of *Haemophilus influenzae* were detected.

However, most centrifugal microfluidic systems used for rapid PCR amplification have limitations, such as limited radial space, incompatibility between manufactured lab-on-a-chip device and multiple systems, and long PCR reaction time.[3,7,90–94] Miao *et al.* created a double-shaft turntable and centrifugation-based disk for amplifying and detecting hepatitis B virus (HBV) target genes.[75] The platform used a secondary rotational axis to control the position of the reaction chamber relative to the centrifugal force and experiments in different reaction areas at three different temperatures were performed. The LOD reached 102 copies per mL, and the amplification time was shortened by 88 min (from 120 min to 32 min). Based on the double-shaft turntable, Li *et al.* attempted to establish a fully integrated, automated HBV DNA detection platform with serum separation and reagent pre-storage.[76] Consequently, they developed a fully automatic disk for the rapid detection of HBV in whole blood, based on a double-shaft centrifugal microfluidic platform. As shown in Fig. 10c, the rotational frequency of the main shaft determines the size of the centrifugal force, and the angular position of the second shaft adjusts the direction of the centrifugal force to yield pumping of fluid in any direction. In this system, HBV DNA extraction reagents and dried PCR reagents are pre-stored on the disk to integrate the whole process of separating plasma from whole blood and lysing, purifying, and amplifying HBV DNA. This disposable disk (which costs approximately USD 5) can process 500 μL of whole blood samples and complete nucleic acid extraction within 15 min (60% of the 20 min extraction time of traditional instruments). An LOD of 102 copies per mL can be achieved within 48 min, indicating that this system is suitable for the rapid detection of HBV.

Since many existing centrifugal microfluidic molecular diagnostic platforms have off-platform operation procedures, such as bacterial cell lysis and nucleic acid purification, a complete system integration is required. Roy *et al.* have proposed an all-thermoplastic, integrated, sample-to-answer, centrifugal

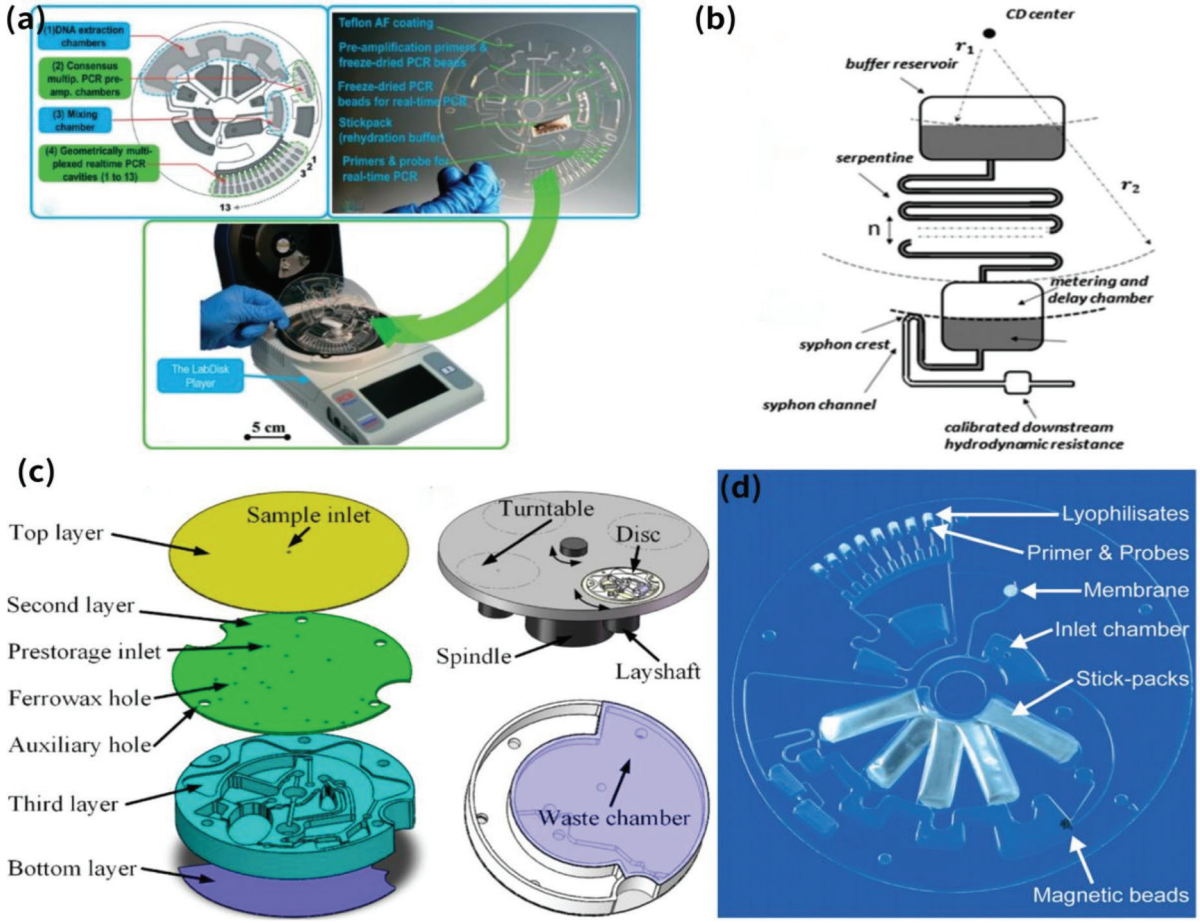

Fig. 10 (a) Design, structure, and final product of a LabDisk-Player.[74] (b) Illustration of the schematic of a timed valve.[77] (c) Illustration of four layers of a chip with a double-shaft and its waste chamber.[76] (d) Illustration of the LabDisk layout with magnetic beads for nucleic acid extraction.[78] CD, compact disk.

microfluidic lab-on-a-disk system for nucleic acid analysis to analyze *Bacillus atrophaeus* subspecies *globigii* spores and have enhanced the reliability of detection results of the entire platform by developing two new microfluidic strategies for reagent mixing and for precise control of liquid delivery and flow on a CD platform (Fig. 10b).[77] Although Roy *et al.* addressed off-disk operations of cell lysis and nucleic acid purification,[77] no centrifugal microfluidic disk for nucleic acid sample-to-answer analysis with fully pre-stored reagents was developed prior to 2016. Stumpf *et al.* developed a fully automated sample-to-answer system for detection of influenza A H3N2 virus in a centrifuge LabDisk with fully pre-stored reagents.[78] All necessary processes based on PCR, pathogen lysis, magnetic bead-based nucleic acid extraction, eluate aliquoting into eight reaction chambers, and real-time RT-PCR, were performed on the same disk (Fig. 10d). A LOD of 2.39×10^4 viral RNA copies per mL was achieved, which is of great significance in clinical diagnosis.

