J. Indian Inst. Sci.
A Multidisciplinary Reviews Journal
ISSN: 0970-4140 Coden-JIISAD

REVIEW ARTICLE

Multidimensional Paper Networks: A New Generation of Low-Cost Pump-Free Microfluidic Devices

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Abstract | Since Andreas Manz first introduced the microchip technology for chemical applications back in the 1990s, the field of 'microfluidics' has expanded widely and microfluidic tools have become ubiquitous in life sciences research. However, pumps and controllers associated with most current microfluidic chips continue to be bulky and costly. A new class of microfluidic devices in which flow channels are composed of multidimensional (2D or 3D) shapes of porous materials is becoming increasingly popular. The ability of porous materials to wick fluids obviates the need for pumps, making such devices portable, low-cost, and ideal for use in low-resource settings. Such devices are broadly referred to as "paper microfluidic devices". The ability to manipulate fluids in paper microfluidics has progressively increased over the past decade and such devices are currently being used to develop highly sensitive and multiplexed low-cost diagnostic/sensing devices. In this article, we review the area of paper microfluidics covering the basic fluid physics, methods of fabrication, flow control tools, applications in diagnostics/sensing, and applications in other emerging areas like tissue engineering and power storage. This review is targeted to a broad audience that does not have prior exposure to the field of paper-based microfluidics. Through this article, we wish to invite researchers from multiple backgrounds to contribute to further development in this new and exciting area of research.

Keywords: *Microfluidics, Point-of-care diagnostics, Paper analytical devices, Tissue engineering, Nucleic acid amplification, Immunoassays, Microfabrication, Valving*

1 Introduction

When microfluidics was first introduced in the 1990s, it brought along an optimism that it would significantly change the way modern analytical chemistry and biology is conducted. The original idea was that microliter quantities of fluids could be precisely manipulated in silicon microchannels fabricated using technologies already developed by the electronics industry[1, 2]. For analytical chemistry, the motivation was that traditional lab-based chemistries involving multiple steps could be condensed into a single fluidic "chip". For biologists, the excitement was in the idea that cells could be cultured under flow-through conditions that better mimicked the in vivo condition and provided better spatiotemporal control over the cellular microenvironment. Miniaturization promised to reduce the quantities of expensive reagents, streamline workflows, and potentially enhance throughput in all these application areas[3]. The terms "lab-on-a-chip" and "micro-total analysis systems"[1] gained popularity during this time. A major development in the field occurred when a method to prototype

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Springer IISc Press

microchannels in elastomeric materials (poly dimethylsiloxane; PDMS) was developed in 1998[4]. This significantly expanded the reach of the technology to the larger academic community because the new method was much faster and safer. Over the years, PDMS-based microfluidic devices have become an indispensable tool in modern analytical chemistry and biology (Table 1, 2).

The true measure of success of any technology is the extent of its utilization in commercial products. After the initial hype of inflated expectations, according to experts, microfluidics as a technology seems to be heading towards a "plateau of productivity"[5], a term defined in the well-known 'Gartner Hype Cycle' for emerging technologies[6]. The Agilent Bioanalyzer capillary electrophoresis system, launched in 1999, was the poster child of the early microfluidics technology and was a big commercial success. Since then, microfluidics has enjoyed large-scale commercial success, not necessarily as a standalone app as was originally envisioned, but as a silent component of larger systems. Some of the major application areas for microfluidics have been pharmaceutical research, drug discovery/delivery, and in vitro diagnostics. Microfluidics, or manipulation of sub-microliter fluid volumes, is now an integral component of commercial next generation sequencers (Nanopore sequencing; Oxford Nanopore Technologies), miniaturized clinical diagnostic systems (ACIX; Achira Labs Pvt Ltd), and several lab analytical instruments. By the year 2021, the global microfluidics market is projected to be worth $8.78 billion[7].

Despite all the success that mainstream microfluidics has enjoyed, one limitation of microfluidic systems has been that while the chip has been miniaturized at an astonishing pace, ancillary equipment required to operate the chip remains bulky. Benchtop pumps, flow controllers, and optical detectors like microscopes or well plate readers are commonly used along with miniaturized microfluidic chips. The need for bulky ancillary equipment has severely restricted the use of microfluidics in "point-of-care" (POC) diagnostics, i.e., in the ability to conduct a medical diagnostic test at or near where the patient is located. In general, current microfluidic technologies fail in low-resource settings that lack high-tech laboratories—scenarios in which POC diagnostic testing is needed the most.

In parallel to modern microfluidic technology, one widget that has silently enjoyed widespread commercial success and has been a game changer for POC testing is the lateral flow immunoassay (LFIA), e.g., a home pregnancy test. While the LFIA does not fit into the traditional definition of microfluidics as popularized by the 1990s revolution, at its core, it is a microfluidic device that moves microliter quantities of fluids through microcapillaries in a porous material. The advantage over traditional microfluidics is that capillary action obviates the need to use bulky pumps and the signal can be read by naked eye. As the need for rapid detection of analytes at the POC has come into focus, influenced partially by a push from funding agencies like the Bill & Melinda Gates Foundation towards decentralized medical diagnostic testing, the LFIA technology has regained the attention of researchers. While LFIAs continue to find new applications, their sensitivity for analyte detection and ability to conduct multiplexed detection is limited compared to those achievable in traditional pressure-driven microfluidic systems.

Technology in which patterned paper is used to distribute minute fluid volumes into multiple detection zones where different analytes can be assayed was introduced in 2007[8]. Such devices are called "microfluidic paper analytical devices" (μPADs) and are becoming increasingly popular. Complementary to μPADs, another class of paper-based devices that enabled sequential delivery of multiple fluids over a test zone was introduced in 2010[9]. These devices enabled conducting multi-step signal-enhanced immunoassays in paper-based devices and are called two-dimensional paper networks (2DPNs)[9–11]. These developments have now led to a field of research called "paper-based microfluidics". The idea is that with innovative designs made from low-cost paper, many of the multi-step assays that were being performed in the traditional microfluidic devices can be conducted in devices that do not require pumps or an electricity source. In addition, paper is easy to print on, easy to cut and shape, and a white background facilitates visual colorimetric readouts. Paper microfluidic devices have now been used to conduct an array of sensing chemistries ranging from simple color change chemistries, immunoassays, ELISA's, and nucleic acid amplification tests, and the excitement level for this new platform is currently very high.

In this review, we aim to provide a broad overview of the field of paper-based microfluidics. While several reviews have been published on this topic[12–15], this review adopts a different tutorial-like approach providing a historical perspective of the field of microfluidics and circumstances that led to the popularity of paper-based

Table 1: Comparison of fabrication techniques.

