Microfluidics: a boon for biological research

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Microfluidics is an emerging new interdisciplinary field that deals with the manipulation of fluids at the microscale and nanoscale. Having its origins in other areas of science and technology, microfluidics is slowly beginning to make radical changes in various fields of biological sciences. The exclusivity of fluid behaviour at the microscale offers a large number of advantages in biological research such as miniaturization of assays, faster sample processing and rapid detection. This article provides a concise overview of the applications of microfluidics technology in some of the major disciplines of biological research. Furthermore, it also mentions the hurdles that microfluidics is facing and the solutions that are envisaged for the future to make it a widely available, reliable and cost-effective technology.

Keywords: Biological research, lab-on-a-chip, microfluidics, microchannels.

BIOLOGICAL research has moved a long way from basic organismal biology to single-cell, single-molecule-based research. In recent years, the field of microfluidics has contributed to the rapid progress of biological research and has created many breakthrough technologies. It is a multidisciplinary field of science that utilizes concepts from physics, chemistry, engineering, mathematics, computing, nanotechnology and biotechnology. The field has made it possible to scale down entire laboratories by manipulating tiny amounts of fluids using channels having micro or nanoscale dimensions.

The physical factors governing fluid behaviour at microscale are distinctive compared to the ones existing at macroscale. At this scale, fluid flow is characterized by low Reynolds number (less than 1). As a result, fluid flow is completely laminar and no turbulence occurs. This leads to an interesting phenomenon in which different fluids can flow parallel to each other in a given microchannel, without any chaotic mixing. Such mixing occurs only through diffusion at the interface of the liquid layers. This allows for the predictable transport of molecules through the microchannels. Fluids in microchannels are also characterized by a high Péclet number, which is the ratio of the rate of advective transport to the rate of diffusive transport. This property enables parallel-flowing fluids to flow longer without mixing.

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Another important physical factor governing fluid transport in microchannels is the process of diffusion. In one dimension, diffusion can be expressed as $d^2 = 2Dt$, where D is the diffusion coefficient of the particle and d is the distance a particle moves in time t. Since distance varies as the square power, diffusion time can be extremely low at the microscale. This property can be used to create concentration gradients having complex profiles within microchannels. A related property is the surface area to volume ratio. Microfluidic devices are characterized by large surface area to volume ratios, which allow macromolecules to quickly diffuse and adsorb to the channel surfaces. This property is particularly useful in immuno-assays such as enzyme linked immunosorbent assay (ELISA) for drastically reducing the sample incubation time (antibodies or antigens)[1]. Additional factors like surface tension, interfacial tension and capillary forces are predominant at the microscale compared to the forces such as gravity, and this contributes to the unique nature of fluid behaviour in microchannels[2,3].

Contrary to traditional experimental approaches, this exclusive fluid behaviour provides an added advantage in biological research, by permitting rapid processing of biological samples, miniaturization of various experimental techniques and by providing a precise control of the reagents. In addition, microfluidics technology offers numerous other advantages: (1) the volume of reagents required is in the order of microlitres or nanolitres, which is valuable when expensive reagents are used; (2) fabrication methods to build microfluidic devices are simple; (3) several analytical functions can be performed on the same device (lab-on-a-chip), and (4) automation of labour-intensive steps is possible by integrating other systems to the device. As a result, microfluidics has made new inroads in many domains of biology such as cell biology, developmental biology, microbiology, diagnostics, genomics, proteomics and drug discovery.

Origins

Microfluidics research initially began with the development of chromatography. Chromatographic techniques like high-pressure liquid chromatography and gas phase chromatography, enabled the detection of analytes using tiny amounts of samples. In the 1980s, the very first micro-pumps and micro-valves for controlling minute fluid volumes were utilized by IBM in the manufacture of

inkjet printers. Later in the 1990s, the United States Department of Defense played an unprecedented role in the advancement of microfluidics technology by developing microfluidic systems for the detection of chemical and biological threats. The major stimulus for increased growth of microfluidics technology, particularly in biological science, was the rapid progression of genomics and molecular biology, which required high-throughput analytical methods having greater sensitivity[4].