For rapid detection of coronavirus, Ji *et al.* have established a microfluidic disk with direct RT-PCR for automatic multiple detection of SARS-CoV-2, influenza A, and influenza B viruses in pharyngeal swab samples.[79] This platform optimizes the selection of DNA polymerase and concentrations of dNTPs and $MgCl_2$. For all three viral RNAs, the LOD reached 200 copies with 2 mL of samples. Compared with the detection results of standard RT-PCR, the detection accuracies of SARS-CoV-2, influenza A, and influenza B were 100%, 99.54%, and 99.25%, respectively. Furthermore, the detection program can complete multiple screening of up to 16 targets for other viral infections within 1.5 h, and detect positive signals within 57 min, which is three times faster than that by traditional RT-qPCR methods.

4.2 Detection based on LAMP or reverse transcription (RT)-LAMP

PCR has a few disadvantages such as the need for thermal cycling steps and the need for thermal cycling steps and trained personnel. LAMP can amplify DNA at a constant temperature,[95] and has a very high sensitivity, whereby 4–6 primers can be recognized by the DNA sequence. Thus, LAMP is widely used for environmental monitoring and virus detection.

Jung *et al.* introduced a small, integrated chemiluminescence detector with microbead-assisted RNA purification and RT-LAMP, which is a micro-device that uses micro-optical detectors for real-time monitoring (Fig. 11a).[80] This detector is innovative as RNA purification can be performed by centrifugal force with optimization of the capillary valve and siphon channel, and purified RNA extracted from waste liquid can also be obtained by changing the rotation direction. The system yields an LOD of 10 copies of viral RNA, which is 10 times better than that of conventional RT-PCR.

Unlike lateral strip flow reported by Kim *et al.*[86] which is time-consuming or the complicated optical detection system used by Jung *et al.*,[80] Sayad *et al.*[81] used a colorimetric detection method with calcein dye to visually detect food-borne pathogens (Fig. 11b). The LOD of this system was 3×10^{-5} ng μL^{-1}, and the device was suitable for point-of-care testing. Furthermore, to overcome inhibition of incomplete removal of chemical lysis reagents on subsequent amplification of cell lysis in many molecular diagnosis procedures, Yan *et al.* have described a centrifugal microfluidic chip that integrates cell lysis, clarification, and LAMP for detecting bacteria.[82] The main advantages of this chip are bacteria lysis was achieved by rotating a pair of magnets (Fig. 11c), and the results can be observed with the naked eye, which is of great significance, especially in resource-limited areas that do not have optical detectors. Compared with the desktop LAMP method, this acquires the same LOD as that of gel electrophoresis for the detection of six types of bacteria by reducing the detection time from 225 min to 70 min.

Innovative reforms, such as valve control and reduced power consumption on centrifugal microfluidic platform, were presented by Liu *et al.* in 2018. Liu *et al.* have described a new laboratory disk platform that uses a membrane resistance (MEMBR) valve for fully automated, sample-to-answer detection of highly pathogenic avian influenza virus (HPAIV).[83] The MEMBR valve is made of different hydrophobic or hydrophilic polycarbonate membranes with superfine pore sizes (Fig. 12). Using MEMBR, accurate analysis has been performed for three HPAIV, namely, H7N3, H7N9, and H9N2 and two other influenza A subtypes, H1N1 and H3N2, within 70 min. Furthermore, to overcome resource limitations such as limited electricity supply, Zhang *et al.* have suggested a fully manual centrifugal microfluidic platform for pathogen diagnostics (inspired by a spinning top, which is one of the old types of toys; Fig. 13a).[84] Researchers can simply pull out the top frame of the operating centrifuge to engender high-speed rotation of the disc to successfully mix pre-loaded reagents. Six different pathogenic bacteria (2×10^2 μL^{-1}) could be successfully analyzed on this disc at the same time without resorting to an external power supply. Fluorescent signal detection can be conducted using a handheld ultraviolet light. To improve the miniaturization and integration of the centrifugal microfluidic platforms, Yao *et al.* have proposed a microfluidic centrifuge disc for the rapid detection of influenza virus subtypes (Fig. 13b).[9] This platform integrates reagent pre-installation, automatic reagent control, and RT-LAMP detection. Two fluorescent dyes, SYBR Green I and calcein, are used for fluorescence detection or visual observation and six types of highly pathogenic influenza viruses, including influenza A viruses H1, H3, H5, H7, H9, and influenza B virus, can be detected; thus, this is an effective method that may be used for preventing large-scale influenza outbreaks.

