Centrifugal microfluidics for biomedical applications

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The centrifugal microfluidic platform has been a focus of academic and industrial research efforts for almost 40 years. Primarily targeting biomedical applications, a range of assays have been adapted on the system; however, the platform has found limited commercial success as a research or clinical tool. Nonetheless, new developments in centrifugal microfluidic technologies have the potential to establish wide-spread utilization of the platform. This paper presents an in-depth review of the centrifugal microfluidic platform, while highlighting recent progress in the field and outlining the potential for future applications. An overview of centrifugal microfluidic technologies is presented, including descriptions of advantages of the platform as a microfluidic handling system and the principles behind centrifugal fluidic manipulation. The paper also discusses a history of significant centrifugal microfluidic platform developments with an explanation of the evolution of the platform as it pertains to academia and industry. Lastly, we review the few centrifugal microfluidic-based sample-to-answer analysis systems shown to date and examine the challenges to be tackled before the centrifugal platform can be more broadly accepted as a new diagnostic platform. In particular, fully integrated, easy to operate, inexpensive and accurate microfluidic tools in the area of *in vitro* nucleic acid diagnostics are discussed.

1.0 Introduction

In the field of microfluidic lab-on-a-chip systems, the centrifugal microfluidic platform has emerged as an advanced technology for biological analysis. "Lab-on-a-CD" systems are the focus of intense research, where complex assays are embedded in fluidic networks on centrifugal microfluidic systems, especially towards the development of *in vitro* diagnostics (IVD). The centrifugal microfluidic platform has the potential to become a standard tool for mainstream diagnostics. Fluidic processing steps such as the mixing of reagents or metering of sample fluids can be automated simply by implementing different spinning profiles. Through the adaption of miniaturization technology, multiple analysis steps can be integrated on a single disc, often towards the development of microfluidic sample-to-answer systems, or micro total analysis systems (μTAS). Moreover, many individual assays can be run simultaneously on the same disc. With the development of optical systems to interface with disc-based assays, centrifugal microfluidic systems allow for operators to not only perform the often complex and timely sample preparation steps required in most assays, but also to rapidly identify biological targets all on the same platform.

In comparison to common chip-based microfluidic systems, centrifugal microfluidic platforms offer many advantages. For fluid propulsion, centrifugal pumping involves a minimal amount of instrumentation, requiring only a simple and compact motor to create the forces needed for fluid manipulation and

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eliminating the need for external syringe pumps. Additionally, centrifugal pumping requires no external interconnects to induce fluid movement. This allows the complete fluidic network (and indeed the inclusive assay) to be contained within a single disc. As centrifugal microfluidics can be mass-produced from inexpensive materials like polycarbonate, they can be manufactured to be disposable in an economical way. Centrifugal pumping also holds advantages over other chip-based pumping, such as electrokinectic methods, because it is not strongly dependent on physicochemical properties of the fluid, such as pH or ionic strength,[1] and does not require large high-voltage power supplies. Additionally, a variety of fluids have been successfully pumped using the microfluidic centrifugal microfluidic platform including aqueous solutions, solvents, surfactants and biological fluids (*e.g.*, blood, mucus, urine, milk).[1]

Beyond pumping of liquids, many other fluidic functions have been successfully integrated on the centrifugal microfluidic platform including valving, decanting, calibration, mixing, metering, sample splitting, and fluid separation. Valving is essential for all microfluidic analysis platforms, as sample fluids and reagents must be properly retained until needed. Multiple types of valves, including passive and active valves, have been developed for the centrifugal microfluidic system and have been successfully implemented. All of these centrifugal microfluidic technologies and functions make the centrifugal microfluidic platform powerful, and insure complete automation to reduce time and error due to handling. In terms of complexity, centrifugal microfluidic technology replaces complex fluidic handling equipment and intricate interconnects. Finally, by reducing experiment size and complexity, costs can be kept to a minimum.[1]

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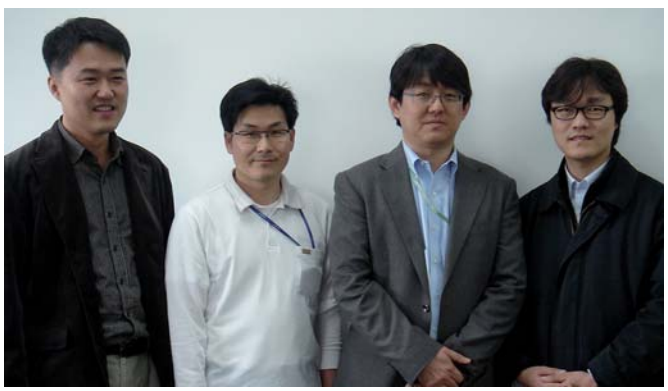

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Although centrifugal microfluidics have many advantages, the simple motor control of fluid handling on a CD has its own inherit limitations. For example, the passive fluid valving operation on a CD strongly depends on the balancing of spin speed and surface tension in most of the conventional centrifugal microfluidic devices utilizing either hydrophobic or capillary valves. But surface tension depends on the surface energy which in the case of a polymer CD is dynamic in nature. That is why recently Park *et al.*[2] explored an active type valving mechanism utilizing laser diodes to melt wax valves avoiding the surface energy changes associated with passive valving. This approach constitutes a solution but it adds other complexities to the realization of the platform (*e.g.*, the need for wax deposition systems and heating actuators). Another example of a technological barrier involves the difficulty in implementing additional forces onto the CD. Say one wants to implement electrical fields on the CD, perhaps to separate cells by dielectrophoresis or to affect osmotic pumping through channels too narrow for centripetal forces to pump. Along this line, Martinez-Duarte *et al.* utilized electric fields on the spinning disc to capture cells by dielectrophoresis.[3] But as before the implementation of this solution renders the platform considerably more complex.

This paper is intended to give a review of the centrifugal microfluidic platform with a focus on biomedical applications, while highlighting the history, recent advances, and potential for future applications. Section 2 gives a historical overview of significant centrifugal microfluidic developments and covers commercial microfluidic centrifugal microfluidic technologies from the biomedical industry. Section 3 describes the principles underlying centrifugal microfluidic functions, such as fluidic transfer and valving that have been adapted for implementation of biological assays. Section 4 discusses many current centrifugal microfluidic research initiatives, and section 5 focuses on centrifugal microfluidic-based sample-to-answer systems. Section 6 outlines some of the challenges still being addressed in the field, particularly in regards to sample-to-answer systems for nucleic acid diagnostics. The final section presents an outlook for the future of centrifugal microfluidics.

2.0 A history of centrifugal microfluidic technology

The field of centrifugal microfluidics began in the late 1960s with the development of the centrifugal analyzer. N. Anderson, from Oak Ridge National Labs (ORNL), developed a clinical chemistry analyzer, which incorporated a rotating disc with a multicuvette assembly, and a stationary optical detector designed for use with a computer.[4,5] The design and operation of the system was simple: the disc was fabricated such that channels and risers (*i.e.* 3D physical barriers) along the radial axis kept samples and reagents separated during fluid loading. During spinning, centrifugal forces drove fluids over the barriers into optical cuvettes positioned on the periphery of the disc. As reactions took place, absorbance changes were monitored with a light source and a photomultiplier tube arranged above and below the cuvettes, respectively. The system was initially used for kinetic assay development.

Work on centrifugal analyzers continued to progress, and miniaturized versions were developed (Fig. 1).[5] Additionally, other optical technologies were incorporated to measure light transmittance, fluorescence, chemiluminescence, and light-scattering properties of several simultaneously initiated reaction mixtures.[5] The implementation of these analysis systems helped to create new clinical laboratory tools for applications in chemistry, toxicology, immunology, and hematology.[5]

By the time Anderson published his landmark paper in 1969,[4] multiple companies were offering prototype versions of the centrifugal analyzer, with the first commercial system introduced by Electro-Nucleonics, Inc. in 1970. In a little over 10 years, the commercial field had grown to five companies that were offering related products: Electro-Nucleonics, Inc.—GEMSAEC and GEMINI, Centri Union Carbide—CentrifiChem, American Instruments—Rotochem, Instrumentation Laboratories, Inc.—Multistat, and Roche—Cobas-Bio.[4]

The next phase in development of centrifugal microfluidics took place in 1989 with the formation of Abaxis, Inc. Abaxis bought the patents from ORNL for their version of the clinical analyzer and began to develop it as a tool for blood analyte analysis.[6] This represented a shift in the utilization of centrifugal microfluidics from a research-oriented tool to a diagnostic platform. In 1995, Abaxis introduced the Piccolo rotor system which integrated sample processing steps required for analyte analysis and incorporated self-contained reagents for each step. The Piccolo system would become the flagship for a range of blood panel products encompassing several areas of medicine.[6]

In 1998, M. Madou and G. Kellogg from Gamera (a US startup company based on centrifugal microfluidic technology) introduced the next generation of centrifugal microfluidics as described in "The LabCD: A centrifuge-based microfluidic platform for diagnostics."[7] The paper outlined basic centrifugal theory of pumping fluids and introduced valving, mixing, sample entry and metering as basic fluidic functions on a centrifugal microfluidic device. Their work represented an expansion of centrifugal technologies into new realms of biological and chemical analysis by introducing microfabrication techniques to create and merge miniaturized fluid networks and microsensors on a single disposable centrifugal microfluidic platform. Madou, Kellogg, and the entire Gamera team were the first to realize that the centrifugal microfluidic platform could be used as an advanced sample-to-answer system.[7] While clinical analyzers had the advantage of being able to perform high-throughput assays in a short time, they often required trained technicians to carry out several operations on different machines. The LabCD offered a unique and attractive platform to overcome limitations of the macro-scale centrifugal systems of that time and opened up the possibility for more advanced tools to be created with applications for drug development through genomics and proteomics, molecular diagnostics, and genetic testing.[7,8] Gamera, later acquired by Tecan in July 2000, would continue to develop the LabCD mainly for assays related to drug development such as ADMET—absorption, distribution, metabolism, excretion and toxicity.[9] Tecan advanced Gamera's system significantly but discontinued their efforts in 2005.