A typical microfluidic device consists of a network of microchannels, inlets for injecting fluids, micropumps and microvalves for fluid manipulation within the chip, outlets for removing fluids, and a coupled detection system for analytical studies (Figure 1). The earliest microfluidic devices were mostly fabricated from silicon, since the technology for patterning silicon wafers through lithographic techniques had already been developed by the electronics industry. Glass and PMMA (polymethylmethacrylate) were used as alternatives to silicon since they offered the ability to integrate optical detection systems due to their transparency. However, patterning microstructures on glass and PMMA was cumbersome due to their brittle nature. More recently, a polymer called PDMS (polydimethylsiloxane) has emerged as the substrate of choice for applications involving biological samples due to its low cost, fast processing, elastic properties, stability under storage conditions, low toxicity and optical transparency. PDMS-based devices can also be fabricated in normal laboratory conditions as opposed to clean-room conditions that are expensive to maintain and access[4,5].

Applications

Genomics

The advent of genomics has revolutionized genetics and led to a better understanding of complex biological systems. The Human Genome Project, which was completed in 2003, kickstarted the meteoric rise of more efficient next generation sequencing (NGS) techniques for rapid DNA (genome) sequencing. The NGS systems have the ability to sequence huge genomes in a matter of hours or days[6]. NGS analysis involves four steps: sample preparation step in which the DNA is randomly fragmented into a library of small segments; target amplification through which the library is linked to adaptor molecules that are clonally amplified to generate millions of molecular clusters; sequencing where the clustered templates are sequenced and data analysis by which the sequence of bases is identified.

One of the bottlenecks in NGS is the preparation of sequencing libraries from DNA or RNA samples, which is a multi-step and lengthy process. An automated solution for preparing sequencer-ready libraries from genomic DNA was demonstrated by Kim *et al.*[7] through a microfluidic platform. By generating discrete fluid droplets containing DNA, this 'sample-in library-out' device integrates all the steps for library preparation, including dispensing of genomic DNA, fragmentation, ligation, amplification and separation[7]. NGS libraries were prepared from minute quantities of human and bacterial genomic DNA (as low as 5 ng).

Bose *et al.*[8] developed a microfluidic platform for trapping lysates from single cells in picotitre wells and capturing cellular RNA on polymeric beads. The device was then scaled up to generate RNA libraries from single cells and perform high-throughput RNA sequencing of hundreds of individual cells. This platform has huge potential which enables researchers to study single-cell transcriptomics more efficiently at a cost of US$ 0.10–0.20 per cell[8]. Similar high-throughput technologies have been released commercially by companies such as Fluidigm® for automating cDNA library preparation from individual cells. Such technologies are poised to push the boundaries of genomics in the years to come.

Diagnostics

One of the most important applications of microfluidics is for point-of-care (POC) testing, i.e. immediate diagnosis at the site of patient care. Miniaturization of diagnostic assays can reduce the cost of healthcare in resource-limited regions and can enable more people to access them.

The applications of paper-based microfluidic devices for diagnostics have drastically increased due to the numerous advantages that paper offers: low cost, wide availability, biocompatibility and its ability to transport liquids via capillary forces without any external assistance. In these devices, precise patterns are made on the surface of paper using polymeric coatings or hydrophobic materials such as wax to form barriers within which a fluid is contained[9,10]. Quantification of analytes through these devices is based on a colorimetric, electrochemical or chemiluminescent output[11,12].

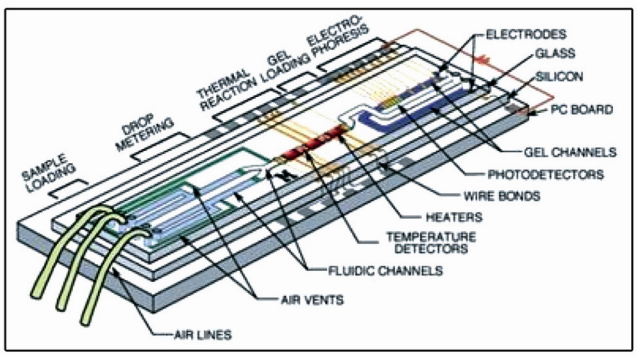

Figure 1. Integrated PCR microfluidic chip for identifying target DNA. (From ref. 18; reprinted with permission from AAAS.)