Fabrication technique	Reagents required	Resolution (μm)		Stored time	Advantages	Disadvantages	References
		Barrier	**Channel**				
Wax printing	Wax	$850 \pm 50\ \mu m$	$561 \pm 45\ \mu m$	–	Simple, rapid (5–10 min) (Lu et al.); suitable for large-scale batch production; Extremely cheap $7/m^2 (Carrilho et al.)	Cannot handle surfactants and organic solvents; Low resolution due to wax penetration	Lu et al.[49] Carrilho et al.[51]
Photolithography	SU-8 2010 photoresist; Low-cost photoresist derived from SU-8 resin; Cyclized poly(isoprene) derivative; Octadecyl -trichlorosilane (OTS)	– – – 233 ± 30 µm	– – – 137 ± 21 µm	Stored at room temperature for 6 months	Rapid, High resolution of microchannels	Complex and involves multiple steps; Involves expensive reagents and equipments (SU-8 is $800/L)	Martinez et al.[42,43] Carrilho et al.[48] He et al.[52]
Wet Etching	Trimethoxyoctadecylsilane (TMOS) solution	–		–	Rapid, simple, free of expensive equipment, metal mask and expensive reagents	Relative low resolution	Cai et al.[54]
Screen Printing	Carbon	–		–	Inexpensive, Ease of fabrication	–	Scida et al.[55]
Wax/polymer Screen Printing	Wax Polystyrene	1200–1800 µm 380 ± 40 µm	550–1000 µm 670 ± 50 µm	Stable even after 6 months' storage	Simple, rapid, expensive wax printers and other accompanying consumables are not needed Flexible device, Single step without requirement of external heat, UV light, clean room	Loss of feature resolution due to wax spreading.	Dungchai et al.[56] Sameenoi et al.[57]
Laser Treatment	Photopolymer Any paper with hydrophobic surface coating	120 µm	80 µm 62 ± 1 µm	–	Non-contact, mask-less and fast; selectively modify the surface structure and properties (hydrophobic to hydrophilic).	Set up cost for laser and other equipment is high. Silica micro-particles needs to be deposited to allow lateral diffusion of fluids	Sones et al.[58] Chitnis et al.[59]

Table 1: continued

Fabrication technique	Reagents required	Resolution (μm)		Stored time	Advantages	Disadvantages	References
		Barrier	Channel				
Plasma Treatment	Alkyl ketene dimer (AKD)	< 1.5 mm in width		–	Cheap Reagents	Requires expensive and fixed-pattern masks as well as dedicated equipment and controlled conditions in labs; Overstretching of the substrate under a mask.	Li et al.[60]
Flexographic Printing	Polystyrene	minimum width of 400 µm of the hydrophobic region		–	Biomolecules and other reagents can also be printed, Ideal for large-scale production.	Requires different printing templates	Olkkonen et al.[61]
Inkjet printing	Alkyl ketene dimer (AKD) Silver Ink Siloxanes	– 200 µm –		–	Low-cost and high-resolution, non-contact, can be scaled up. Involved inexpensive thermal ink jet printer ($ 40)	Horizontal penetration of ink	Li et al.[62] Koo et al.[24] Rajendra et al. [63]
Knife cutting/plotter	_	Open-channel microfluidic feature size 45 µm		–	Rapid, no chemicals were used in the fabrication of the device	Requires specialized or custom-modified patterning equipment, Deformation at the edges of the channel	Fenton et al.[67] Glavan et al.[68]
Embossing	Paper was made omniphobic by carrying out solid–vapor silanization reaction Nonwoven polypropylene (PP) sheet	pressure-driven open-channel microfluidic system (2 mm width, 800 µm depth)		–	Single step process, Mass production. Could be used multiple times (1000)	Better option open channel in the form of PDMS devices, Pressure (294 MPa) applied may damage the channel	Thuo et al.[69] Shin et al.[70]
Laser Cutting		150 µm ~0.4 mm		–	Single step, mass production, high reproducibility	Initial cost of laser cutter is high.	Spicar-Mihalic et al.[71] Nie et al.[72]

Table 2: Comparison of flow control techniques

Study	Control method	Operation of valve	Paper material	Compatibility with chemical reagents
Fu et al.[11]	Geometry control	A time delay in sequential delivery of reagents due to varying length of channels and volume metered pads	Nitrocellulose (Millipore, Billerica, MA), glass fiber (Ahlstrom, Helsinki, Finland) and cellulose (Millipore, Billerica, MA)	Signal-amplified sandwich format immunoassay for the malaria protein PfHRP2
Mendez et al.[34]	Geometry control	Does not involved absorbent pad to change the geometry of downstream of the channel	Millipore Hi-Flow HF135 nitrocellulose (Millipore Corp., Billerica, MA)	
Fu et al.[9]	Geometry control	Controlling fluid transport using the geometry of the channel-network and dissolvable barriers	Nitrocellulose (Millipore, Billerica, MA), An absorbent pad (Millipore, Billerica, MA)	pH detection
Lutz et al.[74]	Geometry control	Controlled arrival time and shut-off time of each reagent in the detection zone	Nitrocellulose membranes (Mylar-backed HiFlow Plus 135, HF13504, Millipore, Billerica, MA) and high fluid capacity cellulose pads (C083, Millipore) were	
Toley et al.[75]	Geometry control	Time delay by using absorbing pads parallel to the channel	Nitrocellulose membranes (FF80HP, GE Healthcare; Waukesha, WI), cellulose CFSP223000 (Millipore, Billerica, MA) or cellulose 320 (Ahlstrom, Helsinki, Finland). glass fiber 8964 (Ahlstrom)	Detection of the malaria protein PfHRP2
Lutz et al.[21]	Chemical control	Programmable flow delays based on the amount of deposited sugar solution	Plastic-backed nitrocellulose (Mylar-backed HiFlow Plus 135, HF13504, Millipore, Billerica, MA), cellulose (C083, Millipore), and glass fiber (Ahlstrom, Helsinki, Finland)	Multi-step malaria assay (ELISA)
Jahanshahi-Anbuhi et al.[23]	Chemical control	Dissolvable polymeric bridge (Shut-off valve based on water-soluble pullulan films) for the time-dependent sequential release of reagent	Whatman #1 filter paper	Fully automated bioactive paper sensor for organophosphate pesticide detection
Koo et al.[24]	Chemical control	ON/OFF switch based on inkjet-printed electrowetting valve for paper fluidic sensors	Nitrocellulose membrane (AE 98 Fast, Whatman), (CF5, Whatman International Ltd., Piscataway, NJ, USA), (CF5, Whatman International Ltd., Piscataway, NJ, USA)	Detection of Saccharomyces cerevisiae rRNA in lateral flow assays
Chen et al.[25]	Chemical control	A trigger and a time delay valve based on the fluidic diode	cellulose paper sheet	
Houghtaling et al.[76]	Chemical control	OFF switch valve based on dissolvable sugar bridge	Glass fiber (Ahlstrom, Helsinki, Finland), polyester (Ahlstrom, Helsinki, Finland), Nitrocellulose (Millipore, Billerica, MA)	

Table 2: continued

Study	Control method	Operation of valve	Paper material	Compatibility with chemical reagents
Noh et al.[77]	Chemical control	Metering the capillary-driven flow rate of fluids within three-dimensional (3D) microfluidic, paper-based analytical devices (μPADs)	The hydrophilic paper is patterned with wax, Whatman chromatography paper	
Toley et al.[20]	Mechanical control	Volume and time-metered ON/OFF, and Diversion switch based on compressed sponge actuation valves	Plastic-backed nitrocellulose FF80HP and AE100, unbacked nitrocellulose (GE Healthcare; Waukesha, WI). Detection strip is nitrocellulose HF120 (Millipore, Billerica, MA), Glass fiber grade 691 (VWR, Radnor, PA). cellulose pads CFSP223000 (Millipore, Billerica, MA) and cellulose 320 (Ahlstrom, Helsinki, Finland)	Detection of the malaria protein PfHRP2
Li et al. [22]	Mechanical control	Electromagnetic normally open and normally closed valve	Filter paper with wax printing paper and tissue-paper bridge are used	Enzyme-based colorimetric reaction performed (ELISA)
Kim et al.[78]	Mechanical control	Solenoid driven pressure Open/Close valves	Chromatography paper (grade 1, GE Healthcare Whatman, Springfield Mill, U.K.), filter papers (grade 1, GE Healthcare Whatman)	Paper-based ELISA with PDVs
Kong et al.[19]	Mechanical control	ON/OFF and Diversion switch valve based on selective wetting of folded paper actuator strips	Chromatography paper (Whatman™, 1 CHR)	Colorimetric assay for the simultaneous detection of glucose, protein, and nitrite from artificial saliva
Li et al.[80]	Mechanical control	Hollow-rivet-assisted movable ON/OFF-switches valve	Wax-printed hydrophobic and hydrophilic papers	ELISA analysis of carcinoembryonic antigen (CEA) cancer diagnosis