The early 2000s saw a rapid development of academic and commercial endeavors to incorporate new assay designs on a centrifugal microfluidic platform. Kido *et al.* first introduced

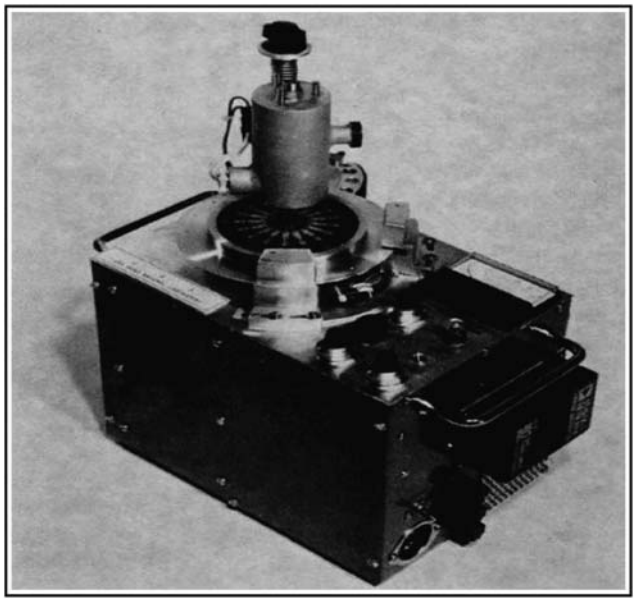

Fig. 1 A miniature fast analyzer platform developed by ORNL with loaded analysis disc; with the disc consisted of two different sides allowing for discrete or metered loading of samples. Figure adapted and reproduced with permission from ref. 5.

disc-based immunoassay microarrays for agriculture and environmental analysis in 2000.[10] In their work, specialized modules were integrated with standard CD drives to set up and analyze protein microarrays.[10] In that same year, Gyros AB was founded and would go on to commercialize a disc-based assay system that automated sandwich immunoassay processing. Their products available on the market are used today as a tool for high-throughput immunoassay analysis.[11] Several other companies would later adapt immunoassay technology on the centrifugal microfluidic platform in various implementations: Quadraspec developed label-free technology for identifying changes in protein arrays,[12,13] while Burstein Technologies[14] and Advanced Array Technology[15] developed array methods on the disc for analyzing nucleic acids (NA).

Although there has been extensive development of centrifugal microfluidic technology, often with collaborations from academia and industry, the market is relatively bare of commercially available products today. While centrifugal microfluidic technology has many advantages as a microfluidic platform, the failure of companies to commercialize products brings to the foreground several barriers to adapting microfluidic platforms for widespread use. Any commercial microfluidic platform needs to bring advantages to the user in terms of cost, throughput, user-friendliness, and accuracy compared to the current gold-standard methods. Even if a microfluidic device with self-contained reagents guarantees a very simple operation protocol and features significantly better accuracy, the cost of both disposable and fixed system plays a key role in determining commercialization success. Even if the newly developed CD system has all of the above-mentioned advantages, it takes time for it to be adapted by the major user groups. With the commercial history of the centrifugal microfluidic platform established, the sections to follow highlight current research advances using centrifugal microfluidic technology.

3.0 Centrifugal microfluidic functions

This section introduces various microfluidic functions that have been implemented on the centrifugal microfluidic systems for biological processing. An explanation of the individual functions serves as an introduction to give the reader insight into how the integration of such systems enables biological processes on a centrifugal microfluidic device. Often, the complex liquid handling involved in biological assays requires the combination of several of the microfluidic functions presented below.

3.1 Centrifugal pumping

In centrifugal pumping, as a disc spins, centrifugal forces induced on sample fluids drive liquids radially outwards from the center toward the edge of the disc. The flow of fluids in a centrifugal platform has been well characterized; centrifugal flow rates depend on the rotational speed, radial location of the fluid reservoirs/channels, channel geometry, and fluidic properties (*e.g.*, viscosity, density, *etc.*) of a sample.[1,16] The average velocity, U, of centrifugally-pumped liquid in a microchannel can be derived from centrifugal theory as:

$$U = \frac{D_h^2 \rho \omega^2 \bar{r} \Delta r}{32 \mu L} \quad (1)$$

where D_h is the hydraulic diameter of the channel (defined as $4A/P$, A is the cross-sectional area and P is the wetted perimeter of the channel), ρ is the density of the liquid, ω is the angular velocity of the disc, $\bar{r}$ is the average distance of the liquid in the channels from the center of the disc, Δr is the radial extent of the fluid, μ the viscosity of the fluid, and L the length of the liquid in the microchannel.[1] The volumetric flow rate, Q, is then defined as $U \cdot A$, where U is from eqn (1). As shown in eqn (1), both the channel geometries and the fluidic properties play a large role in centrifugal pumping, in addition to spin speed.

Madou *et al.* and Duffy *et al.* demonstrated that the flow rates predicted by simple centrifuge theory follow well with experimentally measured flow rates for various kinds of samples including water, plasma, bovine blood, urine, DMSO, and polymerase chain reaction (PCR) products.[1,8] Flow rates ranging from 5 nL s^{-1} to over 0.1 mL s^{-1} have been achieved by various combinations of rotational speed from 400 to 1600 RPM, channel widths from 20 to 500 μm, and channel depths from 16 to 340 μm.[8] The dynamic pumping range of the centrifugal microfluidic platform extends far beyond these limits, as wider channels and higher rotation speeds are easily achieved.[17]

Centrifugal flow rates are relatively insensitive to physicochemical properties such as ionic strength, pH, conductivity, and the presence of various analytes.[8] Thus, the centrifugal microfluidic platform provides a unique pumping mechanism that provides a very large dynamic range in terms of fluidic pumping rates and volumes as well as types/properties of fluids being pumped. This is one of the most important aspects of utilizing the centrifugal microfluidic platform for biological applications, as it allows successful pumping of many different fluids on the same disc.

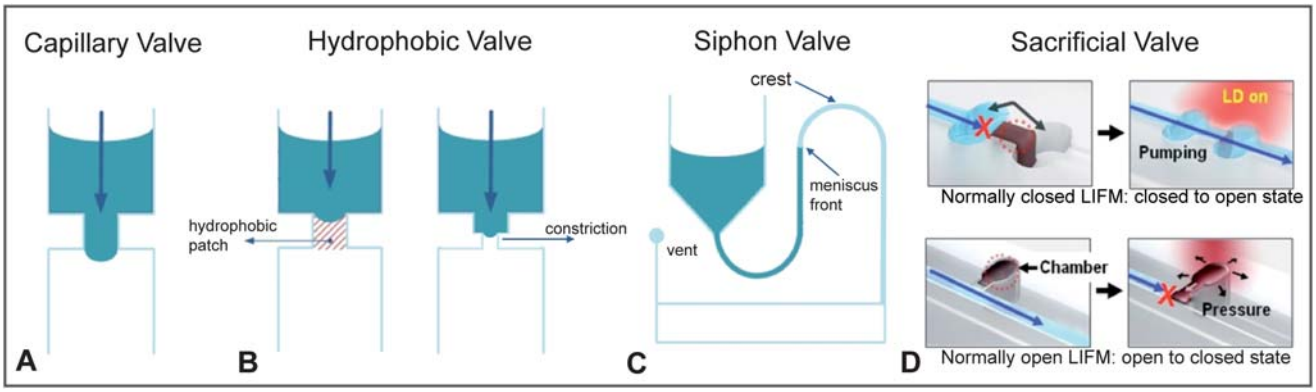

Fig. 2 Centrifugal microfluidics-based valving methods. (A) Capillary valve using a hydrophilic microchannel. Figure adapted and reproduced with permission from ref. 1. (B) Implementation of hydrophobic valving on a disc. Figure adapted and reproduced with permission from ref. 1. (C) Siphon valve. Figure adapted and reproduced with permission from ref. 17. (D) Sacrificial valve made from wax utilizing laser assisted heating for operation. Figure adapted and reproduced with permission from ref. 2.