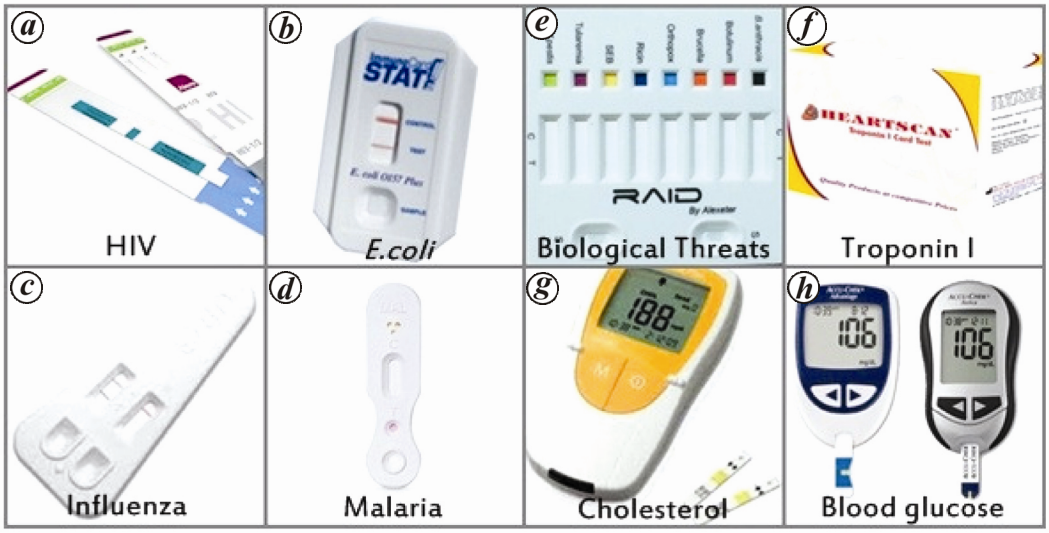

Figure 2. A few commercially available paper-based microfluidic devices. ***a***, Determine™ (courtesy: Alere) to detect all subtypes of HIV; ***b***, ImmunoCard STAT!® *Escherichia coli* O157 Plus (courtesy: Meridian Bioscience Inc.) to detect *E. coli* O157:H7; ***c***, Directigen™ EZ Flu A + B (courtesy: Becton, Dickson and Co.) to detect influenza A and B viral antigens; ***d***, ClearView® Malaria Combo (courtesy: Alere) to detect all four Plasmodium species causing malaria; ***e***, RAID™ 8 (courtesy: Alexeter Technologies) to detect biological threats like ricin, botulinum, etc.; ***f***, Heartscan® Troponin I Card Test (courtesy: Bhat Biotech, India) to detect human cardiac troponin I; ***g***, Accutrend® (courtesy: Roche Diagnostics) to detect cholesterol; ***h***, Accu-Chek® Diabetes Care (courtesy: Roche Diagnostics) to detect blood glucose.

Paper-based microfluidic devices have made a solid impact in the ever-growing market of accurate diagnostic tools. As shown in Figure 2, many devices are available commercially for the detection of various pathogens and biochemical analytes[12]. These devices are inexpensive, disposable and easy to use. Paper-based microfluidic devices producing a colorimetric readout are particularly useful in areas lacking healthcare facilities. Through colour images captured with mobile phone cameras, they could play an important role in the diagnosis of a disease through telemedicine.

A widely used diagnostic tool – ELISA has been extensively demonstrated on microfluidic devices. ELISA utilizes a combination of antibodies, enzymes and proteins for the detection of target antigens in a sample. Although it is considered as a gold standard in protein identification, it requires prolonged incubation, large quantities of expensive samples and reagents, and is labour-intensive. Comparatively, microfluidic ELISA platforms have the ability to shorten incubation times, reduce the volume of reagents, integrate various detection systems and enable automation of the entire assay. Microfluidic immunoassay systems have been demonstrated in the detection of different analytes such as *Cholera toxin*, *Escherichia coli*, *Bacillus globigii* and C-reactive protein[13,14]. Quantification schemes in these systems usually rely on fluorescence due to its high sensitivity, faster response time and ease of coupling the device to fluorescence excitation systems[15,16].