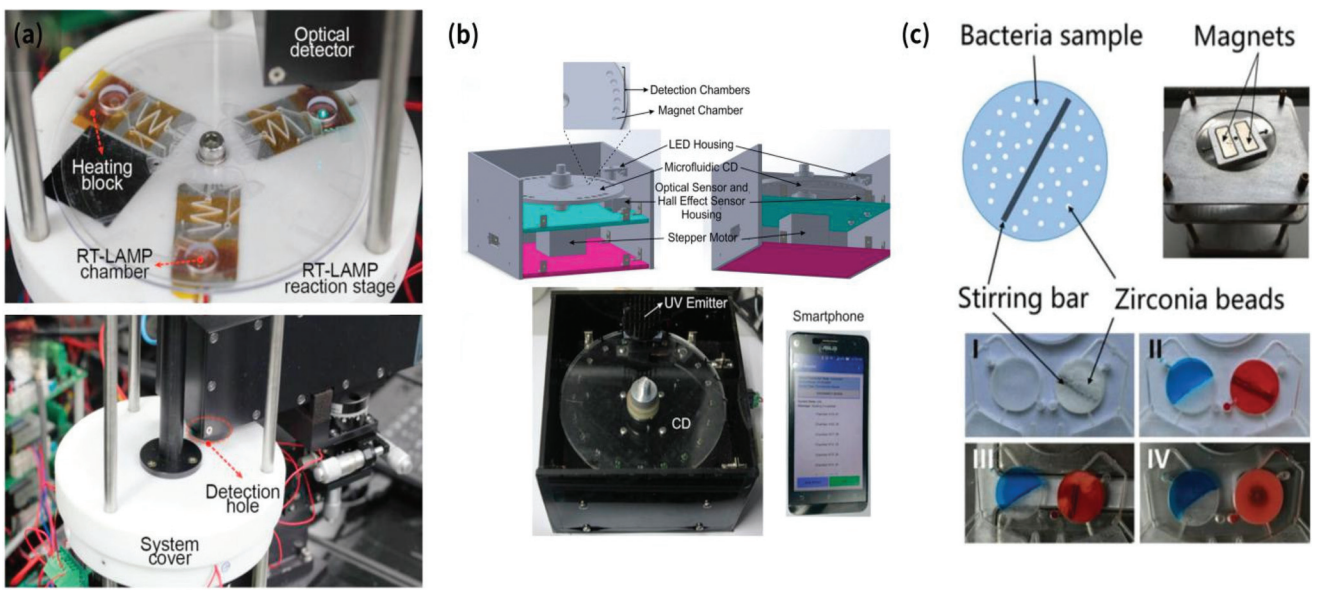

Fig. 11 (a) Image of a chip with an optical detector, heating blocks, and shafts.[80] (b) Image of a smartphone-connected platform with a colorimetric detection system.[81] (c) Illustration of a lysis chamber with beads produced by rotating the magnet (top panel) and flow process of the sample (bottom panel).[82] CD, compact disk; LED, light-emitting diode; UV, ultraviolet; RT-LAMP, reverse transcription-loop-mediated isothermal amplification.

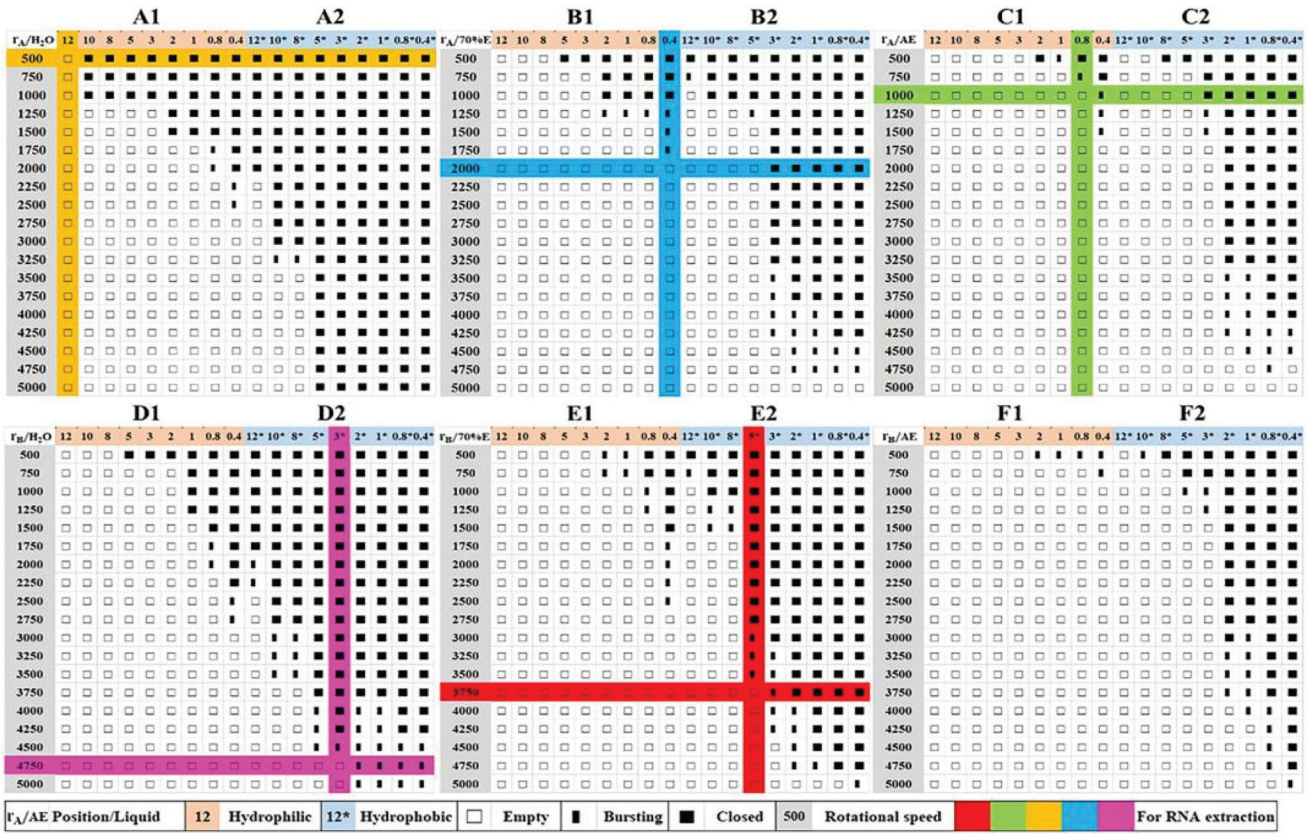

Fig. 12 Schematic of the membrane resistance (MEMBR) valve.[83]

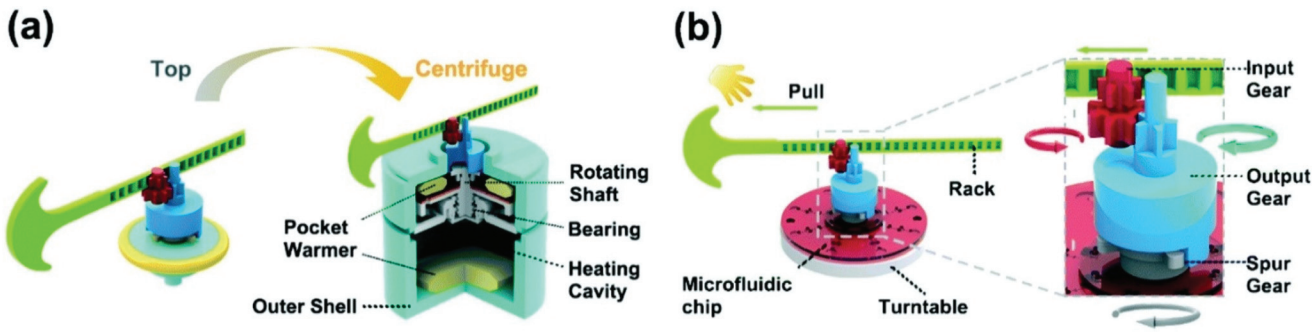

Fig. 13 (a) Illustration of a hand-powered centrifugal microfluidic device (second picture from left) and the old toy, spinning top (first picture from left).[84] (b) Schematic of the rotor module made up of a linear toothed rack, an input gear, an output gear and a spur gear.[84]

4.3 Detection based on RPA

Although LAMP resolves many complications associated with the standard detection system, a temperature of 65 °C should be maintained during the reaction process, which is power consuming. In this case, RPA is a better alternative as it enables the reaction at 37 °C. Compared with PCR, RPA has similar sensitivity and better temperature maintenance and less power consumption.