3.2. Valving

3.2.1 Capillary valves. Valving is essential to centrifugal microfluidics, allowing precise control of fluid movement throughout assay processing. Capillary valves, in particular have been extensively used in many centrifugal microfluidic systems for biomedical applications.[1,16–19] As shown in Fig. 2A, the fundamental principle is based on a balance between capillary pressure (*i.e.*, surface-tension induced pressure, P_s) and centrifugally-induced pressure (P_ω): liquid will not pass through a capillary valve when the centrifugal pressure is less than or equal to the capillary barrier pressure. Capillary pressure increases at the liquid–air interface when the cross section of a hydrophilic capillary expands abruptly.

Duffy *et al.* modeled capillary valving by balancing the pressure induced by centrifugal forces with the capillary pressure P_s.[8] A simplified relation for the critical burst frequency, ω_c (the frequency at which capillary pressure is overcome by centrifugal force and the valve opens) as related to surface tension and geometric parameters is:

$$P_\omega = \rho\omega_c^2\bar{r}\Delta r > a(4\gamma/D_h) + b = P_S \qquad (2)$$

where γ is surface tension, a is a scaling factor for non-spherical droplet shapes, b represents the pressure required to wet the chamber past the capillary valve, and the remaining terms are defined as above.[1] Madou *et al.* have utilized critical burst frequency calculations to achieve sequential operation of multiple, serial capillary valves by simply increasing the spin speed.[19–21] Experimental capillary-valve burst frequencies on a disc have been shown to be in good agreement with calculated burst frequency values.[21]

3.2.2 Hydrophobic valves. Hydrophobic valves, shown in Fig. 2B, rely on either a sudden narrowing in a hydrophobic channel, or functionalized hydrophobic regions in microchannels to impede fluidic movement.[22,23] In both cases, fluid can be forced past these valves when the rotational speed exceeds a certain critical value. Hydrophobic valves have been implemented in several ways for biological assays. For example, Ekstrand *et al.* implemented hydrophobic valves as a way to meter defined liquid volumes and transfer the liquid at high spin speeds, when the centrifugal forces overcome the capillary pressure at the hydrophobic barriers,[22] while Gyros AB uses hydrophobic patches at the entrances of microchannels as a similar method to control the fluidic movement within a centrifugal device.[11]

3.2.3 Siphoning. A disc-based siphon structure, as shown in Fig. 2C, is often used for both liquid valving and transfer.[6,17] Siphoning often relies on the priming of liquid into a siphon channel due to capillary action; therefore the siphon channel surface must be hydrophilic. When the disc is spinning at high speeds, centrifugal forces keep the meniscus front below the crest level of the siphon. When the rotating speed is reduced below a critical value, the channel is primed, and the siphon can transfer the liquid as soon as the meniscus passes the crest point. The liquid flow stops as soon as the liquid level is hydrostatically balanced or there is a discontinuity in the liquid column. Siphon valving in centrifugal microfluidics provides a valving solution for the applications that require higher spin speeds as the first step of the operation (*e.g.*, plasma separation from whole blood).

3.2.4 Sacrificial valves. The passive valves discussed above are controlled simply by changing the rotational speed of the disc, and require no additional moving parts, only deliberate channel geometry designs or surface modifications. However, the passive centrifugal liquid valves are not vapor-tight; there remains a need for vapor-tight valves to prevent vapor migration or moisture absorption if, for example, reagents are to be stored on the disc or high temperature heating steps are required. In addition, the rotational speed must be controlled from slow to fast for the sequential operation of the valves, not *vice versa*.

Recently, Park *et al.* reported a sacrificial valve that is controlled by laser irradiation.[2] As shown in Fig. 2D, both normally-closed laser irradiated ferrowax microvalves (LIFM) and normally-open LIFM have been demonstrated. These sacrificial valves are composed of iron oxide nanoparticles dispersed in paraffin wax. Upon excitation with a laser diode (LD in Fig. 2D), iron oxide nanoparticles within the wax act as integrated nanoheaters, causing the wax to quickly melt at relatively low intensities of laser diode excitation. The valve

operation is independent of the spin speed or the location of the valves and therefore allows for more complex biological assays integrated on the disc.[24]

3.3 Volume metering

Liquid volume metering is an essential function in centrifugal fluidics to achieve proper reagent volumes for diagnostic assays and to ensure reproducible valving processes on a disc. Volume metering on a disc is primarily achieved through the simple use of an overflow channel connected to a fluidic chamber.[2,11] Once the chamber has filled to the radial level of the overflow channel, any additional fluid is routed to a waste chamber.

Steigert *et al.* discuss the significance of "wicking" that occurs along the edges of the disc-based metering chambers, and introduce design principles to reduce its negative effect on metering variation. As part of an integrated colorimetric assay, they reported the metering of 300 nL with a 5% coefficient of variability.[25]

3.4 Mixing

Mixing is necessary in biomedical diagnostic applications in order to homogenize samples and to combine various reagents for downstream analysis. However, mixing is difficult to achieve in microfluidic platforms due to constraints of the microscale domain, namely low Reynolds numbers with laminar flow. This means that there is no convective mixing; fluidic mixing is limited to diffusive mixing which can be a very slow process.[26–29]

In order to overcome this challenge, a number of approaches to achieve rapid mixing have been demonstrated on centrifugal microfluidic platforms.[17,24,30–38] One method uses rapid oscillations of the disc to achieve rapid mixing in low-Reynolds number regimes (*viz.*, oscillations between clockwise and counter clockwise rotation).[32,34,35] The flow inertia and counteracting viscous damping improves rapid mixing in larger-scale fluidic chambers. Additionally, by introducing paramagnetic particles in the liquid and by positioning permanent magnets aligned in non-symmetrical positions underneath the mixing chamber, a magnetic stirring effect can further decrease mixing time.[30,32,39] Ducree *et al.* have shown that the Coriolis pseudo-force is efficient for continuous-flow mixing in "macroscopic" fluid chambers in centrifugal systems at high-speed flow conditions.[36]

3.5 Flow switching

Complex biological assays often require various analytes to be directed to different chambers and/or channels on the disc. A common method for flow switching in a centrifugal device is to utilize the Coriolis force within a Y-shaped structure (a single inlet channel splits into two symmetric outlets).[17] The equation for the Coriolis force is: $F_{coriolis} = 2\rho\omega\vec{v}$ where $\vec{v}$ is the velocity of flow in the rotating disc, and the remaining terms defined above. At lower spin frequencies, ω, the Coriolis force is negligible compared to centrifugal forces and the liquid is evenly distributed between the two outlet channels. However, at higher frequencies, the Coriolis force is large and the flow can be directed to either of the outlet channels, depending upon the direction of the disc rotation.

In addition, flow switching can also be achieved by exploiting surface property differences of the channel structures on the disc. In a centrifugal device developed by Gyros AB, hydrophobic patches at the channels entrances are utilized to direct flow to downstream chambers. Depending on the centrifugal force, the fluid either breaks past the capillary valve, or continues past it and flows into the second hydrophilic channel.[11]

4.0 Current research advances in centrifugal microfluidics technology

With a firm foundation in centrifugal microfluidic principles presented, the following section provides a review of prominent disc-based applications, including various sample preparation techniques, and analysis and detection methods. Other applications such as cell-based assays and organism culturing are also presented.

4.1 Sample preparation

4.1.1 Whole blood processing. The first step in many clinical diagnostic analyses is the separation of red blood cells (RBCs) from blood plasma, as certain cellular components of blood can inhibit NA amplification and interfere with absorbance measurements. Centrifugal microfluidics offer a simple approach to this problem by automating the classical technique of RBC sedimentation through centrifugation. By exploiting the density differences between the RBCs and plasma, centrifugal microfluidic devices are better suited for separation of plasma from whole blood as compared to microfluidic chips driven by pressure, acoustic, or electrokinetic pumping.

Plasma separation from whole blood using a centrifugal microfluidic device has been demonstrated by several research groups.[6,25,40–47] Schembri *et al.*, for example, demonstrated a multiplexed centrifugal microfluidic device capable of processing a 90 μL whole blood sample by separating and then diluting the plasma into 12 separate testing chambers. Additionally, Haeberle *et al.* developed a device to extract 2 μL of plasma from 5 μL of a whole blood sample (Fig. 3). They demonstrated a separation time of about 20 s at a moderate spinning frequency of 40 Hz (2400 RPM). The same group also integrated measurement of the concentration of hemoglobin (Hb)[45] or hematocrit levels[47] in human whole blood samples with plasma separation.

4.1.2 Sample lysis and homogenization. Virtually all molecular diagnostic assays require a step of cell/viral lysis in order to release genomic and proteomic material for downstream processing. Typical lysis methods can be classified into two main groups: chemical/biological methods and physical methods.[52]

Chemical/biological lysis methods use chemical detergents or enzymes to break down membranes. They are the simplest to implement in terms of hardware requirements, but can leave behind residues that may inhibit amplification processes (*e.g.*, PCR). Additionally, chemical lysis efficiency is not sufficient, especially for Gram-positive bacteria and tissues, which are known to be difficult to lyse.[52] Physical lysis methods, which include manual grinding, freeze/thaw cycles, sonication, and mechanical disruption, among others, require additional

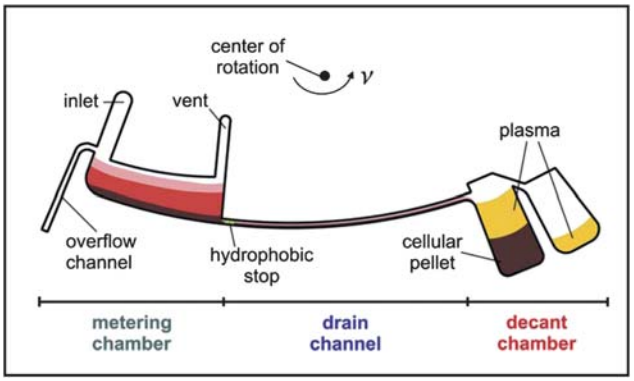

Fig. 3 The fluidic layout for continuous plasma separation from 5 μL of whole blood developed by Haeberle *et al.* The separation time is about 20 s at moderate spinning frequencies of 40 Hz. Figure adapted and reproduced with permission from ref. 40.