Conventional methods for detecting pathogens from food, water and clinical samples are microscopy and plate cultures, which are laborious and often inconclusive. As a superior and faster means of pathogen identification, integrated microfluidic chips have been developed to perform polymerase chain reaction (PCR) and electrophoresis[17,18]. Microfluidic chips are capable of accepting a crude sample, amplifying the target DNA, performing electrophoresis to separate the DNA fragments, and identifying the target DNA – all in a single integrated platform (Figure 2). An essential part of PCR is the thermal cycling process, which requires temperatures as high as 94°C. To achieve such high temperatures in a compact microfluidic device, micro-heaters are usually integrated into the chip. Detection schemes utilize fluorescent probes or dyes that interact with the amplified and separated DNA. Many PCR-based microfluidic devices have been designed for the detection of pathogens such as *Bacillus anthracis*, *Bordetella pertussis*, *Mycobacterium tuberculosis*, *E. coli*, *Salmonella typhimurium* and HIV from various sources[19].

Developmental biology

Developmental biology deals with understanding the cellular, molecular and genetic processes involved in the growth and development of a plant or animal from a single cell. The study of animal development using microfluidics is still in its infancy. An experiment was initiated to study the effect of fluctuating environmental conditions on the development of *Drosophila melanogaster* (fruit fly) embryos[20]. A microfluidic device was

constructed in which two aqueous streams of different temperatures were in continuous laminar flow. The fly embryos were suspended in the cross-section of the microfluidic channel, and the anterior and posterior ends of the embryo were exposed to both aqueous streams. The development rate of the embryo in both these streams was studied, and it was concluded that a compensatory mechanism exists in the embryonic cells to counter the effects of fluctuating temperature conditions.

Microfluidic platforms have also been developed for the large-scale study of *D. melanogaster* embryos. The high-throughput automated platform positions the embryos vertically along their dorsoventral axis. This enables an efficient quantitative analysis of the patterns formed on the dorsoventral region, which serve as indicators of embryo morphogenesis[21].

During plant reproductive development, pollen tubes encounter chemical guidance signals from the ovules which allow the sperm cells to reach the egg cells and achieve fertilization. These guidance signals cannot be studied effectively in existing bioassays carried out in petri dishes due to isotropically diffusive environments, and hence microfluidic devices have been utilized to mimic the plant microenvironment during pollen tube growth[22]. Yetisen *et al.*[22] also demonstrated that the pollen tubes can sense minute changes in the chemical concentration gradient secreted by the unfertilized ovules and preferentially move towards it.

Ghanbari *et al.*[23] demonstrated the ability of pollen tubes to navigate through various microchannel geometries. Following the immobilization of pollen cells within the microchannels, pollen tube growth was imitated by providing chemical cues. By introducing obstacles, the pollen tubes were forced to change their direction of growth and follow the shape of the microchannels. The study showed that pollen tubes grow in straight lines as long as they do not run into any mechanical obstacles[23]. These technological developments demonstrate the ability of microfluidic devices to replicate the plant microenvironments more accurately without the need for bulky and expensive experimental set-ups.

Microbiology and cell biology

Microfluidics has numerous applications in the closely related disciplines of microbiology and cell biology. A widely pursued area of research in microbiology is the promotion and prevention of formation of biofilms, which are assemblages of microbial cells associated with a surface. When a particular microorganism forms a biofilm, it acts as a protective structure, thus shielding the microorganism from the immune system or any antimicrobial agent. Such biofilms are responsible for chronic infections in humans and most importantly, fouling of medical implants and prosthetics[24]. A microfluidic device was used to control and monitor the environment of biofilms caused by *Candida albicans*, the organism responsible for candidiasis. A device with dielectric sensors was created to measure the real-time response of *C. albicans* biofilms to different shear stresses and antibiotic concentrations[25]. The findings suggested that an increased shear stress led to considerable changes in the biofilm formation patterns, whereas the addition of an antibiotic – amphotericin B resulted in two distinct forms of dynamic behaviour of the biofilm.

Flow cytometry is an indispensible tool in many microbiological and cell biological studies. It is used to identify individual cells from a mixed cell sample based on parameters such as cell size, fluorescent markers and granularity. A laser-based detection system is utilized to obtain quantitative or qualitative information on cell populations. Flow cytometric systems have been miniaturized through microfluidics and microfabrication (Figure 3) for the detection of various analytes such as red blood cells, CD4+ T cells, natural killer cells, etc.[26,27]. Researchers have also utilized microfluidic flow cytometers for the quantification of bacterial samples from different sources[28].