Lutz *et al.* developed a fully automated nucleic acid analysis system based on RPA to detect *mecA*, an antibiotic resistance gene of *Staphylococcus aureus*.[85] They developed a novel foil-based centrifugal microfluidic cartridge, which included pre-stored liquid, dry reagents, and a commercial centrifugal analyzer enabling 37 °C incubation and real-time fluorescence detection. The system can detect and amplify 20 copies of *mecA* from a sample within 20 min, making this system highly appealing for point of care testing such as the screening of methicillin resistant bacteria in hospital. To eliminate the defects associated with manual DNA extraction, Kim *et al.* integrated DNA extraction, RPA, and signal detection on a chip to detect salmonella in milk samples.[86] The platform innovatively

used laser diodes for valve control, cell lysis, and heating and lateral flow strips for detection. Finally, the LODs of 10 cfu mL^{-1} for salmonella in phosphate buffered saline and 102 cfu mL^{-1} in milk, respectively, were obtained within 30 min.

4.4 Detection based on other methods

In addition to PCR, LAMP, and RPA, other innovations in nucleic acid amplification assays have been achieved. Many instruments are prone to product transfer during DNA amplification; this causes aerosol contamination, which in turn leads to false positive results and difficulty in obtaining quantitative nucleic acid amplification in real time. To address this, Chen *et al.* developed a reagent-loaded, automated, centrifugal microfluidic system for high sensitivity point-of-care nucleic acid detection and used it to detect *Pseudomonas aeruginosa.*[96] By using an integrated RAA-Cas12a system, they resolved the problem of aerosol pollution and other difficulties in quantitative analysis to greatly improve the detection sensitivity within 1.5 hours. The integrated system is so called the Cas12a-assisted straightforward microfluidic equipment for the analysis of nucleic acid (CASMEAN).

Although Zhang *et al.* used manual centrifugal microfluidics for rapid detection,[84] the current manual centrifuge is associated with instrument customization and low speed. Using a "buzzer toy" as inspiration, Li *et al.* created a fully manual, centrifugal, miniaturized nucleic acid amplification test platform, which integrates sample preparation, strand exchange amplification (SEA), and visual fluorescence detection.[87] This manual centrifugal microchip[11] can achieve a high rotational speed by simply dragging a looped rope, without relying on any other additional equipment. This is also the first combination and introduction of a new isothermal amplification method—denaturation bubble-mediated strand exchange amplification (SEA).[97] This platform can perform DNA or RNA detection (for instance, that of *Vibrio parahaemolyticus*) with only a pair of primers and polymerase, within 1 h. Here, the final LOD of *Vibrio parahemolyticus* was 10^3 cfu g^{-1}. On adopting an enrichment procedure, the LOD reached 10° cfu g^{-1}. Such a sample-to-answer operating platform that operates only by manually providing power sources is greatly helpful in resource-limited areas. Additionally, Siegrist *et al.* have creatively used a magnetically actuated bead-beating system for sample lysis to extract nucleic acid from *Bacillus subtilis* spores and other clinical samples.[98] Nucleic acid extraction efficiency of this platform is equivalent to that obtained from bead-beating lysis in a tube. Furthermore, Strohmeier *et al.* have used a one-cartridge system, which enables both lysis and nucleic acid purification to process up to 200 mL of sample.[99]

5. Conclusions and future perspectives

Here, we reviewed the latest developments in centrifugal microfluidic platforms in immunoassays, biochemical analyses, and molecular diagnosis in recent years. The structure, principle, performance, efficiency, and innovation of each platform have been discussed in different sections. A centrifugal microfluidic system is used for the extraction and purification of nucleic acids, quantitative detection of nucleic acids, separation of plasma and blood cells, filtration and extraction of biological particles, various immunoassays, and drug screening. Centrifugal microfluidic technology provides new insights and development opportunities for health monitoring, molecular diagnosis, and biochemical analysis. Interestingly, these platforms display a similar or even higher sensitivity than traditional platforms. In addition, their production costs are lower, which is beneficial for rapid monitoring in resource-limited areas.

However, there are still many difficulties and shortcomings that need to be addressed. In terms of detection methods, since most of them are based on optics, it is critical to minimize the light interference from the external environment (some platforms based on fluorescence monitoring system are susceptible to interference from external light, resulting in deviations.). Furthermore, the existing external optical equipment and instruments tend to be expensive, so we can consider the development of low-cost optical equipment and instruments to help the promotion of point of care testing. In terms of system integration, the trade-offs between miniaturization, low cost, and multi-functional integration should also be focused on, and the control of external devices can learn from valving methods reviewed above. What's more, different kinds of detection devices can be promisingly integrated into microfluidic chips, which will be a great benefit for patients in remote areas.

In the future, rapid monitoring of the centrifugal microfluidic platform can be effectively combined with big data analysis and 5G technology to provide more accurate and comprehensive diagnosis results to people in resource-limited areas, and medical data can be efficiently transmitted to 5G cloud servers with ultra-low delay. Both individuals and hospitals can keep abreast of the latest diagnosis results and medical data, which is of great help to the early diagnosis and treatment of diseases. Taken together, molecular diagnosis, immunoassay, and biochemical analysis platforms based on centrifugal microfluidics will serve as important diagnostic tools in the future.

Conflicts of interest

There are no conflicts to declare.

Acknowledgements

The authors acknowledge the financial support from the Shenzhen Science and Technology Program (KQTD20170809110344233) and Shenzhen Bay Laboratory (SZBL2019062801005).