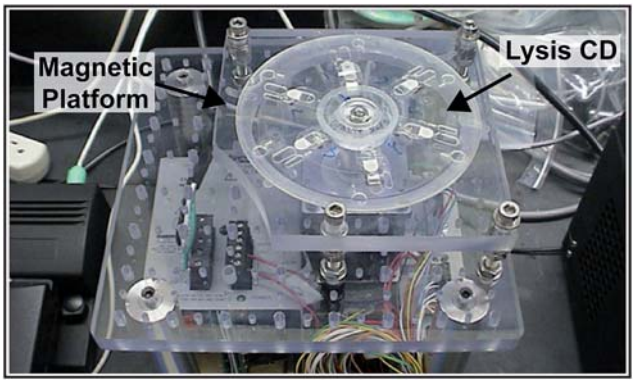

Fig. 4 Picture of the magnetic assisted cell lysis system developed by Kido *et al.* Figure adapted and reproduced with permission from ref. 30.

instrumentation. However, such systems leave behind little or no residues, and are often faster and more efficient than chemical/biological techniques.[52] Indeed, mechanical lysis is the most effective method for breaking down cells that have thick cell walls, such as Gram-positive microbes, and for successfully extracting intact DNA.[53,54] The flexibility and advantages of the centrifugal microfluidic platform make it attractive for implementing different types of lysis techniques. Several methods of disc-based cell lysis have been developed as summarized in Table 1.

Early implementations of lysis on a centrifugal microfluidic device relied on chemical lysis where alkaline buffers were introduced that decomposed the cell walls to release DNA. Kellogg *et al.* employed heating with thermoelectric devices to assist in chemical lysis and to denature proteins inhibitory to PCR reactions.[48] Klepárnik *et al.* utilized an applied voltage to generate hydroxyl ions at an anode to create a high enough alkali concentration to drive chemical lysis.[49]

Mechanical lysis has been performed using bead beating systems on a disc. Kim *et al.* combined cells and beads in a lysis chamber and used the forces created during centrifugation to break apart the cell membranes.[50] Lysis occurred due to collisions and shearing between the beads and the cells and through friction shearing along the lysis chamber walls. Additionally, by alternating the spin direction of the disc, further impaction and collisions occurred due to Coriolis effects in the lysis chambers. This was the first microfluidic lysis system where the lysis mechanism was fully dependent on centrifugally-induced forces.

Kido *et al.* introduced magnetic actuation to assist bead beating lysis (Fig. 4). In their system, a stationary platform embedded with permanent magnets was added below the spinning CD. Additional magnetic blades were placed inside the CD lysis chambers along with grinding-bead media. As the disc was spun, the magnetic blades were actuated due to the changing

Table 1 Examples of sample lysis implemented using centrifugal microfluidic platforms

Reference	Lysis method	Lysis Time	Samples tested (volume)	Comments
	Chemical			
Kellogg *et al.*[48]	• Alkaline lysis/heat	• N/A	• *E. coli* (5 μL)	Fully integrated cell lysis and PCR.
Klepárnik *et al.*[49]	• Alkaline lysis	• 3–5 min	• Single cardiomyocyte cell (2 μL)	Single-cell lysis followed by electrophoresis on a disc.
	Mechanical			
Kim *et al.*[50]	• Bead milling (collisions and shearing)	• 5–7 min	• CHO-K1, *E. coli*, Yeast (300–550 μL)	65% lysis efficiency as compared to conventional lysis methods.
Kido *et al.*[30]	• Magnetic assisted bead beating (impaction)	• 8 min	• *E. coli*, Yeast (70 μL)	Clarification step included.
Siegrist *et al.*[51]	• Advanced magnetic assisted bead milling (collisions/shearing/impaction)	• 6 min	• *Bacillus subtilis* spores and clinical samples (nasopharyngeal aspirates) for respiratory virus	Designed for sample distribution and integration
	Laser			
Cho *et al.*[24]	• Localized heat shock and mechanical shock due to collision	• 30 s	• Whole blood spiked with Hepatitis B virus (100 μL)	Fully integrated plasma separation, virus separation, purification, concentration, and DNA extraction

magnetic field, which caused greater impaction than suspended beads alone. The system also performed clarification, where lysis by-products were centrifuged in a chamber, and the DNA-laden supernatant transferred from the waste through siphoning.[30] Both systems were tested with yeast to demonstrate their viability for tough to lyse cells.

Recently, Siegrist *et al.* created an advanced magnetic assisted lysis platform that incorporated the lysing mechanisms of both previous CD based bead beating systems.[51] In that work, the magnetic lysis chamber was redesigned to work on a fuller sector of the CD. This allowed for bead collision and friction shearing lysis events to occur (as in Kim *et al.*[50]), while at the same time incorporating magnetic actuation to increase the force of the lysis events. The lysis system was validated using *Bacillus subtilis* spores and clinical samples (nasopharyngeal aspirates) for respiratory virus detection.

Cho *et al.* introduced laser-induced lysis in their integrated centrifugal platform for DNA extraction from pathogens in whole blood.[24] To test the system, plasma was separated from a whole blood sample spiked with a target virus. The virus-loaded plasma was then mixed with magnetic beads pre-modified with antibodies specific to the target. The magnetic beads captured the virus and were concentrated in a small chamber on the disc. An laser was then used to induce lysis. Lysis is thought to occur from both localized heat shock and mechanical shock due to collisions from the heated beads and the cells. The lysis efficiency was found comparable to that of the conventional bench-top methods based upon chemical lysis.[24] This was the first demonstration of fully integrated system for virus DNA extraction starting from whole blood.

4.2 Nucleic acid amplification (PCR)

When performing NA analysis, amplification of the target material is often performed using enzymatic-based amplification assays that require heating or thermocycling of the reaction volume, with the most widely adopted form being the polymerase chain reaction (PCR). PCR thermocycling often poses a challenge to microfluidic devices, as the 95 °C upper cycle temperatures required can cause sample loss, evaporation, and/or leakage.

Kellogg *et al.* demonstrated disc-based PCR that included sample preparation functions for analysis of *E. coli*.[48] Thermocycling was achieved *via* direct contact with a spinning platform housing a Peltier thermoelectric device for thermocycling and a thermistor for temperature measurement/control. Fast thermocycling (± 2 °C s^{-1}) of a standard 25 μL PCR reaction volume was achieved. Sample loss was avoided as follows: as the sample evaporated during thermocycling (and spinning), it condensed upstream of the PCR chamber, and was subsequently recuperated by spinning the condensed material back into the PCR chamber. After this PCR step, detection was performed off-CD.

TECAN reported that their LabCD-PCR Plus platform could process 16 simultaneous DNA extraction and amplification reactions.[59] The entire process was completed within 30 min, starting with 2–10 μL samples.

Another centrifugal PCR system has been reported by Mårtensson *et al.* that utilizes spinning to induce Coriolis-based mixing during PCR, and to facilitate both fluidic and thermal homogeneity of the sample.[61] Heating was performed using infrared radiation and, in a fashion similar to the one in the commonly used centrifuge-based RotorGene® qPCR bench-top instrument, cooling was facilitated by spinning the disc as air was pumped in the system.[62]

Recently Sundberg *et al.* reported on a spinning disc based system to perform digital PCR, a technique that is used to quantify original levels of genomic material.[63] In digital PCR, a sample is diluted and distributed into small chambers so that each vessel has either one or no DNA complexes present. After distribution, PCR is performed, and dyes are added that bind to double-stranded DNA. A reader can then analyze each chamber to see the results of the PCR reactions. By counting the chambers that have positive results, an operator can infer the number of genomic templates from the original sample. Sundberg *et al.* used a disc with 1000 nano-liter-sized wells in which the sample was compartmentalized through centrifugation. A rapid air thermocycler created the temperature conditions needed for PCR and CCD cameras where adapted for fluorescence detection.[63]

4.3 Analyte detection

4.3.1 Immunoassays on centrifugal microfluidic device. Immunoassays have become standard tools in proteomics used to evaluate drug targets, for identification of biomarkers, to aid in clinical diagnostics, and to give insights into disease biology. The tests rely on the specific detection of either the antibody or antigen, and are commonly performed by labeling the antibody/antigen of interest through various means such as fluorescent or enzymatic labels, colloidal gold, radioisotopes, or magnetic labels.