Compared to conventional single-cell techniques such as fluorescent activated cell sorting (FACS) and micromanipulation on agar, microfluidics offers a unique opportunity for studying and monitoring single cells in real time. A microfluidic device was used to study the

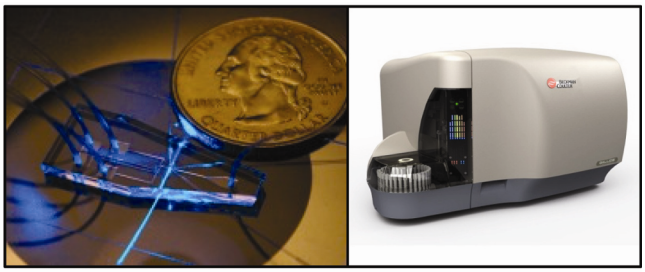

Figure 3. A microfluidic flow cytometry chip (left) (reprinted with permission from Mao *et al.*[27] ©2012, AIP Publishing LLC) and a Gallios™ flow cytometer by Beckman–Coulter (right).

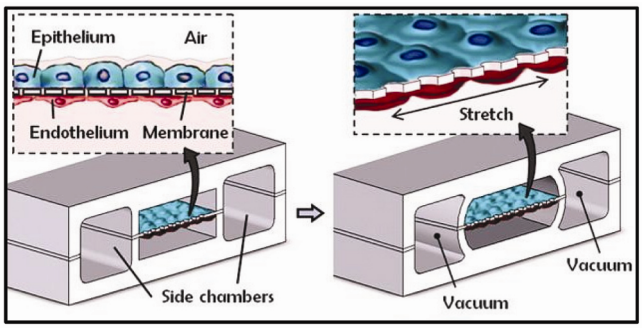

Figure 4. Lung-on-chip device to mimic and study critical lung functions. (From ref. 34; reprinted with permission from AAAS.)

ageing process in individual yeast cells by analysing the single-cell gene expression levels of *RAS2* and *HSP104* over time[29]. *RAS2* is known to extend the lifespan of yeast when overexpressed, and decrease its lifespan when it is underexpressed. *HSP104* plays an important role in yeast heat shock-induced lifespan extension. In this study, discrete yeast cells were 'trapped' in the device and expression analysis of *RAS2* tagged to *YFP* (yellow fluorescent protein) and *HSP104* tagged to *GFP* (green fluorescent protein) was done using fluorescent microscopy. The study continuously monitored the expression of these genes over many hours and was able to demonstrate inter-cell variation in the expression of both the genes.

Microfluidics technology has also been extensively used in understanding many other domains of cell biology, including the cytoskeletal elements[30], cell adhesion properties[31] and separation of motile and non-motile sperms[32].

Organs-on-chips

Over the years, *in vitro* cultures have been the major tools for studying physiological processes. However, cell culture models fail to replicate the features of organs such as tissue–tissue interfaces, spatio-temporal gradients and mechanical microenvironments (such as shear stress)[33]. Animal models are time-consuming, expensive, and have a multitude of legal and ethical issues. 'Organs-on-chips' have been designed as a better way to replicate biological processes. They utilize microfluidic principles to mimic the physiological environment within a living system and integrate all the processes required for a bioassay. Microfluidic devices have been designed using polymers to create biomimetic systems, which can replicate functions of critical organs.

A groundbreaking device was designed to replicate critical lung functions on-chip[34]. The tissue–tissue interface of the alveolus was replicated in this microdevice by culturing human alveolar epithelial cells and pulmonary capillary endothelial cells on opposite sides of a thin PDMS membrane (Figure 4). To simulate physiological breathing movements, vacuum was used in the hollow side chambers to cyclically stretch and relax the cell layers. A culture medium was pumped through the bottom microchannel, containing the capillary endothelial cell layer, to simulate the flow of blood through the lung microvasculature. This device was used effectively to mimic the human inflammatory response to pathogens and study the toxic effects of airborne particles, chemicals or drugs. Similar organs-on-chip have been created to study the proximal tubule of kidney, osteoblast, gastrointestinal tract and human heart functions[33,35,36]. These devices can also be used to visualize cellular responses in real time by high-resolution microscopic imaging.