microfluidics. In addition, in our opinion, this review covers the broadest range of topics under the purview of paper-based microfluidics compared to all previously published reviews. We will cover the fundamental physics governing flow in paper microfluidics, common device geometries, and various fabrication methods. We will then review innovative strategies that have been developed to control and automate fluid flow in paper microfluidic devices. This will be followed by a broad overview of the applications of paper-based microfluidics in biomedical engineering. Some upcoming areas like tissue engineering, plasma separation, and energy storage will also be covered. As will become clear through the various sections that follow, the development of paper-based microfluidic devices for biomedical applications is a highly multidisciplinary field. We hope that this review will attract researchers from a diverse background to contribute towards the development of integrated paper microfluidic systems.

2 Analysis of Publications Related to Microfluidics

A brief history of microfluidics showing major developments in the field is presented in Fig. 1a. The earliest technology used silicon micromachining to create microfluidic channels and led to the conception of micro-total analysis systems (μTAS)[1, 16]. However, silicon micromachining was cumbersome and not accessible to many. Rapid prototyping of microfluidic channels in PDMS was invented in 1998 and it radically enhanced the penetration of microfluidics in the scientific community[4]. Another notable advancement was the invention of multi-layer PDMS devices in 2000[17] that enabled active valving and massive parallelization of bioassays[18]. The modern revolution in paper-based microfluidics was triggered by μPADs (dotted red rectangle) in 2007 and enabled multiplexed detection from small sample volumes[8]. Two-dimensional paper networks introduced in 2010 provided a way to conduct more sensitive multi-step assays in paper[9]. From 2013 onward, several new methods of flow control and valving for paper-based microfluidics have been introduced and this has led to increasingly new application areas[19]–[25].

A rapid increase in the number of publications related to microfluidics ensued from the advent of PDMS-based microfluidic fabrication

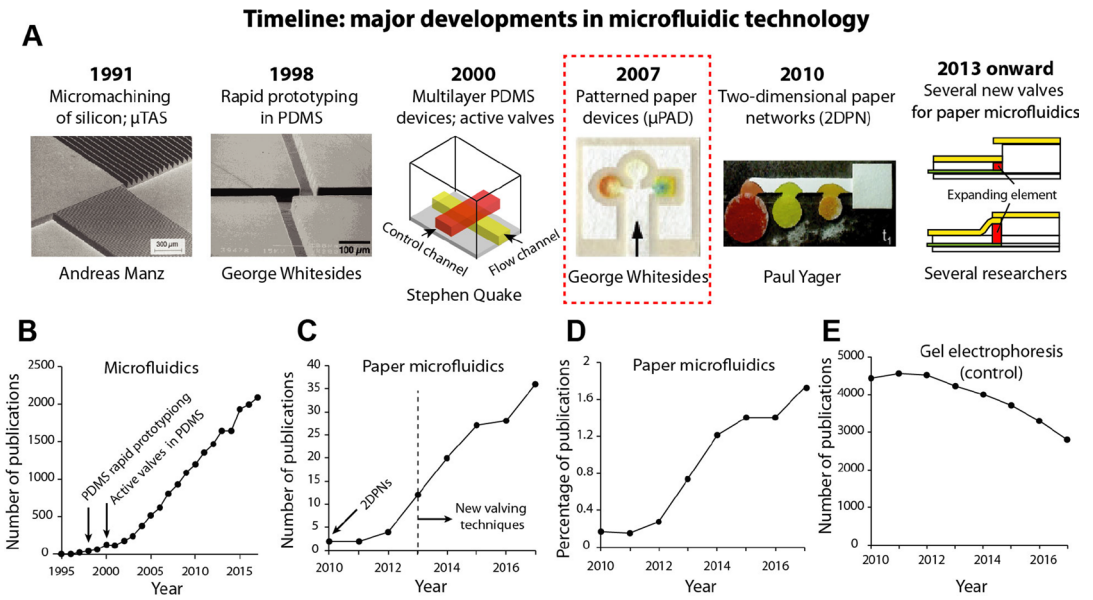

Figure 1: A brief history of microfluidics and analysis of number of publications over the years. **a** Timeline of major technological breakthroughs that popularized microfluidics in the scientific community. **b–d** Number of publications on the topic "microfluidics" since 1995 (**b**); publications on the topic "paper-based microfluidics" since 2010 (**c**), percentage of total publications on microfluidics focused on paper-based microfluidics since 2010 (**d**), and publications on the topic "gel electrophoresis" since 2010 (**e**), used as a control to show that increase in publications on microfluidics is not only because of an increase in the number of scientific journals and research universities 1991: silicon micromachining, reproduced from ref [16] with permission from Elsevier; 1998: rapid prototyping in PDMS, reproduced from ref [4] Copyright (2010) American Chemical Society; 2000: multilayer PDMS fabrication; 2007: microPADs, reproduced from ref [8] with permission from Elsevier; 2010: 2DPNs, reproduced from ref [9] with permission from Elsevier.

in 1998 (Fig. 1b).[1] The number of articles related to microfluidics continues to increase at a high rate (Fig. 1b). Simultaneously, there has been a rapid increase in the number of publications related to paper-based microfluidics since the year 2010 (Fig. 1c), amounting to 36 publications in the year 2017. Moreover, the percentage of the total publications on microfluidics that are related to paper-based microfluidics went up to 1.72% in the year 2017 from 0.17% in the year 2010 (Fig. 1d). This marks a 10-fold increase in the contribution of paper-based microfluidics to the field over a period of 7 years. Although still a niche, paper-based microfluidics are rapidly making a mark. We acknowledge that an analysis of the number of publications of this sort must always be accompanied by a word of caution. It could be argued that a general increase in the number of scientific journals and number of research universities worldwide over the last decade alone could account for the rapid increase in the number of publications and that this increase is independent of topic area. To rule this possibility out, we analyzed the number of publications related to "gel electrophoresis", a common technique used in most biology-related labs, as a control. Despite an increase in the number of scientific journals, there has been a decrease in the number of publications on this topic since 2010 (Fig. 1e) owing to little/no new developments in this area. In contrast, publications related to paper-based microfluidics seem to be rapidly increasing in number.

3 Physics of Fluid Flow in Paper-Based Microfluidics

Unlike duct flow in traditional positive pressure-driven microfluidic devices, flow in paper materials is driven by capillary pressure generated by pores within the paper. The generated capillary

[1] An analysis of the number of publications related to microfluidics was conducted using Web of Science. The following keywords, in quotes, were used under topic search: (i) "Microfluidics" (Fig. 1b), (ii) "paper microfluidics" OR "paper analytical devices" (Fig. 1c, d), and (iii) "gel electrophoresis" (Fig. 1e).