While immunoassays have been successfully used for detection and quantification of proteins, the tests can be labor intensive and time consuming. Often, a series of mixing, incubation and washing steps are required that can take hours to days to complete. Long incubation times are a bottleneck for the process and the tedious and lengthy protocols often result in errors and inconsistent results.[19]

Integration of immunoassays into microfluidic networks allows for more precise control of fluids throughout the assay process while also reducing reagent consumption. Additionally, the small chamber and channel dimensions reduce incubation times by facilitating improved mass-transport due to shorter diffusion lengths.[19] Microfabrication has been utilized to create new detection means for immunoassays where capture antibodies have been immobilized on 2D microarrays or concentrated on 3D bead based systems, improving target capture rates and incorporating multiple tests in a single device. Disc-based immunoassays have been a focus of many academic and commercial efforts because of the attractiveness of the miniaturization and multiplexing advantages of the centrifugal microfluidic platform, along with the ability to integrate optical detection hardware. Table 2 shows recent examples of the immunoassays demonstrated on a centrifugal platform.

The first immunoassays developed for the centrifugal microfluidic platform were enzyme-linked immunosorbent assays/enzyme immunoassays (ELISA/EIA), where enzymes were used to produce a detectable signal from an antibody–antigen complex. Several versions of ELISA on the disc have been

Table 2 Examples of immunoassays implemented using centrifugal microfluidic platforms

Reference	Integrated with Sample Prep?	Target	Sample Type/Volume	Assay Time	Limit-of-Detection	Detection Method		Binding Surface
Lai *et al.*[19]	Yes	• Rat IgG	• 10 μL	• <1 h	• 5 mg L^{-1}	• Enzymatic	*2D*	• PMMA
Zhao *et al.*[12]	No	• Rat IgG • Mouse IgG	• N/A	• N/A	• 100 ng L^{-1}	• Label-Free		• Ta_2O_5/SiO_2
Morais *et al.*[55]	No	• Chlorpyriphos • Metolachlor • 2,4,5-TP	• N/A	• 60 min	• 0.02–0.62 μg L^{-1}	• Enzymatic		• Polycarbonate
Honda *et al.*[56]	Yes	• AFP • IL-6 • CEA	• 200 nL	• 50 min for 104 parallel reactions	• 0.15 pmol L^{-1} • 1.25 pmol L^{-1} • 1.31 pmol L^{-1}	• Fluorophore	*3D*	• Bead columns: polystyrene, silica, sepharose
Riegger *et al.*[57]	Yes	• Hepatitis A • Tetanus	• Human serum • 20 μL	• N/A	• 215 mIU mL^{-1} • 158 mIU mL^{-1}	• Fluorophore		• Polystyrene beads
Nagai *et al.*[58]	No	• Secretory IgA	• 1 μL	• 30 min	• N/A	• Enzymatic		• Glass beads
Lee *et al.*[42]	Yes	• HBsAg • Anti-HBs	• Human blood • 150 μL	• 30 min	• 0.51 ng mL^{-1} • 8.6 mIU mL^{-1}	• Enzymatic		• Polystyrene beads

developed using both 2D and 3D reaction substrates (*i.e.*, by functionalizing the disc-surface or using functionalized bead beds, respectively).

Lai *et al.* demonstrated a fully integrated multiplexed ELISA for rat IgG from a hybridoma cell culture, running 24 simultaneous assays at once (Fig. 5A).[19] The centrifugal microfluidic design fabricated in PMMA included nine sets of chambers which held the various reagents and buffers and a series of passive valves which released the fluids at increasing spin speeds. All reactions took place in a detection chamber which was analyzed with a fluorescence microscope.[19] Morais *et al.* developed an ELISA for atrazine on a polycarbonate CD surface, where absorption of the immuno-reagents took place on the CD surface itself (Fig. 5B).[55] In their work, the various reagents where spotted directly on the disc surface; there were no microchannels or chambers and all fluid handling was done sequentially by an operator. Furthermore, quantitative detection was accomplished by using the standard CD reader laser beam ($\lambda =$ 780 nm) and a planar type photodiode integrated in the CD drive to measure the transmittance through the surface of the disc.[55]

Nagai *et al.* created a competitive ELISA on a disc-type device for measurement of biomarkers for mental stress.[58] The simple system made out of PDMS included a loading chamber connected to a purification chamber and a detection chamber. The sample with targeted antigen was first mixed with enzyme-labeled antibodies in the loading chamber. The solution was then passed through over a single glass bead functionalized with the antigen for purification. The purified solution was finally passed into the detection chamber, which was pre-loaded with substrate for the enzyme. The resulting reaction was measured using a fluorescent imaging scanner.[58]

Lee *et al.* from Samsung reported on a fully integrated ELISA on a disc for biomarkers of the Hepatitis B virus (HBV) starting from whole blood.[42] The centrifugal microfluidic system utilized the LIFM valves described previously to hold reagents and transfer fluids. After isolating HBV antibodies in plasma from whole blood, the sample was passed into a reaction chamber that contained a bed of functionalized polystyrene beads functionalized with capture antigens and separate antigens labeled with enzyme. The bead bed served to improve capture efficiency, increasing surface area and improving mass transport efficiency. The solution was mixed under rotation, then subsequent washing steps were performed and the substrate was added. Finally, the fluid was moved to a detection chamber and absorbance measurements were taken by a custom built detection unit (Fig. 5C).

In addition to typical ELISA assays, fluorescent immunoassays (FIA) have been introduced on a centrifugal microfluidic device. Riegger *et al.* investigated color-multiplexed fluorescence immunoassays by using sets of beads modified with different quantum dots or dyes functionalized with specific capture proteins.[57] The beads were aggregated in a detection chamber, and the sample, with fluorescently-labeled detection antibodies, was passed over the bead bed and analyzed for fluorescence intensity; each modified bead and complex produced a unique signal.[57]

Honda *et al.* also demonstrated fluorescent immunoassays on a multiplexed CD developed by Gyros as shown in Fig. 5D.[56] Gyros AB introduced their GyroLab workstation and Bioaffy

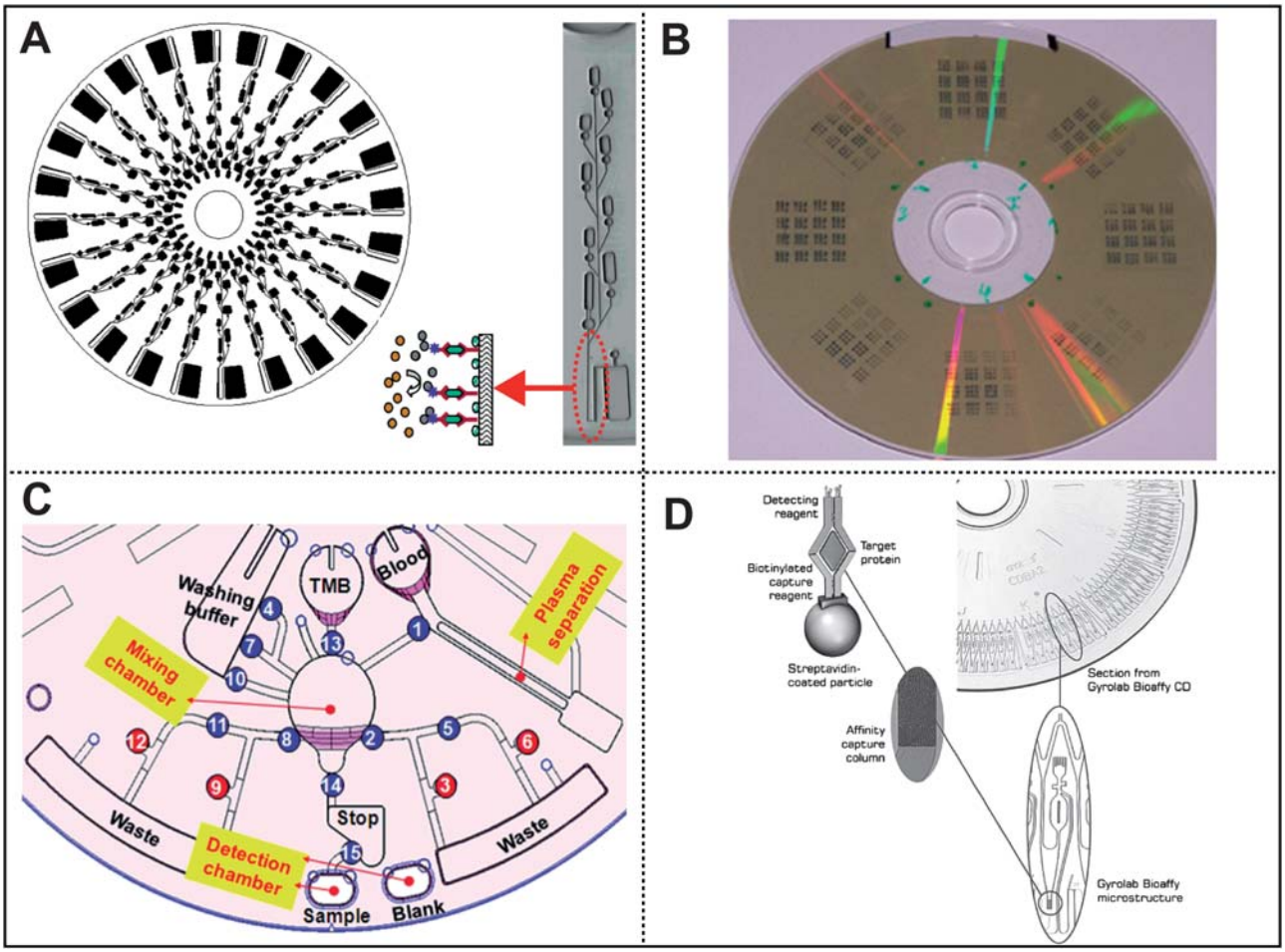

Fig. 5 (A) A fully integrated multiplexed immunoassay with 24 experiments on a centrifugal microfluidic platform. Figure adapted and reproduced with permission from ref. 19. (B) Protein microarray spotted on standard CD substrate. Figure adapted and reproduced with permission from ref. 60. (C) An integrated ELISA on a disc from Samsung. Figure adapted and reproduced with permission from ref. 42. (D) A schematic diagram to show the reaction principle of the Gyros immunoassay platform using pre-packed column. Figure adapted and reproduced with permission from ref. 11, 56.