Drug discovery

The discovery and development of new drugs and therapeutics to treat various diseases is one of the most active areas of research around the globe. Pharmaceutical companies invest billions of dollars to identify, test and successfully release a novel drug compound in the market. Microfluidics has accelerated research at every stage of the drug discovery and development process. It has emerged as a valuable tool in this domain by miniaturizing assays and enabling high-throughput screening of potential drug candidates.

The drug discovery process consists of target selection, lead identification and optimization, and pre-clinical studies. The first step in drug discovery is to identify a 'target', which is a protein or gene that can be affected by a drug molecule. With the recent advances in microfluidic technology, protein targets have been characterized using microfluidic devices which can express, separate, label and quantify proteins to the level of single-cells. Researchers have developed microfluidic chips capable of separating the proteins through micellar electrokinetic chromatography (MEKC)[37], capillary zone electrophoresis[37,38] and isoelectric focusing[39] in less than 20 min.

To study drug–target interactions, the structure of proteins needs to be determined. Protein crystallization is the bottleneck in solving the structure of drug targets by X-ray diffraction. Microbatch and vapour diffusion are the most commonly adopted methods to crystallize proteins. In recent years, microfluidic platforms have evolved into an indispensable tool for rapid, large-scale screening and crystallization of thousands of proteins with low sample consumption (nanolitre and picolitre). Automated microfluidic screening systems have been used for the crystallization of proteins of therapeutic interest such as thaumatin, bovine liver catalase, glucose isomerase and other membrane proteins[40].

A novel microfluidic device called SlipChip[41] was developed for the crystallization of membrane proteins. The device has two glass plates in close contact. Different solutions for crystallization were loaded in the wells of the bottom plate, and a protein sample was loaded in the channels of the top plate. The top plate was then 'slipped' or moved to mix protein samples with crystallization solutions to form crystallization trials. Another noteworthy technique is the development of X-ray transparent microfluidic chips that enable on-chip protein crystallization and characterization using X-ray diffraction[42]. These devices eliminate manual harvesting and mounting of protein crystals and enable the collection of X-ray diffraction data from multiple crystals on-chip.

In the next stage of drug discovery, a 'lead' or drug molecule that can interact with the target is identified. Many microfluidic devices have been designed for ligand-binding studies to investigate the interaction of

drugs with specific targets[43,44]. A recent study using droplet microfluidics demonstrated the interaction of human serum albumin (HSA) and an anti-coagulant drug warfarin using droplet microfluidics[45]. In this study, magnetic beads were coated with HSA in a solution following which ^{14}C-labelled warfarin was added and allowed to reach equilibrium. Using a microfluidic device, discrete droplets of fluid containing the magnetic beads (and consequently, HAS–warfarin complexes) were created. Using magnets, the droplets were separated from the solution containing the free drug. The concentration ratio between free and bound warfarin was calculated using a scintillation counter. This enabled researchers to determine the affinity constant for the binding of the drug with the target. Such devices can enable high-throughput screening of drug targets, minimize interaction times and improve sensitivity.

The last stage of drug discovery is pre-clinical studies in which the drug candidate is tested for safety and efficacy in animal models. Microfluidic systems are now being used as an alternative to animal models for pre-clinical studies. An organ-on-chip was designed to mimic the microenvironment in cancer cells for drug screening[46]. The microenvironment in cancerous cells is highly complicated, consisting of various signalling molecules and mechanical cues that are responsible for cancer initiation, metastasis and drug resistance[47]. Different cancer cell lines were cultured in separate areas within the device and the cytotoxic effect of an anti-cancer drug (Tegafur®) was studied on each cell line. The findings suggested that the microfluidic platform provided a realistic microenvironment for studying cancerous cells, similar to the one existing *in vivo*.

Advancement of microfluidics in the Indian context

New microfluidic technologies for studying biology are being developed by research groups in various institutions across India. Researchers from the Indian Institute of Science, Bengaluru have demonstrated the technique of imaging flow cytometry (IFC), that combines the principles of digital microscopy and flow cytometry, to image and analyse red blood cells (RBCs) flowing through microfluidic channels[48]. A microfluidic device working on the same concept was designed to diagnose malaria in less than 30 min. The malarial parasite, *Plasmodium* infects RBCs and the infected RBCs display morphological features that are different from normal RBCs. Through IFC and a custom-built smartphone application, it was possible to differentiate between infected and normal cells, and obtain a quantitative value of the parasitemia levels[49]. Thus, the combination of IFC and microfluidics could possibly be a low-cost alternative to conventional detection schemes, such as clinical microscopy.