References

1 M. Tang, G. Wang, S.-K. Kong, H.-P. Ho, N. A. Yusoff, N. Soin, *et al.*, A Review of Biomedical Centrifugal Microfluidic Platforms, *Micromachines*, 2016, **7**(2), 26.
2 C. A. Burtis, J. C. Mailen, W. F. Johnson, C. D. Scott, T. O. Tiffany and N. G. Anderson, Development of a miniature fast analyzer, *Clin. Chem.*, 1972, **18**(8), 753–761.
3 R. Gorkin, J. Park, J. Siegrist, M. Amasia, B. S. Lee, J.-M. Park, *et al.*, Centrifugal microfluidics for biomedical applications, *Lab Chip*, 2010, **10**(14), 1758–1773.
4 Lab-on-a-disk as a potential microfluidic platform for dengue NS1-ELISA, in *2009 IEEE Symposium on Industrial Electronics & Applications*, ed. N. A. Yusoff, N. Soin and F. Ibrahim, 2009 4-6 Oct, 2009.
5 Y. Ukita, S. Kondo, T. Azeta, M. Ishizawa, C. Kataoka, M. Takeo, *et al.*, Stacked centrifugal microfluidic device with three-dimensional microchannel networks and multifunctional capillary bundle structures for immunoassay, *Sens. Actuators, B*, 2012, **166**, 898–906.
6 A. I. Barbosa and N. M. Reis, A critical insight into the development pipeline of microfluidic immunoassay devices for the sensitive quantitation of protein biomarkers at the point of care, *Analyst*, 2017, **142**(6), 858–882.
7 J. Ducree, S. Haeberle, S. Lutz, S. Pausch, F. von Stetten and R. Zengerle, The centrifugal microfluidic bio-disk platform, *J. Micromech. Microeng.*, 2007, **17**(7), S103–SS15.
8 R. Burger, D. Kirby, M. Glynn, C. Nwankire, M. O'Sullivan, J. Siegrist, *et al.*, Centrifugal microfluidics for cell analysis, *Curr. Opin. Chem. Biol.*, 2012, **16**(3–4), 409–414.
9 Y. Yao, X. Chen, X. Zhang, Q. Liu, J. Zhu, W. Zhao, *et al.*, Rapid Detection of Influenza Virus Subtypes Based on an Integrated Centrifugal Disc, *ACS Sens.*, 2020, **5**(5), 1354–1362.
10 C. M. Miyazaki, D. J. Kinahan, R. Mishra, F. Mangwanya, N. Kilcawley, M. Ferreira, *et al.*, Label-free, spatially multiplexed SPR detection of immunoassays on a highly integrated centrifugal Lab-on-a-Disc platform, *Biosens. Bioelectron.*, 2018, **119**, 86–93.
11 M. S. Bhamla, B. Benson, C. Chai, G. Katsikis, A. Johri and M. Prakash, Hand-powered ultralow-cost paper centrifuge, *Nat. Biomed. Eng.*, 2017, **1**(1), 0009.
12 C.-T. Lin, S.-H. Kuo, P.-H. Lin, P.-H. Chiang, W.-H. Lin, C.-H. Chang, *et al.*, Hand-powered centrifugal microfluidic disc with magnetic chitosan bead-based ELISA for antibody quantitation, *Sens. Actuators, B*, 2020, **316**, 128003.
13 Y.-S. Park, V. Sunkara, Y. Kim, W. S. Lee, J.-R. Han and Y.-K. Cho, Fully Automated Centrifugal Microfluidic Device for Ultrasensitive Protein Detection from Whole Blood, *J. Visualized Exp.*, 2016, (110), e54143.
14 S. Okamoto and Y. Ukita, Automatic microfluidic enzyme-linked immunosorbent assay based on CLOCK-controlled autonomous centrifugal microfluidics, *Sens. Actuators, B*, 2018, **261**, 264–270.
15 J. Yang, K. Liu, M. Yang and G. Wang, An immunoturbidimetric assay for specific proteins identification from whole blood based on multi-layered centrifugal microfluidic chip, in *Eleventh International Conference on Information Optics and Photonics. Proceedings of SPIE*, ed. H. Wang, 112092019.
16 E. M. Arjmand, M. Saadatmand, M. R. Bakhtiari and M. Eghbal, Design and fabrication of a centrifugal microfluidic disc including septum valve for measuring hemoglobin A1c in human whole blood using immunoturbidimetry method, *Talanta*, 2018, **190**, 134–139.
17 T.-H. Kim, K. Abi-Samra, V. Sunkara, D.-K. Park, M. Amasia, N. Kim, *et al.*, Flow-enhanced electrochemical immunosensors on centrifugal microfluidic platforms, *Lab Chip*, 2013, **13**(18), 3747–3754.
18 Z. Gao, Z. Chen, J. Deng, X. Li, Y. Qu, L. Xu, *et al.*, Measurement of Carcinoembryonic Antigen in Clinical Serum Samples Using a Centrifugal Microfluidic Device, *Micromachines*, 2018, **9**(9), 470.
19 R. Uddin, M. Donolato, E.-T. Hwu, M. F. Hansen and A. Boisen, Combined detection of C-reactive protein and PBMC quantification from whole blood in an integrated lab-on-a-disc microfluidic platform, *Sens. Actuators, B*, 2018, **272**, 634–642.
20 Z. Noroozi, H. Kido, R. Peytavi, R. Nakajima-Sasaki, A. Jasinskas, M. Micic, *et al.*, A multiplexed immunoassay system based upon reciprocating centrifugal microfluidics, *Rev. Sci. Instrum.*, 2011, **82**(6), 064303.
21 K. Wang, R. Liang, H. Chen, S. Lu, S. Jia and W. Wang, A microfluidic immunoassay system on a centrifugal platform, *Sens. Actuators, B*, 2017, **251**, 242–249.
22 B. S. Lee, J.-N. Lee, J.-M. Park, J.-G. Lee, S. Kim, Y.-K. Cho, *et al.*, A fully automated immunoassay from whole blood on a disc, *Lab Chip*, 2009, **9**(11), 1548–1555.
23 B. S. Lee, Y. U. Lee, H.-S. Kim, T.-H. Kim, J. Park, J.-G. Lee, *et al.*, Fully integrated lab-on-a-disc for simultaneous analysis of biochemistry and immunoassay from whole blood, *Lab Chip*, 2011, **11**(1), 70–78.
24 T. Abe, S. Okamoto, A. Taniguchi, M. Fukui, A. Yamaguchi, Y. Utsumi, *et al.*, A lab in a bento box: an autonomous centrifugal microfluidic system for an enzyme-linked immunosorbent assay, *Anal. Methods*, 2020, **12**(40), 4858–4866.
25 B. Johannsen, L. Mueller, D. Baumgartner, L. Karkossa, S. M. Frueh, N. Bostanci, *et al.*, Automated Pre-Analytic Processing of Whole Saliva Using Magnet-Beating for Point-of-Care Protein Biomarker Analysis, *Micromachines*, 2019, **10**(12), 833.
26 A. Voller, A. Bartlett and D. E. Bidwell, Enzyme immunoassays with special reference to ELISA techniques, *J. Clin. Pathol.*, 1978, **31**(6), 507–520.
27 Y. Liu, D. Yang, T. Yu and X. Jiang, Incorporation of electrospun nanofibrous PVDF membranes into a microfluidic chip assembled by PDMS and scotch tape for immunoassays, *Electrophoresis*, 2009, **30**(18), 3269–3275.
28 D. C. Duffy, H. L. Gillis, J. Lin, N. F. Sheppard and G. J. Kellogg, Microfabricated centrifugal microfluidic systems: Characterization and multiple enzymatic assays, *Anal. Chem.*, 1999, **71**(20), 4669–4678.