CD, as a commercial two part platform to automate sandwich immunoassay processing.[11] The GyroLab workstation handles all the operator steps from sample preparation to detection; it includes a robotic station for liquid transfer from microtiter plate to CD, a CD spinner which performs the assay, and a detector for laser induced fluorescence. The Bioaffy disc automates the capture of proteins and antigens for the assay. Utilizing a multiplexed design with bead beds embedded in the disc many different antigens can be tested on a single disc. Gyros currently offers several versions of their CD for the detection of low and high concentrations of analytes. Their most miniaturized discs, the Bioaffy 20 HC and Bioaffy 200, have 112 microstructures for multiplexed analysis, with each experiment processing just several hundred nanolitres of sample in under an hour.

In addition to traditional immunoassays with specific labels, new players have investigated "label-free" immunoassays on-a-CD. Recently, Zhao *et al.* demonstrated spinning disk interferometry, which uses changes in the refractive index of beams of light to identify the presence of bound proteins. In short, two beams of light are directed at a CD substrate with one beam striking a surface with a protein layer and another beam striking a surface without a protein layer. As the change in refractive index is directly proportional to the amount of mass on the surface, one can measure the presence of bound protein. The system's sample preparation steps are carried out on a separate platform using robotic fluidic handling. The disc is then moved onto optical detection platform for analysis. Quadraspec, a new startup company, is working to commercialize this technology.[13] A review of their work about interferometry on disc based systems has been reported recently.[64]

It should be noted that other detection methods offer potential for protein identification on a CD. Recently Magalhães *et al.* used surface plasmon resonance (SPR) based biosensors to quantify the amount of the C-reactive protein in human blood serum.[64] This technology is being adapted for detection of a range of blood panels in centrifugal microfluidic platform in a startup company called Biosurfit.[65]

4.3.2 DNA microarray hybridization. Nucleic acid microarrays, and DNA microarrays in particular, have become important tools for genetic analysis, gene expression profiling, and diagnostics. In the latter application, microarrays allow for higher-order multiplexed scanning and detection of NA targets, beyond the typical 4–6 target multiplexing limit when using

common real-time PCR assays. Many advantages can be obtained by moving the fluidics of a DNA microarray hybridization assay into the micro-domain, and further speed-of-hybridization advantages are gained when flow is involved. Centrifugal microfluidic platforms have been utilized in various ways for microarray applications.

As mentioned previously, two early-stage companies explored DNA microarray applications using centrifugal microfluidic devices: Burstein Technologies and Advanced Array Technology (AAT). Burstein Technologies in 2002 developed a modified CD drive as a laser-scanning microscope for detection of DNA microarray hybridization using their BioCompact Disc platform.[14] Biotinylated DNA probes created using off-CD sample preparation and PCR steps were introduced into a CD array chamber containing immobilized DNA capture probes. Streptavidin-labeled microspheres were then added to label hybridized array spots, and hybridization verified by scanning the CD-surface microarray with the CD-drive laser and analyzing the reflected light.[14]

AAT created its own Bio-CD platform for analysis of DNA microarrays based on a "hybrid" disc: the inner diameter was used as a normal data CD to store numerical and operational control information, and a second scanning laser system was used to image the DNA microarrays on an outer-radius CD.[15] Like in the Burstein system, biotinylated DNA probes were introduced to label hybridized probes. The AAT Bio-CD system, however, added streptavidin-labeled colloidal gold particles followed by a treatment of Silver Blue Solution to aid in detection. The solution causes silver metal to deposit on the gold particles, making the hybridization-positive microarray spots refract incident laser light.[12] As with Burnstein, ATT never commercialized its centrifugal microfluidic technology, but AAT was acquired by Eppendorf in 1999.

More recently, Peytavi *et al.* used a centrifugal platform to increase the fluorescent hybridization signal and reduce the total hybridization time as compared to common, passive hybridization arrays Fig. 6A.[66] Microfluidic polydimethylsiloxane (PDMS) units were aligned and passively bound to glass slides equipped with pre-spotted DNA capture probes; the slide units with the attached PDMS fluidic structures were then mounted onto a centrifugal holder disc. Serial capillary valves in the PDMS units were used to control the subsequent release of sample, wash, and rinse by increasing the disc spin speed. A ~10-fold increase in hybridization signal was obtained as compared to a passive hybridization assay, and the entire system ran in 15 min at room temperature, as compared to hours for the passive assay.

Chen *et al.* used an intersecting, radial-spiral channel approach to perform both printing and hybridization of DNA microarrays in a centrifugal microfluidic device.[67–69] The device consisted of a PDMS disc with channels sealed to a matching glass disc. As shown in Fig. 6B, a dense set of hybridization assays (384 × 384) were demonstrated on such a centrifugal microfluidic platform.[67] The use of a spiral channel design allows for intersecting fluidic-communication between many channels while still allowing centrifugal pumping from the disc-center towards the rim of the disc, thus overcoming limitations of radially-oriented channels. The 384 capture probe lines (96 different probes with 4 replicates) were first printed utilizing the centrifugal microfluidic platform, and then 384 sample solutions (96 different samples in 4 replicates) were introduced for hybridization *via* intersection with the printed capture probe lines. Hybridization of both oligonucleotides (30 and 50 mer) and PCR products (~260 bp) was accomplished within minutes.[69]

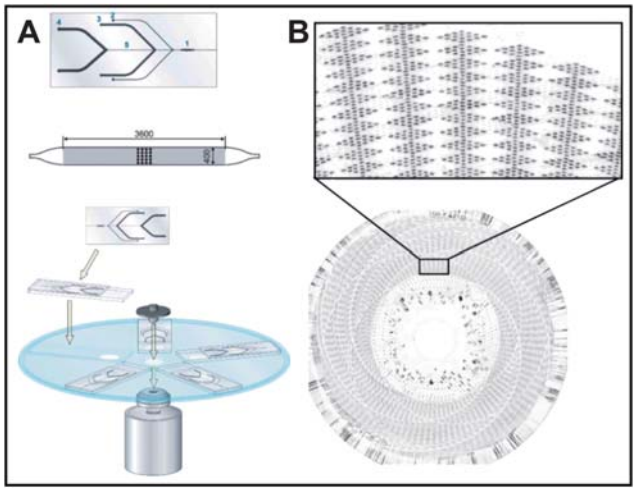

Fig. 6 Centrifugal microfluidic systems for DNA microarray applications. (A) A disc-based microfluidic platform for rapid microarray hybridization, showing the PDMS units on glass slides and mounted on a slide holder. Figure adapted and reproduced with permission from ref. 66. (B) A DNA microarray platform for capture probe printing and hybridization assays, created in a double-spiral channel format on a centrifugal microfluidic device. Figure adapted and reproduced with permission from ref. 67.

As in disc-based immunoassay microarray platforms,[10,55,60,70] there have been approaches involving standard audio CDs as the substrate for DNA microarray hybridization assays.[71] Various methods to chemically modify the polycarbonate surface of audio CDs have been to print and immobilize of DNA capture probes for hybridization assays. In particular, Bañuls *et al.* demonstrated single nucleotide polymorphism (SNP) detection using a microarray hybridization assay with capture probes spotted on the PC surface of a conventional CD.[71] A conventional CD-player and laser were used for CD spinning and fluorescence excitation;[71] additional photosensor hardware was added to allow fluorescence detection.

4.3.3 Colormetric detection of biochemical markers. Colorimetric detection has been used in clinical chemistry applications to detect biochemical markers such as calcium, creatinine, glucose, and electrolytes, in addition to various blood protein panels. The adaptation of colorimetric detection to the centrifugal platform presents both advantages and challenges. An advantage is that the centrifugal microfluidic platform is well suited to automated, multiplexed mixing of the reagents necessary for colorimetric assays. A challenge is that the centrifugal platform, with thin, flat devices, must overcome the shortened path length inherent to all miniaturized systems. Since absorbance measurements are linearly proportional to optical path length (Beer's law), decreasing the path length limits the sensitivity of the detection system.