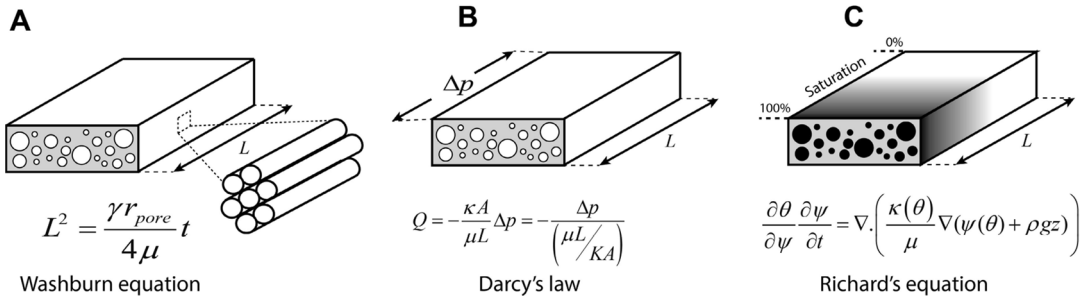

Figure 2: Mathematical models for fluid flow through paper materials. **a** Washburn equation, **b** Dacry's law, and **c** Richard's equation. The Richard's equation is the only equation that accounts for partial saturation.

pressure is a function of pore size and liquid–air surface tension. Thus, the rate of fluid flow in paper varies according to the material of construction, porosity, and pore size distribution[26]. A thorough understanding of how these parameters affect flow rates is obviously essential to designing paper microfluidic devices. There are two widely used equations to model fluid flow in porous materials: (a) the Lucas–Washburn equation and (b) Darcy's law; they assume uniform cross section of the paper device and a fully saturated advancing fluid front, respectively[27]. A third model called the Richard's equation, which accounts for partial saturation of the fluid front in multidimensional domains has recently been used[28, 29]. We briefly describe the three models below.

3.1 Lucas–Washburn Equation

This model was originally derived for flow through a single capillary assuming Hagen–Poiseuille flow through a rigid and isotropic cylinder. Its use has been extended to paper by assuming that paper is composed of a bundle of parallel capillary tubes[30] (Fig. 2a). Washburn equation predicts the position of the wetting front as a function of time as follows[10, 31]:

$$L^2 = \frac{\gamma r_{\text{pore}}}{4\mu} t$$

where L is the distance traversed by the fluid front, r_{pore} is the average radius of the pore, γ is the effective surface tension of the fluid-air interface (including the dependence on contact angle), and μ is the dynamic viscosity of the fluid. According to this equation, the position of the fluid front in a straight (1D) porous membrane is proportional to the square root of time[19, 27], and the velocity decreases with distance because of viscous resistances. The square root time dependence of the advancing fluid front has been experimentally validated in many diagnostic membranes and a lumped proportionality constant is often experimentally derived for each diagnostic membrane[10, 11, 19, 32]. A major limitation of this method, however, is that it is limited to 1D domains.

3.2 Darcy's Law

Darcy's law[10, 33] is a phenomenologically derived Ohm's law-like model that relates pressure drop across a porous material to the average flow rate linearly as follows:

$$Q = -\frac{\kappa A}{\mu L}\Delta p = -\frac{\Delta p}{\left(\mu L / \kappa A\right)}$$

where Q is the volumetric flow rate, Δp is the pressure difference over length, L, κ is the permeability, and A is the cross-sectional area of the paper strip. The equation can be rearranged to reveal $\mu L / \kappa A$ as equivalent to electrical resistance and Δp equivalent to voltage (Fig. 2b). Darcy's law when used for modeling flow in the case of imbibition requires the solution of a moving boundary problem and is difficult to implement. Mendez et al.[34] have presented a comprehensive numerical solution to such a moving boundary problem. However, because of the mathematical and computational complexity involved in the solution, the use of Darcy's law in the paper microfluidics community has been restricted to modeling flow through fully saturated domains[11].

From the above discussion, it is noteworthy that both the Washburn equation and Darcy's law assume that a sharp fluid front exists and that the porous material behind the wetting front is fully saturated[33, 35]. However, from traditional models of fluid flow in porous media like soil, it is well known that the advancing fluid front is partially

saturated. This fact has largely been ignored by the paper microfluidics community[28, 36].

3.3 Richard's Equation

The phenomenon of partial saturation occurs when the porous material contains pores of multiple sizes. A direct consequence of partial saturation is that the capillary pressure induced by the material and the permeability of the material change with the extent of saturation. In contrast to Washburn equation and Darcy's law, the Richard's equation[28, 29] can be used to describe the motion of a fluid in partially saturated porous media[37] (Fig. 2c). This has been extensively used to model fluid flow in soil-based samples[38] and other porous materials[29]. The functional form of the Richard's equation most relevant to modeling flow in paper microfluidics is

$$\frac{\partial\theta}{\partial\psi}\frac{\partial\psi}{\partial t} = \nabla.\left(\frac{\kappa(\theta)}{\mu}\nabla(\psi(\theta)+\rho gz)\right)$$

This equation assumes that the capillary pressure, ψ, and permeability, κ, of the paper material are functions of saturation, θ, which represents the extent of saturation in the material. The term ρgz is the gravitational head, which can be neglected for paper strips placed on horizontal surfaces. This is a partial differential equation that can be solved for ψ as a function of space and time. To solve the equation, the functional relationships $\psi(\theta)$ and $\kappa(\theta)$ must be known. Recently, Perez-Cruz et al [28] demonstrated the use of the Richard's equation in modeling imbibition through 2D shapes of filter paper. However, the parameters relating the permeability and capillary pressure to saturation were obtained by fitting experimental data to the mathematical model because of the complexity in obtaining such parameters experimentally. Experimental measurement of $\psi(\theta)$ and $\kappa(\theta)$ has been demonstrated in other fields of study, e.g., in soil dynamics, but the paper microfluidics community is yet to adopt such methods[38, 39].

4 Common Geometries and Definitions

The aim of this section is to introduce the reader to some common geometries of paper networks that have become popular and to define some important terminologies. As researchers from a plethora of backgrounds have become interested in paper-based microfluidics over the last few years, there has been an inconsistency in the use of terminologies. Here, we provide a historic context of the terminologies as well as their current connotation.

4.1 Lateral Flow Assay

A lateral flow assay, by definition, is composed of a paper strip in which fluid flows in the plane of the paper along its length, i.e., laterally, as opposed to perpendicular to the plane like in filtration. In the field of diagnostics, the phrase "lateral flow assay" is often used synonymously with "lateral flow immunoassay" (LFIA). An LFIA is a specific kind of diagnostic device in which the sample flows through a test zone where antibodies for an analyte are immobilized, into a waste collection pad (Fig. 3a). LFIAs are restricted to 1D fluid flow. One must note, however, that a lateral flow assay need not necessarily be an LFIA. A large amount of recent literature features the use of a lateral flow assay for detection of nucleic acids[40, 41].

4.2 μPADs

These are a specific kind of paper microfluidic devices featuring distribution of one fluid (sample) into multiple test zones (Fig. 3b), where color changes occur corresponding to the concentration of analyte being assayed in each test zone. μPADs, as described in the original publication in 2007, featured 2D flow[42] and became popular for their ability to assay multiple analytes in small sample volumes. Within a few years, these designs were extended to include 3D flows by layering multiple layers of paper[43]–[45].

4.3 P-ELISA

These refer to paper ELISA (enzyme-linked immunosorbent assay) devices. Introduced in 2010, these devices are reminiscent of well plates (Fig. 3c) and contain circular porous regions where capture antibodies are spotted[46]. The sample, wash buffers, and signaling reagents are pipetted into these regions manually at fixed time intervals. These fluids flow perpendicular to the plane of the paper into a waste pad. It must be noted that because this design was first proposed by the Whitesides group, which first introduced μPADs, almost all other research groups that subsequently developed P-ELISA devices continued to refer to these devices as a type of μPAD.