29 S. Lai, S. N. Wang, J. Luo, L. J. Lee, S. T. Yang and M. J. Madou, Design of a compact disk-like microfluidic platform for enzyme-linked immunosorbent assay, *Anal. Chem.*, 2004, **76**(7), 1832–1837.
30 S. Guo, R. Ishimatsu, K. Nakano and T. Imato, Automated chemiluminescence immunoassay for a nonionic surfactant using a recycled spinning-pausing controlled washing procedure on a compact disc-type microfluidic platform, *Talanta*, 2015, **133**, 100–106.
31 J. Siegrist, R. Gorkin, L. Clime, E. Roy, R. Peytavi, H. Kido, *et al.*, Serial siphon valving for centrifugal microfluidic platforms, *Microfluid. Nanofluid.*, 2010, **9**(1), 55–63.
32 C. E. Nwankire, G. G. Donohoe, X. Zhang, J. Siegrist, M. Somers, D. Kurzbuch, *et al.*, At-line bioprocess monitoring by immunoassay with rotationally controlled serial siphoning and integrated supercritical angle fluorescence optics, *Anal. Chim. Acta*, 2013, **781**, 54–62.
33 S. J. Oh, B. H. Park, G. Choi, J. H. Seo, J. H. Jung, J. S. Choi, *et al.*, Fully automated and colorimetric foodborne pathogen detection on an integrated centrifugal microfluidic device, *Lab Chip*, 2016, **16**(10), 1917–1926.
34 X. Meng, Y. Zhu, Y. Chen, Y. Lu, Y. Xu and J. Cheng, Conditional siphon priming for multi-step assays on centrifugal microfluidic platforms, *Sens. Actuators, B*, 2017, **242**, 710–717.
35 Z. Cai, J. Xiang, B. Zhang and W. Wang, A magnetically actuated valve for centrifugal microfluidic applications, *Sens. Actuators, B*, 2015, **206**, 22–29.
36 Z. Cai, J. Xiang, H. Chen and W. Wang, Membrane-based valves and inward-pumping system for centrifugal microfluidic platforms, *Sens. Actuators, B*, 2016, **228**, 251–258.
37 I. T. Gug, M. Tertis, O. Hosu and C. Cristea, Salivary biomarkers detection: Analytical and immunological methods overview, *TrAC, Trends Anal. Chem.*, 2019, **113**, 301–316.
38 P. J. F. Rantonen and J. H. Meurman, Viscosity of whole saliva, *Acta Odontol. Scand.*, 1998, **56**(4), 210–214.
39 W. Schramm, T. M. Annesley, G. J. Siegel, J. C. Sackellares and R. H. Smith, Measurement of phenytoin and carbamazepine in an ultrafiltrate of saliva, *Ther. Drug Monit.*, 1991, **13**(5), 452–460.
40 K. L. Helton, K. E. Nelson, E. Fu and P. Yager, Conditioning saliva for use in a microfluidic biosensor, *Lab Chip*, 2008, **8**(11), 1847–1851.
41 D. Malamud, Saliva as a diagnostic fluid, *Dent. Clin. North Am.*, 2011, **55**(1), 159–178.
42 L. Zhao, L. Sun and X. Chu, Chemiluminescence immunoassay, *TrAC, Trends Anal. Chem.*, 2009, **28**(4), 404–415.
43 G. Czilwik, S. K. Vashist, V. Klein, A. Buderer, G. Roth, F. von Stetten, *et al.*, Magnetic chemiluminescent immunoassay for human C-reactive protein on the centrifugal microfluidics platform, *RSC Adv.*, 2015, **5**(76), 61906–61912.
44 S. M. T. Delgado, D. J. Kinahan, F. S. Sandoval, L. A. N. Julius, N. A. Kilcawley, J. Ducree, *et al.*, Fully automated chemiluminescence detection using an electrified-Lab-on-a-Disc (eLoaD) platform, *Lab Chip*, 2016, **16**(20), 4002–4011.
45 S. M. T. Delgado, D. J. Kinahan, L. A. N. Julius, A. Mallette, D. S. Ardila, R. Mishra, *et al.*, Wirelessly powered and remotely controlled valve-array for highly multiplexed analytical assay automation on a centrifugal microfluidic platform, *Biosens. Bioelectron.*, 2018, **109**, 214–223.
46 S. M. T. Delgado, J. G. Korvink and D. Mager, The eLoaD platform endows centrifugal microfluidics with on-disc power and communication, *Biosens. Bioelectron.*, 2018, **117**, 464–473.
47 C. E. Nwankire, A. Venkatanarayanan, T. Glennon, T. E. Keyes, R. J. Forster and J. Ducree, Label-free impedance detection of cancer cells from whole blood on an integrated centrifugal microfluidic platform, *Biosens. Bioelectron.*, 2015, **68**, 382–389.
48 J. Zhang, Q. Guo, M. Liu and J. Yang, A lab-on-CD prototype for high-speed blood separation, *J. Micromech. Microeng.*, 2008, **18**(12), 125025.
49 J.-N. Kuo and X.-F. Chen, Plasma separation and preparation on centrifugal microfluidic disk for blood assays, *Microsyst. Technol.*, 2015, **21**(11), 2485–2494.
50 D. J. Kinahan, S. M. Kearney, M. T. Glynn and J. Ducree, Spira mirabilis enhanced whole blood processing in a lab-on-a-disk, *Sens. Actuators, A*, 2014, **215**, 71–76.
51 M. Amasia and M. Madou, Large-volume centrifugal microfluidic device for blood plasma separation, *Bioanalysis*, 2010, **2**(10), 1701–1710.
52 J.-N. Kuo and X.-F. Chen, Decanting and mixing of supernatant human blood plasma on centrifugal microfluidic platform, *Microsyst. Technol.*, 2016, **22**(4), 861–869.
53 Z. Cai, J. Xiang and W. Wang, A pinch-valve for centrifugal microfluidic platforms and its application in sequential valving operation and plasma extraction, *Sens. Actuators, B*, 2015, **221**, 257–264.
54 A. Shamloo, A. Selahi and M. Madadelahi, Designing and modeling a centrifugal microfluidic device to separate target blood cells, *J. Micromech. Microeng.*, 2016, **26**(3), 035017.
55 C.-H. Liu, C.-A. Chen, S.-J. Chen, T.-T. Tsai, C.-C. Chu, C.-C. Chang, *et al.*, Blood Plasma Separation Using a Fidget-Spinner, *Anal. Chem.*, 2019, **91**(2), 1247–1253.
56 R. Martinez-Duarte, R. A. Gorkin III, K. Abi-Samra and M. J. Madou, The integration of 3D carbon-electrode dielectrophoresis on a CD-like centrifugal microfluidic platform, *Lab Chip*, 2010, **10**(8), 1030–1043.
57 B. J. Tauro, D. W. Greening, R. A. Mathias, H. Ji, S. Mathivanan, A. M. Scott, *et al.*, Comparison of ultracentrifugation, density gradient separation, and immunoaffinity capture methods for isolating human colon cancer cell line LIM1863-derived exosomes, *Methods*, 2012, **56**(2), 293–304.
58 D. J. Kinahan, S. M. Kearney, N. A. Kilcawley, P. L. Early, M. T. Glynn and J. Ducree, Density-Gradient Mediated Band Extraction of Leukocytes from Whole Blood Using Centrifugo-Pneumatic Siphon Valving on Centrifugal Microfluidic Discs, *PLoS One*, 2016, **11**(5), e0155545.