At the Indian Institute of Technology (IIT) Bombay, novel work on paper-based microfluidics has been carried out. Researchers have developed a paperfluidic chip that measures salivary pH, to estimate its buffering capacity, and also tests susceptibility to dental caries by checking for salivary reductase. This cheap, disposable and easy to manufacture POC device can be used to screen the oral health of persons having limited access to good healthcare facilities[50].

Researchers at IIT (Delhi) have worked on the theoretical and modelling aspects of microfluidic immunosensors. They analysed the uncertainty induced in model predictions of a T-sensor-based competitive diffusion immunoassay. A stochastic quantification method was used to determine uncertainty in diffusion constants, binding reaction rate constants and inlet flow speed[51].

Indian technology companies like Achira Labs® and Bigtec Labs® are also actively engaged in developing diagnostic platforms using the principles of microfluidics. Achira Labs (www.achiralabs.com) develops 'Fab Chips' in which silk yarns weaved into a cloth are coated with reagents for an immunoassay. The test results are in the form of a visual readout and can be used for detecting pathogens such as HIV and syphilis[52]. The company recently launched its first product, 'ACIX-100', which uses patented microfluidics technology to test blood serum samples. Bigtec Labs (www.bigteclabs.com) is developing microfluidic devices for on-chip detection of pathogens. These devices are capable of sample preparation, mixing, screening and detection on the same platform. In addition, several institutions and industries across India are carrying out research in the field of microfluidics and its applications.

Challenges of working with microfluidic devices

Although miniaturized technologies such as microfluidics offer numerous benefits, there are a few major challenges associated with them. First, these devices are prone to physical failure[53]. For example, at high fluidic pressures, device-bonding and fluidic interconnects tend to fail. Secondly, device performance is affected due to the coupling of different energy domains (electrical, fluidic, thermal, etc.) and the mechanisms for device failure due to this reason are not yet fully understood[54]. Thirdly, when working with biological materials such as cells, microchannels tend to get clogged irreversibly. Fourthly, the design and integration of various liquid-handling elements in microfluidic devices such as reservoirs are complex. It is crucial that the analytes are not exposed to extreme conditions such as high electrical charges or temperature fluctuations[53,55].

PDMS-based microfluidic devices in particular have their own set of challenges. Trapped air bubbles in the PDMS elastomer must be removed completely by

desiccation; otherwise they may render the device unusable for the intended application. Furthermore, PDMS tends to swell when exposed to organic solvents and therefore its usage is limited to aqueous solutions[56]. PDMS also has high permeability to gases, and is unsuitable for experiments involving oxygen-sensitive reactions. Under extreme thermal conditions, PDMS can also deform easily[57]. The advantages offered by microfluidic devices outweigh these drawbacks and therefore, researchers around the world are working towards circumventing these problems to make microfluidics a widely used technology in biological research.

Future directions

Evidently, microfluidics offers numerous advantages in nearly every conceivable area of biological science. It has led to a paradigm shift in the way biological research is performed. In spite of this, microfluidics research is considered to be in its early stages since there are some issues that need to be addressed. Currently, microfluidics research is restricted to certain laboratories due to three reasons: (i) the technology for fabricating such devices is not widespread; (ii) microfluidic devices are not user-friendly, since they employ numerous and complex integrated components for fluid manipulation and analyte detection and (iii) their ability to replace certain 'gold standards' of biomolecule detection and analysis is questionable, since their reliability is yet to be satisfactorily demonstrated. Corporations and academia must collaborate and take up the task of solving these problems so that this domain is not restricted to the release of prototypes and proof-of-concept displays by laboratories. Hence, microfluidics technology must be further developed so that it becomes cost-effective, reliable and accessible to users who are not experts.

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ACKNOWLEDGEMENT. We thank Dr Sai Siva Gorthi (Department of Instrumentation and Applied Physics, Indian Institute of Science, Bengaluru) for his valuable suggestions and inputs.

Received 8 September 2015; revised accepted 5 January 2017

doi: 10.18520/cs/v112/i10/2021-2028