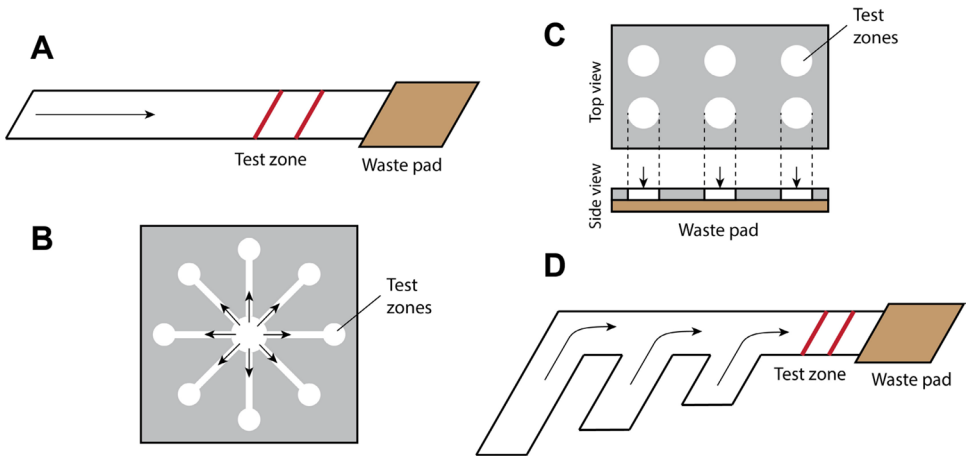

Figure 3: Common geometries used in paper-based microfluidic devices. **a** The traditional lateral flow assay featuring 1D flow. **b** Micro-paper analytical devices (μPADs) featuring flow of one fluid to multiple test zones. **c** Paper ELISA (P-ELISA) devices, featuring multiple reaction zones reminiscent of a well plate and flow perpendicular to the plane of the paper. **d** Two-dimensional paper networks (2DPNs) featuring flow of multiple fluids sequentially over a test zone. Arrows represent direction of flow.

5 2DPNs

These are a specific kind of paper microfluidic devices featuring flow of multiple fluids sequentially over a test zone (Fig. 3d). These gained popularity because sequential delivery enabled conducting more complex chemistries, e.g., signal-enhanced immunoassays [9].

5.1 Other Usage of Terminologies

Over the years, several of these terminologies have been used interchangeably. For example, any device that uses a paper-like porous material to wick fluid has commonly been referred to in literature as a 'microfluidic paper analytical device (μPAD), 'paper analytical device', or a 'paper-based microfluidic device'. However, in this article, we will stick to the original definitions of the terms as stated above.

5.2 A note on 2D vs 3D geometries

Following the advent of the early paper microfluidic designs highlighted in Fig. 3, several new paper microfluidic designs emerged that were essentially 3D variations of the original 2D designs. For example, the original 2D μPADs[47] were followed by 3D μPADs[43], and the original 2DPNs were followed by introduction of mechanical valves into such devices making them 3D[32]. It must be noted that the dimensionality of flow does not make a design particularly suitable for a specific type of assay. Instead, three-dimensionality adds fluidic capability and may enable handling higher number of samples or larger fluid volumes or introducing on–off valves/switches, at the cost of increasing device complexity. One must always design the simplest device that does the job. Thus, we propose that 3D devices only be considered where 2D devices do not provide the required fluid handling capability.

6 Fabrication Methods

The recent upsurge in the use of paper-based microfluidics has featured the development of many new methods of fabricating paper devices. In this section, we will briefly review the various fabrication techniques, which we have classified into two categories: i) chemical methods and ii) physical methods. A direct comparison of fabrication methods is presented in Table 1.

6.1 Chemical Methods

These methods generally involve the formation of hydrophobic regions on an otherwise hydrophilic porous paper substrate.

6.1.1 Wax Printing

Wax printing is a rapid, efficient, and inexpensive technique that has been used for fabricating μPADS. In 2009, two independent studies were carried out by Carrilho et al.[48] and Lu et al.[49] on wax printing. The fabrication process involved two steps: (i) hydrophobic solid wax was printed on the paper substrate, and (ii) wax was melted such that it penetrated the porous structure of the paper and formed hydrophobic barriers resulting

in well-defined microchannels on the paper. This process involves only a solid wax printer and a hot plate or an oven. However, the resolution of wax printing is coarse due to wax penetration in lateral directions, thereby reducing the sharpness of the hydrophobic boundaries. Carrilho et al.[48] established a model to account for the spread of the molten wax. Three different ways of patterning wax were introduced by Lu et al.[49]: (i) painting with a wax pen, (ii) printing with an inkjet printer followed by painting with a wax pen, and (iii) printing by a wax printer directly. Due to its simplicity, this method is well suited for large-scale batch production of μPADS. However, one of the biggest disadvantages of wax-printed devices is that they cannot handle surfactants and organic solvents which can dissolve wax.

6.1.2 Photolithography

In the first demonstration of a μPAD, Martinez et al.[8] used photolithography to pattern millimeter-sized hydrophilic channels surrounded by hydrophobic polymer. Chromatographic paper was patterned with SU-8 2010 photoresist using well-established methods used for fabricating PDMS microfluidic devices. μPADs fabricated using this method had very high resolution but the fabrication process was complex and involved many steps. It also resulted in reduced flexibility of the substrate. To circumvent the high cost of this photolithographic method, Martinez et al.[50] developed a low-cost variant of this method called fast lithographic activation of sheets (FLASH) where they impregnated the paper substrate with low-cost, home-made, photoresist derived from SU-8 resin. The method was based on photolithography but required just a UV lamp and a hot plate (patterning could be done by sunlight as well). No clean room or special facilities were required. In another method, Carrilho et al.[51] used an inexpensive photoresist formulation made from cyclized poly(isoprene) derivative that allowed rapid (15 min) prototyping of paper-based 96 and 384 microzone plates. He et al.[52] demonstrated a novel method for fabricating paper-based microfluidic devices by means of the coupling of hydrophobic silane [octadecyl trichlorosilane (OTS)] to paper filters followed by deep UV-lithography. One publication evaluated a novel and facile fabrication method for the μPADs using flash foam stamp lithography and compared it with other techniques such as wax printing and inkjet printing and found it to be convenient, quick and economic[53].

6.1.3 Wet Etching

Cai et al.[54] demonstrated a novel, simple and cost-effective method for fabricating μPADs based on the selective wet etching of hydrophobic filter paper. The procedure involved two steps—first the hydrophilic filter paper was made hydrophobic by immersing in 2.0% trimethoxyoctadecylsilane (TMOS) solution, and next, a paper mask penetrated with NaOH solution (containing 30% glycerol) was aligned on top of the hydrophobic filter paper, allowing the etching of the silanized filter paper by the etching reagent. The region covered with mask became hydrophilic while unmasked regions remained hydrophobic. This method did not involve any expensive equipment, a metal mask, or expensive reagents. However, this method had relatively low resolution, which could be improved by printing etching agents on the hydrophobic paper using an inkjet printer (Fig. 4b).

6.1.4 Screen Printing

Screen printing is an inexpensive method that has been used to print carbon electrodes directly on cellulose paper to perform bipolar electrochemistry[55]. A single pair of driving electrodes was used to control an array of 18 screen-printed bipolar electrodes (BPEs) simultaneously. The electro-generated chemiluminescence signal produced from the large array of BPEs was stable and reproducible. These results demonstrated the feasibility of coupling bipolar electrochemistry in microfluidic paper-based analytical devices (μPADs) to perform highly multiplexed, low-cost measurements.