The Abaxis Piccolo system analyzes concentrations of target analytes such as cholesterol or glucose using a portable system

equipped with absorbance measurement hardware.[6,41] Ducree *et al.* have demonstrated fully-integrated colorimetric assays for the determination of alcohol[25] or glucose[43] concentration in whole blood. In contrast to the perpendicular style detection cuvettes on the centrifugal platform developed by Abaxis, the absorbance was measured in this case using a horizontal type detection chamber in which the absorbance path length is 10 times longer, and thus the colorimetric detection sensitivity was enhanced. The total process from whole blood sample input to alcohol detection can be completed in less than 3 min.

4.4 Other applications

Beyond molecular-scale applications based on centrifugal microfluidic platforms, cell and organism-based applications have been demonstrated as well. Cell-based assays (*viz.*, cell viability assays) have been carried out for drug discovery applications using a disc system to reduce the often labor-intensive operations required for both cell culture and screening.[72] A complete bacterial viability assay, based on an off-the-shelf assay kit (LIVE/DEAD BacLight Bacterial Viability Kit from Molecular Probes, Inc.) was automated using a centrifugal microfluidic device to study G-force effects on cells.[1]

Martinez-Duarte *et al.* reported on the capture and separation of yeast cells from latex beads using dielectrophoresis (DEP) on a centrifugal microfluidic device.[3,73] Their setup utilizes self-contained 3D carbon electrodes integrated on the centrifugal microfluidic platform with connections to a stationary power source (Fig. 7A). The work illustrates not only how to combine electrical and centrifugal forces on a single platform but also illuminates the potential for the centrifugal microfluidic platform to aid in DEP studies.

Finally, Kim *et al.* went beyond cell culturing and reported a centrifugal microfluidic system for the culturing of *C. elegans* roundworms and for gene expression studies of these worms when exposed to hypergravity (Fig. 7B).[31,74] This system consisted of a PDMS disc with integrated growth, waste, and feeding chambers. Using centrifugally controlled fluidics, the disc automated feeding and waste removal methods to assist in extended studies involving altered gravity.

The cell and organism-based applications of centrifugal microfluidic platforms are interesting, yet remain a relatively unexplored focus of centrifugal microfluidic devices. Such applications hold much promise as research tools, especially in the pharmaceutical field. As cell-based research areas, such as tissue engineering and control of stem cell differentiation, continue to grow, the centrifugal microfluidic platform will find many more applications in these areas.

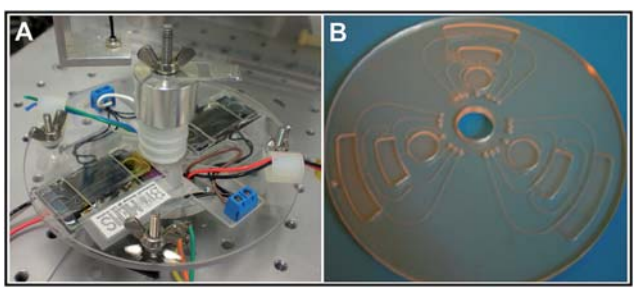

Fig. 7 (A) The spinning DEP setup; DEP chips are embedded in the disc for analysis. Figure adapted and reproduced with permission from ref. 3. (B) The culturing platform for *C. elegans*; the innermost chamber holds nutrients, the middle serves as the culturing chamber, and the chamber near the edge is the waste reservoir. Figure adapted and reproduced with permission from ref. 31.

5. Integrated sample-to-answer analysis systems

While many individual biological analysis steps, functions, and systems have been demonstrated using centrifugal microfluidic platforms, there are relatively few examples of integrated sample-to-answer systems (whether on the market or under development). Many working definitions of sample-to-answer systems are in use today, but the authors strictly define it here as an integrated system (disposable disc + permanent spin-stand hardware) capable of accepting a non-processed biological sample/specimen (*e.g.*, blood, saliva, urine, *etc.*) and outputting information useful for medical diagnostic/monitoring purposes. The few centrifugal microfluidic sample-to-answer examples available are reviewed in this section.

The Piccolo clinical blood analyzer system from Abaxis, discussed above, was one of the first examples of a disc-based sample-to-answer system, and performs panel testing directly on whole blood samples (Fig. 8A).[41] Currently, Abaxis sells Piccolo discs for both medical and veterinary diagnostics, including tests such as analyte panels for cholesterol, metabolites and electrolytes, as well as panels for renal, kidney, and liver function.[6]

The Abaxis disc disposable consists of several injection-molded plastics parts, made of polymethylmethacrylate (PMMA) and ABS (acrylonitrile, butadiene, and styrene). During manufacturing, lyophilized beads with the appropriate colorimetric reagents in addition to liquid diluents are added on the disc, and the plastic parts are ultrasonically welded afterwards.[41] Operation of the Piccolo system is described here, but a more detailed account can be found in the literature.[41] In a first step, the operator adds several drops of capillary or venous whole blood (~100 μL) to the disc, and the disc is then placed on the hardware spin-stand system. During mounting and capping of the disc, a specialized pouch is pierced and diluents are released into the disc system. Next, the appropriate program parameters are chosen, and the operator initiates the system.[41] These are the only manual steps required of the operator, save for removal and disposal of the used disc post-analysis.

After the operator initiates the system, sample processing begins by varying the spin-speed and spin-direction of the disc. First, the disc is spun at a high-speed which meters the diluents in metering chambers. Simultaneously, the blood sample is metered in separate chambers, and the high-speed centrifugation causes plasma to be separated from the blood cells. The spin-speed is then reduced, which draws fluids (both the sample plasma and diluent aliquots) into mixing chambers through siphoning. The spin-speed is then varied to mix the plasma and diluents. The spinning is slowed again, allowing a final siphon to pull fluid from the mixing chambers to distribution channels, which fill a series of mini-cuvettes; each cuvette contains the lyophilized reagents specific to the panel being run. When the diluted plasma enters the cuvette, the reagents reconstitute, and the disc is spun in a mixing pattern to homogenize the fluid. Finally, an off-disc

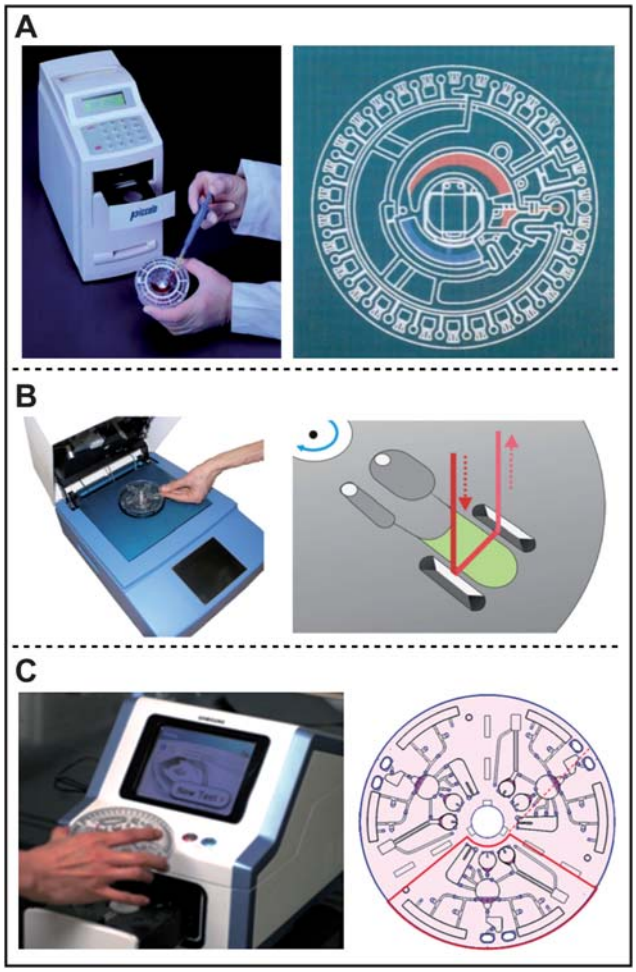

Fig. 8 Examples of disc based sample-to-answer systems. (A) The Piccolo clinical blood analyzer system from Abaxis. Figure adapted and reproduced with permission from ref. 41. (B) The blood analyzer system from IMTEK. Figure adapted and reproduced with permission from ref. 44. (C) The integrated ELISA assay system from Samsung. Figure adapted and reproduced with permission from ref. 42.

spectrophotometer is used to perform the colorimetric absorbance measurements. In addition, reference solutions are read from the disc reference cuvettes such that calibration is performed simultaneously. After ~12 min of processing time, a quantitative result is obtained. This system from Abaxis is a prime example of how centrifugal microfluidic technologies can be used to implement an accurate and effective sample-to-answer system for medical diagnostics.