59 H.-K. Woo, V. Sunkara, J. Park, T.-H. Kim, J.-R. Han, C.-J. Kim, *et al.*, Exodisc for Rapid, Size-Selective, and Efficient Isolation and Analysis of Nanoscale Extracellular Vesicles from Biological Samples, *ACS Nano*, 2017, **11**(2), 1360–1370.

60 Y. Zhang, J. Xiang, Y. Wang, Z. Qiao and W. Wang, A 3D printed centrifugal microfluidic platform for spilled oil enrichment and detection based on solid phase extraction (SPE), *Sens. Actuators, B*, 2019, **296**, 126603.

61 X. Su, Y. Xu, H. Zhao, S. Li and L. Chen, Design and preparation of centrifugal microfluidic chip integrated with SERS detection for rapid diagnostics, *Talanta*, 2019, **194**, 903–909.

62 L. Wang, M.-C. Kropinski and P. C. H. Li, Analysis and modeling of flow in rotating spiral microchannels: towards math-aided design of microfluidic systems using centrifugal pumping, *Lab Chip*, 2011, **11**(12), 2097–2108.

63 J. Steigert, T. Brenner, M. Grumann, L. Riegger, S. Lutz, R. Zengerle, *et al.*, Integrated siphon-based metering and sedimentation of whole blood on a hydrophilic lab-on-a-disk, *Biomed. Microdevices*, 2007, **9**(5), 675–679.

64 O. Strohmeier, M. Keller, F. Schwemmer, S. Zehnle, D. Mark, F. von Stetten, *et al.*, Centrifugal microfluidic platforms: advanced unit operations and applications, *Chem. Soc. Rev.*, 2015, **44**(17), 6187–6229.

65 S. Pahlow, A. Maerz, B. Seise, K. Hartmann, I. Freitag, E. Kaemmer, *et al.*, Bioanalytical application of surface- and tip-enhanced Raman spectroscopy, *Eng. Life Sci.*, 2012, **12**(2), 131–143.

66 K. Hering, D. Cialla, K. Ackermann, T. Doerfer, R. Moeller, H. Schneidewind, *et al.*, SERS: a versatile tool in chemical and biochemical diagnostics, *Anal. Bioanal. Chem.*, 2008, **390**(1), 113–124.

67 T. L. Wang, F. Y. Peng, H. H. Lu, Y. T. Hung, T. J. Fang and H. K. Chiang, *et al.*, *Surface enhanced Raman spectra in biochemical molecules and cell detection*, 2004. pp. 57–58.

68 W. Zhao, S. Tian, L. Huang, K. Liu, L. Dong and J. Guo, A smartphone-based biomedical sensory system, *Analyst*, 2020, **145**(8), 2873–2891.

69 M. Focke, F. Stumpf, G. Roth, R. Zengerle and F. von Stetten, Centrifugal microfluidic system for primary amplification and secondary real-time PCR, *Lab Chip*, 2010, **10**(23), 3210–3212.

70 M. Focke, F. Stumpf, B. Faltin, P. Reith, D. Bamarni, S. Wadle, *et al.*, Microstructuring of polymer films for sensitive genotyping by real-time PCR on a centrifugal microfluidic platform, *Lab Chip*, 2010, **10**(19), 2519–2526.

71 J. H. Jung, S. J. Choi, B. H. Park, Y. K. Choi and T. S. Seo, Ultrafast Rotary PCR system for multiple influenza viral RNA detection, *Lab Chip*, 2012, **12**(9), 1598–1600.

72 O. Strohmeier, N. Marquart, D. Mark, G. Roth, R. Zengerle and F. von Stetten, Real-time PCR based detection of a panel of food-borne pathogens on a centrifugal microfluidic "LabDisk" with on-disk quality controls and standards for quantification, *Anal. Methods*, 2014, **6**(7), 2038–2046.

73 A. Sedighi, V. Whitehall and P. C. H. Li, Enhanced destabilization of mismatched DNA using gold nanoparticles offers specificity without compromising sensitivity for nucleic acid analyses, *Nano Res.*, 2015, **8**(12), 3922–3933.

74 G. Czilwik, T. Messinger, O. Strohmeier, S. Wadle, F. von Stetten, N. Paust, *et al.*, Rapid and fully automated bacterial pathogen detection on a centrifugal-microfluidic LabDisk using highly sensitive nested PCR with integrated sample preparation, *Lab Chip*, 2015, **15**(18), 3749–3759.

75 B. Miao, N. Peng, L. Li, Z. Li, F. Hu, Z. Zhang, *et al.*, Centrifugal Microfluidic System for Nucleic Acid Amplification and Detection, *Sensors*, 2015, **15**(11), 27954–27968.

76 L. Li, B. Miao, Z. Li, Z. Sun and N. Peng, Sample-to-Answer Hepatitis B Virus DNA Detection from Whole Blood on a Centrifugal Microfluidic Platform with Double Rotation Axes, *ACS Sens.*, 2019, **4**(10), 2738–2745.