6.1.5 Wax/Polymer Screen Printing

Dungchai et al.[56] reported a low-cost, simple and rapid fabrication method for μPADs which involved two steps: (i) patterning wax by rubbing through the screen onto paper filters, and (ii) melting the wax into the paper using a hot plate to form hydrophobic barriers. The final widths of the hydrophobic barrier and hydrophilic channel were found to be in the range of 1200–1800 μm and 550–1000 μm, respectively, at the optimal melting temperature and time. This method does not require expensive wax printers and the accompanying consumables. In addition, fabrication can be carried out without the use of a clean room, UV lamp, organic solvents or complex instruments. However, this method also suffers from loss of feature resolution due to wax spreading. A similar fabrication technique was used by Sameenoi et al.[57] to fabricate μPADs by replacing

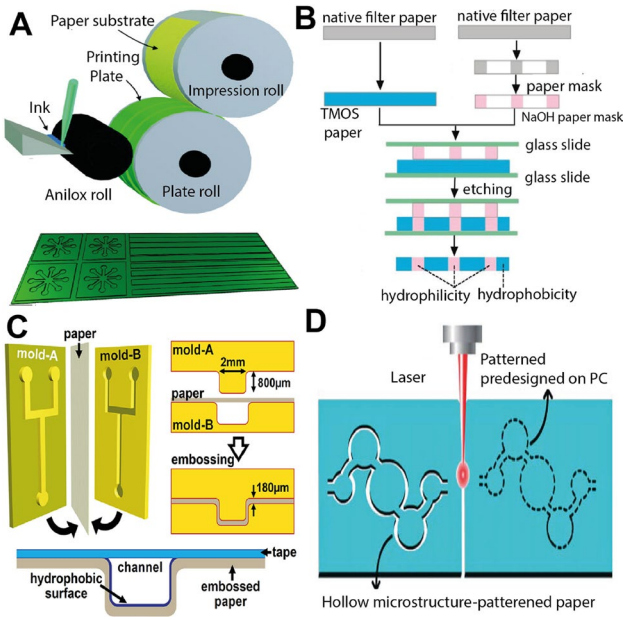

Figure 4: Schematic illustration of some fabrication techniques. **a** Flexographic printing. **b** Wet etching. **c** Embossing. **d** Laser cutting **a** adapted with permission from Ref. [61]. Copyright (2010) American Chemical Society **b** adapted from Ref. [54], with the permission of AIP Publishing **c** adapted with permission from Ref. [69]. Copyright (2014) American Chemical Society. **d** reproduced from Ref. [72] with permission of The Royal Society of Chemistry.

wax with polystyrene solution. The device formed was flexible and could be used for applications where the device may require bending or folding.

6.1.6 Laser Treatment

Laser treatment is a non-contact, mask-less and non-lithographic method which involves polymerization of a photopolymer. The laser-based procedure is used to create hydrophilic fluidic channels on a paper substrate that has been previously impregnated with a hydrophobic material. The technique demonstrated by Sones et al.[58] also offered the possibility to control the patterning process by tuning laser parameters such as wavelength, pulse duration and repetition rate. In their study, they found the minimum width for the hydrophobic barriers that successfully prevented fluid leakage was ~ 120 μm and the minimum width of the fluidic channels that can be formed was ~ 80 μm. Chitnis et al.[59] were able to selectively modify the surface structure and properties (hydrophobic to hydrophilic) of several papers using a CO_2 laser. This process is suited for rapid prototyping at preliminary stages as well as final device optimization.

6.1.7 Plasma Treatment

This novel method of fabrication of μPADs involves making the paper hydrophobic using a hydrophobization agent followed by plasma treatment in the presence of a photomask to make the treated regions hydrophilic. Li et al.[60] used alkyl ketene dimer (AKD) along with *n*-heptane as the hydrophobization agent on Whatman filter paper. μPADs fabricated using this technique retained the flexibility of paper as well as surface topography. The major advantage of this method was that it also allowed the building of simple functional components such as control switches, microfilters, and microreactors. But a known problem with this method is overstretching of the substrate under a mask. However, this overstretching could be controlled by optimizing the plasma treatment intensity and time.

6.1.8 Flexographic Printing

Flexographic printing involves the printing of polystyrene polymer that forms liquid guiding boundaries and allows the formation of hydrophobic barriers in the substrate. Other hydrophobizing agents can also be used such as alkyne ketene dimer, poly(methyl methacrylate), and cross-linked polyvinyl alcohol. Olkkonen et al.[61] reported that a minimum width of 400 μm of the hydrophobic region was required to obtain a

leak-free structure. Ink-spreading in flexographic printing was found to be negligible. A significant advantage of flexographic printing is that biomolecules and other reagents required in analytical and diagnostic tests can also easily be printed on paper substrates. Flexographic printing can be done in a roll-to-roll process ideal for large-scale production (Fig. 4a).

6.1.9 Inkjet Printing

Inkjet printing is a low-cost and high-resolution technique based on dot-on-demand technology, which enables jetting of ink droplets onto cellulose paper. It involves printing of hydrophilic–hydrophobic contrast on the substrate. It is a non-contact printing process, which offers the advantage of minimum cross-sample contamination. Inkjet printer prints hydrophobizing 'ink' only on one surface of the paper, which penetrates the paper structure. Li et al.[62] printed alkenyl ketene-dimer-heptane solution onto untreated filter paper using a reconstructed inkjet printer. This method can also be used to print desired patters of reagents and offers enormous potential for mass production of microfluidic sensors at a low cost. Koo et al.[24] fabricated electrowetting valves for paper fluidic devices by inkjet printing and spraying conductive hydrophobic electrodes beside conductive hydrophilic electrodes. By changing an electrode from hydrophobic to hydrophilic state, a valve was created. Rajendra et al.[63] demonstrated thermal inkjet printing of silicone precursors (siloxanes) onto porous filter papers that were rapidly converted into hydrophobic silicone resin barriers. The resulting barriers resisted penetration by surfactant solutions and even by lower surface energy solvents, which are frequently used in biological assays. Abe et al.[64] also used the inkjet printing method for fabrication of entire microfluidic multi-analyte chemical sensing devices made from paper suitable for quantitative analysis.

Various other techniques were also used by various research groups for fabricating paper-based microfluidic devices such as chemical vapor phase deposition[65], PDMS-printing using x–y plotters[66], etc. However, we will not be presenting the details of those methods here.

6.2 Physical Methods

As the name suggests, these methods involve physically modifying/cutting the paper material to form flow channels.

6.2.1 Knife Cutting/Plotter

Fenton et al.[67] demonstrated a low-cost method of fabricating multiplexed lateral flow assays by shaping thin-sheet porous media in two dimensions using a computer-controlled X–Y knife plotter. Other than bio-reagents, no chemicals were used in the fabrication of the device. Glavan et al.[68] fabricated pressure-driven, open-channel microfluidic systems with features sizes as small as 45 μm carved in omniphobic paper using electronic craft cutting tool/engraving tool. Vapor phase silanization of paper was done after carving with alkyl or fluoroalkyl trichlorosilane making it hydrophobic while preserving its high gas permeability and mechanical strength. The carved conduits were sealed with tape and were found to be capable of guiding transporting liquids in the low-Reynolds regime.