Using blood-based clinical chemistry analysis similar to that utilized by Abaxis, Ducrée *et al.* from the Institute of Microsystems Technology (IMTEK), at the University of Freiburg in Germany have shown successful integration of blood-plasma separation and colorimetric-based analyte detection systems.[44] The centrifugal microfluidic system developed accepts raw blood samples, meters the sample to a defined volume, mixes the sample with a colorimetric detection agent, and then separates plasma as the color develops (Fig. 8B). After centrifugation of the blood, absorbance is measured as the disc spins; the total process takes less than 3 min. The disc, made of machined cyclic olefin copolymer (COC), consists of integrated monolithic optics to enable colorimetric detection from above the disc. The system was successfully demonstrated using assays to determine either blood-alcohol content from a droplet of blood[25] or blood-glucose levels from several μLs of blood.[44] While the reagents required for the assays were not stored on the disc (they required manual addition of liquid), this shows an excellent example of the integration of various centrifugal microfluidic systems and established biological assays to develop a complete, integrated, sample-to-answer system.

More recently, Lee *et al.* from Samsung demonstrated an integrated sample-to-answer system utilizing an ELISA assay for the detection of Hepatitis from whole blood.[42] A system was developed that combines blood-plasma separation with a suspended-bead based ELISA assay. The centrifugal microfluidic device was made of injection molded PMMA parts, and bonding was performed using a UV-cure adhesive (Fig. 8C). The laser-actuated ferrowax valves previously demonstrated by the Samsung team were utilized to provide fluidic control for steps that included blood separation, mixing of plasma with functionalized beads, multiple bead-washing steps, and filling of a chamber for absorbance measurements. A complete hardware spin-stand system was also developed that included hardware for disc spinning and absorbance measurements (photodiodes and LEDs), lasers for ferrowax valve actuation, and thermal management of the enclosed system shown in Fig. 8C.

This sample-to-answer centrifugal microfluidic ELISA system was able to complete an entire Hepatitis assay directly from whole blood (150 μL volume) within 30 min with a limit-of-detection similar to standard ELISA assays but within 25% of the time. This work represents the first case of a disc-based sample-to-answer system for whole blood testing beyond just the Abaxis chemistry panels. It shows an excellent integration of various biological steps and centrifugal microfluidic technologies to create an efficient and automated system. Moreover, advanced centrifugal microfluidic manufacturing techniques were utilized that are amenable to mass manufacturing, and the entire hardware system was packaged in a portable-scale footprint. However, as with the sample-to-answer work from Ducrée *et al.* discussed above, this device also requires the manual additional of reagents (beyond just that of the sample) before processing.

6. Advanced sample-to-answer centrifugal microfluidics

As seen by the recent advances in sample-to-answer systems for clinical diagnostics presented above, the application of centrifugal microfluidics in that area is slated for future growth. One potential embodiment of the next generation of centrifugal microfluidics will involve even more complex assays on the CD. In particular, nucleic acid analysis has been identified as an area for ongoing research.

In general, the development of an integrated, sample-to-answer, nucleic acid diagnostic system is considered by most the "holy grail" of the μTAS field. By moving away from the traditional means of phenotypic recognition to real-time identification of pathogens *via* the use of molecular diagnostics, tests can be made more rapid and specific. Moreover, the use of molecular diagnostics drastically reduces the time and costs required for

a diagnosis (from days to hours) while greatly increasing the specificity and accuracy.

While individual process steps towards nucleic acid analysis and diagnostics have been demonstrated on centrifugal microfluidic platforms, there remains hurdles to overcome before an integrated sample-to-answer nucleic acid system can be achieved. Many of these hurdles are not reserved solely for centrifugal microfluidic platforms, but are endemic to all microfluidic systems. However, as discussed previously, the advantages of the centrifugal microfluidic system make it a promising solution to bridge the gap in this field.

Typical NA analysis consists of a sample preparation step (including cellular/viral lysis), NA amplification (*e.g.*, PCR), and detection (*e.g.*, *via* DNA/RNA microarray). As discussed above, each of these systems has been independently demonstrated using centrifugal microfluidic technology, showing many of the advantages in bringing the current bench-top methods into the centrifugal microfluidic domain. The integration of these steps, however, remains complex.

As with any biological diagnostics process, the main source of complexity and variability comes from the sample itself (blood, saliva, mucus, urine, *etc.*), which can vary drastically in terms of both fluidic characteristics (*e.g.*, viscosity, density, surface tension) and biological characteristics (*e.g.*, analyte concentration, inhibitor concentration). In particular, the initial sample volume required for NA analysis presents unique challenges. In blood for example, genomic DNA exists at concentrations around 10^6–10^7 DNA mL^{-1} and pathogenic DNA at concentrations as low as 10–10^2 DNA mL^{-1}. This means that systems must work with samples as large as several mLs to obtain the amount of DNA required for detection. In contrast, for clinical chemistry and immunoassays applications, sample volumes of less than μLs are usually sufficient.

The integration of macro and micro systems for fluid manipulation remain another challenge for integrated systems; targets must be isolated from mL volumes then purified and concentrated to μL volumes. Furthermore, different sample types dictate the necessity for distinctive sample preparation steps. However, centrifugal microfluidic technology has shown itself to be very adaptable in the area of sample preparation, thus making it a leading technology choice for the development of an NA sample-to-answer system.

Besides the inherent difficulties with sample preparation, there are problems with reagent handling required for assays in microfluidic systems. Automated handling through isolated reagent packs and robotics to the disc has been demonstrated, and eliminates the need for reagent storage on the disc; however, this option drastically increases the complexity and cost of the system and limits its use in point-of-care settings. Another option is to store reagents on the disc, either in liquid or solid form (*e.g.*, through drying, lyophilization). However, long-term storage of reagents on the disc requires robust liquid and vapor valving. Additionally, methods of preparing reagents are inherently difficult for advanced assays. For example, lyophilization can be difficult for the delicate enzymes and reagents required for NA analysis.

Another hurdle to overcome towards integrated NA diagnostic systems is the complex and expensive hardware platform required for complete analysis. Although it is often presented that inexpensive disc rotors and optical systems like those found in widely available computer drives can be implemented for centrifugal microfluidic analysis systems, there remains additional instrumentation requirements for propulsion and detection. While stronger motors are widely available to spin the discs at higher speeds required for processes like blood separation or for bead-beating cell lysis, all current detection systems use some form of adapted optics. These optical systems, like those required for fluorescence detection, include light sources, detectors, filters, and electronics. Moreover, heaters/coolers will be required to perform thermal control for sensitive steps such as lysis, NA amplification, and NA hybridization. Finally, the entire system needs to be packaged to include thermal management systems and user interface systems. All motor control, analysis instrumentation, optical systems and other equipment must be integrated and built in a cost effective way.

In regards to the assay platform itself, many of the centrifugal microfluidic systems discussed are excellent examples of separating the disposable from the permanent hardware system, which helps to keep the cost-per-test low. Additionally, the motor allows the disc to shift easily for alignment with off-disc hardware. This minimizes the need for multiplexed hardware systems, thus reducing costs and complexities. However, other issues, such as injection molding and FDA approval for the devices, remain challenges to development of advanced centrifugal microfluidic systems.

While the key components are in place for a disc-based NA sample-to-answer system, the complex task of robust and efficient integration of both the disposable disc itself and the hardware platform must be carefully considered before success can be achieved.

It should be noted that the barriers presented in this section are not limited to NA analysis. Changing the assay type will change the sample preparation and analysis steps; however, the considerations outlined above are generic. For example, if disc based immunoassays will ever be accepted for diagnostics, biological issues of sample complexity and reagent storage as well as engineering issues of mass production and instrumentation must still be dealt with. Additionally, while new detection technologies, like those based on interferometry or SPR, potentially lessen the burden on analysis by reducing extensive analysis steps and decreasing the complexity of analysis equipment, they cannot eliminate the fundamental sample preparation steps that are universal to all assays from clinical samples (*e.g.*, isolation of biomolecule targets from the bulk samples through lysis and purification and preparation for detection). In summary, the continued work on individual sample preparation systems for varied clinical specimens can potentially serve to answer technological issues for centrifugal based assays in general.

7.0 Conclusions

A review of centrifugal microfluidic technologies has been presented, with an emphasis on biomedical applications both in industry and academia. Various centrifugal microfluidic functions such as valving, metering, mixing, and flow-switching were discussed, in addition to a broad range of applications in which those components and their combinations were incorporated for biological applications. As discussed, the centrifugal microfluidic platform provides many advantages over other microfluidic

platforms, making it an excellent candidate to tackle complex biological analysis problems, particularly in the area of *in vitro* diagnostics (IVD).

While many advances in centrifugal microfluidic disc technology have been made, the full potential of the platform has yet to be realized. With the exception of the few established companies like Abaxis and Gyros, the market is relatively bare of centrifugal microfluidic technologies and is limited to the applications of blood panel analysis and characterization of antibodies and proteins for drug development. While no direct leaders have emerged in the disc-based NA diagnostics field, several prominent groups in industry and academia are working to develop not only advanced disc-based NA analysis devices, but true sample-to-answer devices for NA IVDs as well (*e.g.*, Madou group, Samsung, IMTEK). Moreover, new areas of interest like cell-based applications for the centrifugal microfluidic platform will likely emerge as valuable research tools in the near future. There are many areas where centrifugal microfluidic technology can find new applications, and the areas already established for centrifugal microfluidic devices will continue to strengthen. Expanded R&D efforts in both industry and academia are expected to "burst" this barrier in the near future and bring the centrifugal microfluidic platform to its full potential.

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