77 E. Roy, G. Stewart, M. Mounier, L. Malic, R. Peytavi, L. Clime, *et al.*, From cellular lysis to microarray detection, an integrated thermoplastic elastomer (TPE) point of care Lab on a Disc, *Lab Chip*, 2015, **15**(2), 406–416.

78 F. Stumpf, F. Schwemmer, T. Hutzenlaub, D. Baumann, O. Strohmeier, G. Dingemanns, *et al.*, LabDisk with complete reagent prestorage for sample-to-answer nucleic acid based detection of respiratory pathogens verified with influenza A H3N2 virus, *Lab Chip*, 2016, **16**(1), 199–207.

79 M. Ji, Y. Xia, J. F.-C. Loo, L. Li, H.-P. Ho, J. He, *et al.*, Automated multiplex nucleic acid tests for rapid detection of SARS-CoV-2, influenza A and B infection with direct reverse-transcription quantitative PCR (dirRT-qPCR) assay in a centrifugal microfluidic platform, *RSC Adv.*, 2020, **10**(56), 34088–34098.

80 J. H. Jung, B. H. Park, S. J. Oh, G. Choi and T. S. Seo, Integrated centrifugal reverse transcriptase loop-mediated isothermal amplification microdevice for influenza A virus detection, *Biosens. Bioelectron.*, 2015, **68**, 218–224.

81 A. Sayad, F. Ibrahim, S. M. Uddin, J. Cho, M. Madou and T. Kwai Lin, A microdevice for rapid, monoplex and colorimetric detection of foodborne pathogens using a centrifugal microfluidic platform, *Biosens. Bioelectron.*, 2018, **100**, 96–104.

82 H. Yan, Y. Zhu, Y. Zhang, L. Wang, J. Chen, Y. Lu, *et al.*, Multiplex detection of bacteria on an integrated centrifugal disk using bead-beating lysis and loop-mediated amplification, *Sci. Rep.*, 2017, **7**, 1460.

83 Q. Liu, X. Zhang, L. Chen, Y. Yao, S. Ke, W. Zhao, *et al.*, A sample-to-answer labdisc platform integrated novel membrane-resistance valves for detection of highly pathogenic avian influenza viruses, *Sens. Actuators, B*, 2018, **270**, 371–381.

84 L. Zhang, F. Tian, C. Liu, Q. Feng, T. Ma, Z. Zhao, *et al.*, Hand-powered centrifugal microfluidic platform inspired by the spinning top for sample-to-answer diagnostics of nucleic acids, *Lab Chip*, 2018, **18**(4), 610–619.

85 S. Lutz, P. Weber, M. Focke, B. Faltin, J. Hoffmann, C. Mueller, *et al.*, Microfluidic lab-on-a-foil for nucleic acid

analysis based on isothermal recombinase polymerase amplification (RPA), *Lab Chip*, 2010, **10**(7), 887–893.

86 T.-H. Kim, J. Park, C.-J. Kim and Y.-K. Cho, Fully Integrated Lab-on-a-Disc for Nucleic Acid Analysis of Food-Borne Pathogens, *Anal. Chem.*, 2014, **86**(8), 3841–3848.

87 M. Li, A. Ge, M. Liu, B. Ma, C. Ma and C. Shi, A fully integrated hand-powered centrifugal microfluidic platform for ultra- simple and non-instrumental nucleic acid detection, *Talanta*, 2020, **219**, 121221.

88 K. B. Barken, J. A. J. Haagensen and T. Tolker-Nielsen, Advances in nucleic acid-based diagnostics of bacterial infections, *Clin. Chim. Acta*, 2007, **384**(1–2), 1–11.

89 S. Yang and R. E. Rothman, PCR-based diagnostics for infectious diseases: uses, limitations, and future applications in acute-care settings, *Lancet Infect. Dis.*, 2004, **4**(6), 337–348.

90 M. Madou, J. Zoval, G. Jia, H. Kido, J. Kim and N. Kim, Lab on a CD, *Annu. Rev. Biomed. Eng.*, 2006, **8**, 601–628.

91 O. Strohmeier, A. Emperle, G. Roth, D. Mark, R. Zengerle and F. von Stetten, Centrifugal gas-phase transition magnetophoresis (GTM) - a generic method for automation of magnetic bead based assays on the centrifugal microfluidic platform and application to DNA purification, *Lab Chip*, 2013, **13**(1), 146–155.

92 G. Wang, H.-P. Ho, Q. Chen, A. K.-L. Yang, H.-C. Kwok, S.-Y. Wu, *et al.*, A lab-in-a-droplet bioassay strategy for centrifugal microfluidics with density difference pumping, power to disc and bidirectional flow control, *Lab Chip*, 2013, **13**(18), 3698–3706.

93 M. Amasia, M. Cozzens and M. J. Madou, Centrifugal microfluidic platform for rapid PCR amplification using integrated thermoelectric heating and ice-valving, *Sens. Actuators, B*, 2012, **161**(1), 1191–1197.

94 B. H. Park, J. H. Jung, H. Zhang, N. Y. Lee and T. S. Seo, A rotary microsystem for simple, rapid and automatic RNA purification, *Lab Chip*, 2012, **12**(20), 3875–3881.

95 N. Y. Jayanath, N. Loc Thai, V. Thu Thi and T. Lam Dai, Development of a portable electrochemical loop mediated isothermal amplification (LAMP) device for detection of hepatitis B virus, *RSC Adv.*, 2018, **8**(61), 34954–34959.

96 Y. Chen, Y. Mei, X. Zhao and X. Jiang, Reagents-Loaded, Automated Assay that Integrates Recombinase-Aided Amplification and Cas12a Nucleic Acid Detection for a Point-of-Care Test, *Anal. Chem.*, 2020, **92**(21), 14846–14852.

97 C. Shi, F. Shang, M. Zhou, P. Zhang, Y. Wangb and C. Ma, Triggered isothermal PCR by denaturation bubble-mediated strand exchange amplification, *Chem. Commun.*, 2016, **52**(77), 11551–11554.

98 J. Siegrist, R. Gorkin, M. Bastien, G. Stewart, R. Peytavi, H. Kido, *et al.*, Validation of a centrifugal microfluidic sample lysis and homogenization platform for nucleic acid extraction with clinical samples, *Lab Chip*, 2010, **10**(3), 363–371.

99 O. Strohmeier, S. Keil, B. Kanat, P. Patel, M. Niedrig, M. Weidmann, *et al.*, Automated nucleic acid extraction from whole blood, B. subtilis, *E. coli*, and Rift Valley fever virus on a centrifugal microfluidic LabDisk, *RSC Adv.*, 2015, **5**(41), 32144–32150.