6.2.2 Embossing

Thuo et al.[69] introduced a new technology that enabled paper to be used as a pressure-driven open-channel microfluidic system. They sandwiched paper between two complimentary shaped dies and compressed it into a channel by applying pressure (~ 0.2 kg/cm^2). Paper was made omniphobic by carrying out solid–vapor silanization reaction. Dies for embossing were generated using 3D printing (Fig. 4c). Shin et al. [70] developed barriers resistant to organic solvents and surfactants. This technique involved applying pressure on a nonwoven polypropylene (PP) sheet using a steel mold. The embossed region acted as a physical barrier which prevented the flow of liquid. They reported minimum embossing pressure required for the leakproof barrier as 294 MPa. The main advantage of embossing is that it is a single step process and suitable for mass production.

6.2.3 Laser Cutting

Spicar-Mihalic et al. [71] fabricated paper-based microfluidic devices using a commercially available CO_2 laser cutter. This machine allowed controlled through-cutting and ablative etching of nitrocellulose substrates. In addition, laser cutters can be used to cut a variety of components that are useful in the fabrication of paper-based devices like cellulose wicking pads, glass fiber source pads, and Mylar-based substrates. It involves only a single step as compared to various other fabrication techniques which require multiple steps. Minimum feature size obtained by CO_2 laser cutter was 150 μm. The depth of cut could

easily be modified by varying the laser power, speed, and a number of passes. Nie et al.[72] fabricated hollow structures in paper to create μPADs with the help of commercially available mini type laser cutting/engraving. The average width of the hollow structure obtained was ~ 0.4 mm. This method enjoys high reproducibility and is suitable for large-scale production of μPADs (Fig. 4d).

7 Flow Control/Valving Techniques

The lateral flow assay (LFA; Fig. 3a) is an ingenious format for rapid and easy-to-use diagnostics, but it is fundamentally limited to assay chemistries that can be reduced to a single chemical step. However, multi-step paper-based assays, such as enzyme-linked immunosorbent assay (ELISA) involve more complex sequences that are not easily implemented as a one-step dip and read operation. Up until 2013, microfluidic paper-based devices lacked crucial components for fluid manipulation. Since 2013, however, several new methods have been developed to control fluid flow and automate multi-step reactions in paper (Fig. 1a). These methods represent a set of paper fluidic control tools, i.e., analogs to valves in conventional microfluidics that could be used to manipulate fluids within paper for precise timing of reagent delivery and metering of reagent volumes. Methods to control flow in paper-based microfluidic devices can broadly be classified into three categories: (i) geometry-based methods, (ii) chemical-based methods, and (iii) mechanical methods. A direct comparison of flow control/valving techniques is presented in Table 2.

7.1 Geometry-Based Methods

This strategy involves controlling flow rates by simply changing the geometry of channels. This can be accomplished by varying the length or the width of the channels[73] [Fig. 5a (i)] and using multiple volume-metered source pads. Fu et al [11] used different lengths of channels and different sizes of volume-metered pads to release different volumes of fluids in channels. Lutz et al.[74] controlled the shut-off time of flow by submerging paper legs of different lengths into a volume-limited common well, which disconnected from the legs after a pre-defined volume of fluid passed. Toley et al.[75] used a shunt (absorption pad) to the main fluid flow path, which produced a tunable time delay (3–20 min) in the main path. The time delay could be varied by changing the dimensions of the shunt, i.e., thickness and length of the shunt material [Fig. 5a (ii)]. Mendez et al.[34] experimented with changing the shape of a downstream wicking region. They mimicked the sustained flow provided by a thicker wicking pad usually attached at the end of a lateral flow strip by replacing the wicking pad with fan shapes of the membrane itself. These fan-shaped devices provided a continuous increase in un-wetted pore volume which caused a deviation from Lucas–Washburn dynamics and led to quasi-stationary flows in channels [Fig. 5a (iii)].

7.2 Chemical Methods

This strategy involves the use of chemicals to create temporary barriers or temporary flow channels and allows the user to control the flow rates and/or create time delays. Lutz et al.[21] created tunable delays in paper by drying different amounts of sugar in the channels. Solid sugar blocked flow and flow resumed after sugar dissolved [Fig. 5b (ii)]. Koo et al.[24] developed valves based on the principle of electrowetting of dielectrics, i.e., a hydrophobic electrode could be turned into a hydrophilic electrode by passing electric current. They successfully used it to stall fluid flow at the hydrophobic electrode and initiate it by passing current through the electrode. Houghtaling et al.[76] developed dissolvable sugar bridges as shut-off valves with a tunable range of passage volumes [10–80 μL; Fig. 5b (i)]. Similarly, Jahanshahi-Anbuhi et al.[23] used erodible polymeric bridges made of water-soluble pullulan films serving as time-controlled shut-off valves. Chen et al.[25] developed a single-use fluidic diode, i.e., a method in which fluid could only flow in one direction. The diode consisted of a hydrophilic region (surfactant) placed next to a hydrophobic region. Fluid approaching from the hydrophobic side did not pass the diode but fluid approaching from the hydrophilic side flowed through [Fig. 5b (iii)]. Noh et al.[77] developed a method of controlling the flow rate of fluid through paper channels by controlling the amount of paraffin wax deposited in the channels.

7.3 Mechanical Methods

This strategy involves mechanical motion to connect or disconnect channels. Toley et al.[20] designed one of the first set of multi-functional valves. i.e., on-switches, off-switches and flow-diversion switches that can be programmed to actuate automatically. The fundamental mechanism to actuate the valve involved displacement of one end of the paper channel, thereby causing connection or disconnection with other channels, triggered by the arrival of fluid at the actuators. Compressed sponges that expand when wet were

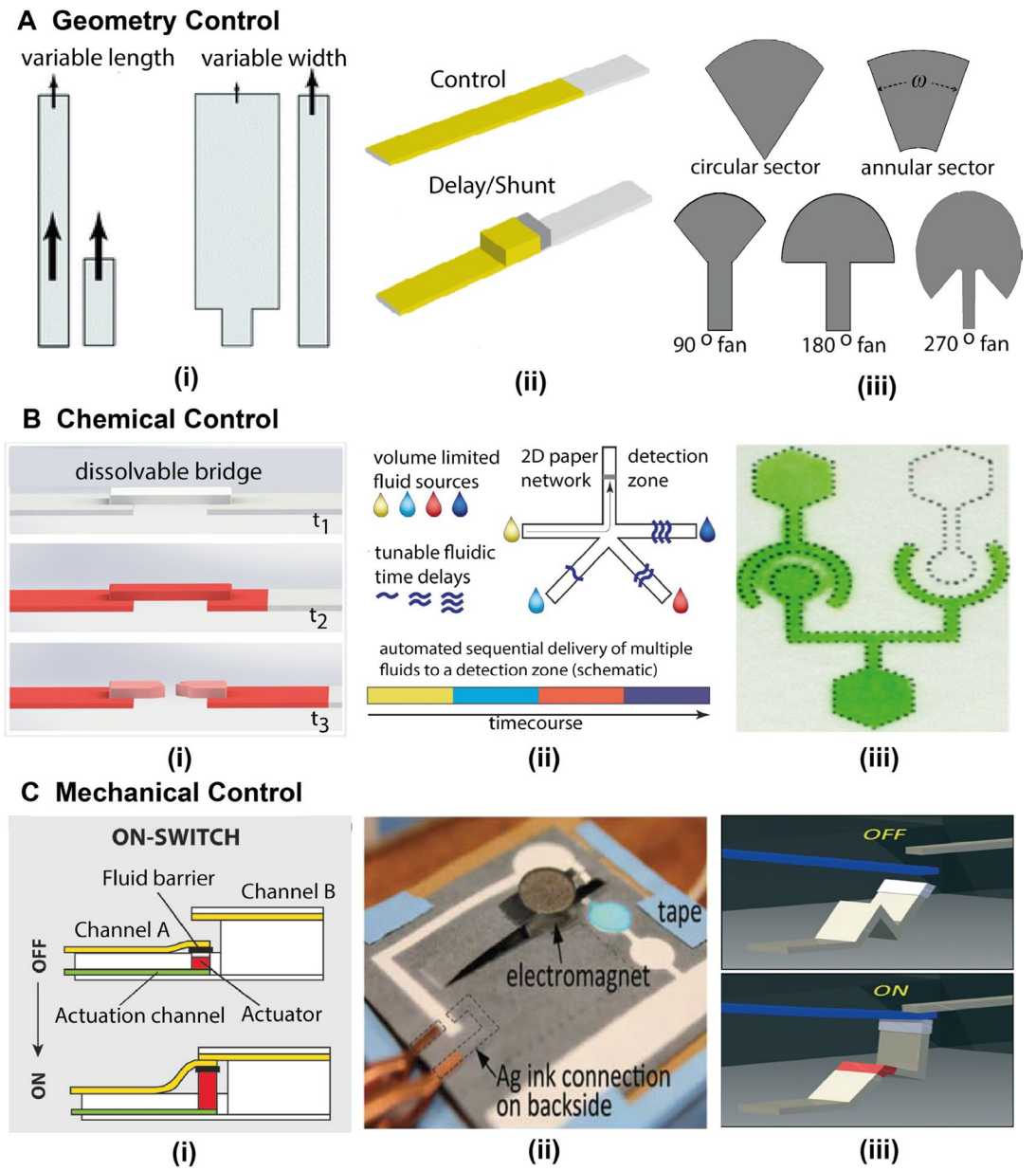

Figure 5: Schematic illustration of various valving techniques developed for paper microfluidic. **a**. Geometry control. **b.** Chemical control. **c.** Mechanical control **(i)** Variable length and width adapted from Ref. [73] with permission of The Royal Society of Chemistry. **(ii)** Tunable delay shunts. Adapted with permission from Ref. [75]. Copyright (2013) American Chemical Society. **(iii)** 2D complex shapes. Adapted with permission from Ref. [34] Copyright (2009) American Chemical Society. **b (i)** dissolvable bridge valve. Adapted with permission from Ref. [76] Copyright (2013) American Chemical Society. **(ii)** Dissolvable fluidic time delay. Adapted from Ref. [21] permission of The Royal Society of Chemistry. **(iii)** a fluidic diode valve. Adapted from Ref. [25] with permission of The Royal Society of Chemistry **c. (i)** compressed sponge actuation valve. Adapted from Ref. [20] with permission of The Royal Society of Chemistry. **(ii)** Electromagnetic valve. Adapted from Ref. [22] with permission of The Royal Society of Chemistry. **(iii)** Folded paper actuator valve. Adapted from Ref. [79] with permission of The Royal Society of Chemistry.

used as actuators [Fig. 5c (i)]. Both time-metered and volume-metered valves were demonstrated. Li et al.[22] developed one of the first magnetic timing valves using an electromagnet and ferromagnetic nanoparticle-embedded PDMS. This device contains a paper timing channel with an ionic resistor, which can detect the event of a solution flowing through the resistor and trigger an electromagnet to open or close a paper bridge valve [Fig. 5c (ii)]. Kim et al. [78] developed a novel and

bifunctional mechanical paper fluidic control system comprising a linear push–pull solenoid and an Arduino Uno microcontroller. The solenoid pressure-driven valve (PDV) enables not only ON–OFF control but also controlling flow velocity. Kong et al.[79] developed a control valve by folding chromatography paper multiple times to crate crests and troughs. The folded paper could be moved by addition of fluid at critical places, which actuated valves [Fig. 5c (iii)]. Li et al.[80] fabricated manually actuated valves by incorporating hollow rivets that could be used as hinges to rotate paper channels to connect or disconnect with other channels.

To summarize, all three strategies of valving have certain advantages and disadvantages. Geometry-based valving methods are inexpensive, involve less complexity, and do not release chemicals into the flow, but their functionality is limited. Chemical-based methods enable slightly enhanced functionality, but release of chemicals into channels may hinder the chemistry being performed. Mechanical methods are the most versatile method of valving but involve moving parts that may be more difficult to fabricate. The use of electronic components to implement valves in paper-based microfluidics seems to be a new trend and may enable more sophisticated timing control.

8 Applications in Sensing/Diagnostics

The primary motivation for the development of paper-based microfluidic devices over the past decade has been cost reduction associated with using simple materials and elimination of ancillary equipment, which has tremendous advantages in rapid and low-cost diagnostics. It is, therefore, easy to imagine that paper microfluidic devices have found diverse applications in the general area of sensing/diagnostics. In this major section of this review, we will discuss the applications that paper-based devices have found in conducting three types of diagnostic assays: (i) multiplexed color change chemistries, (ii) signal-enhanced immunoassays, and (iii) nucleic acid amplification tests.

Before we proceed to describing the different devices, it is worthwhile to briefly discuss the different detection mechanisms compatible with paper. One of the primary advantages of using paper as a substrate for detection is that its base white color provides an excellent background for high-contrast imaging. However, several other methods of signal detection have been used, primarily to enhance the sensitivity of signal readout. One classic readout method is electrochemical, the most well-known example of which is the commercially available glucometer[81]. In addition, several other methods such as fluorescence[82], chemiluminescence[83, 84], electrochemiluminescence[85, 86], and surface-enhanced Raman spectroscopy (SERS) have successfully been used. A comparison of these detection methods is outside the scope of this review as these methods are independent of the use of paper. However, it is noteworthy that these different detection mechanisms can, in theory, be coupled to any kind of assay, e.g., immunoassays or nucleic acid detection assays. Combinations of the type of assay performed and the detection method used have led to the development of many novel diagnostic device designs.

8.1 Multiplexed Color Change Chemistries

The idea of assaying multiple analytes in a single sample in paper-based devices was popularized by the original μPAD designs (Fig. 3b). Such devices have recently been used extensively for a wide range of applications. The working concept behind most of these devices is essentially the same, i.e., distribution of a sample fluid volume into multiple detection zones by wicking. We categorize these devices into multiple application areas: (i) clinical diagnostics, (ii) pharmaceutical analysis, and (iii) environmental analysis.

8.1.1 Clinical Diagnostics

Some clinically relevant body fluids used for bioanalytical sensing are urine, saliva, sputum and blood; the target analytes in these samples could be a host of biomarkers ranging from proteins, hormones, small molecules like glucose, uric acids etc.[87–89]. Developments in the field of sensing clinically relevant analytes are briefly discussed below.

The first described μPAD was designed for multiplexed detection of glucose and bovine serum albumin (BSA) spiked in urine samples [90, 91] [Fig. 6a (i)]. Bruzewicz et al. reported a variant of this design that could be fabricated using an X–Y plotter pen [Fig. 6a (ii)] [66]. In another extension of this work, multiple analytes were detected in 3D 'universal μPADs' [Fig. 6a (iii)] [44]. For an extensive study on urine analysis, the reader is referred to the review paper by Lepowsky et al.[92]. Blood is another extensively used sample for clinical analysis. Blood is composed of blood cells (RBCs, white blood cells, platelets, etc.) and plasma (which contains the biomolecules and

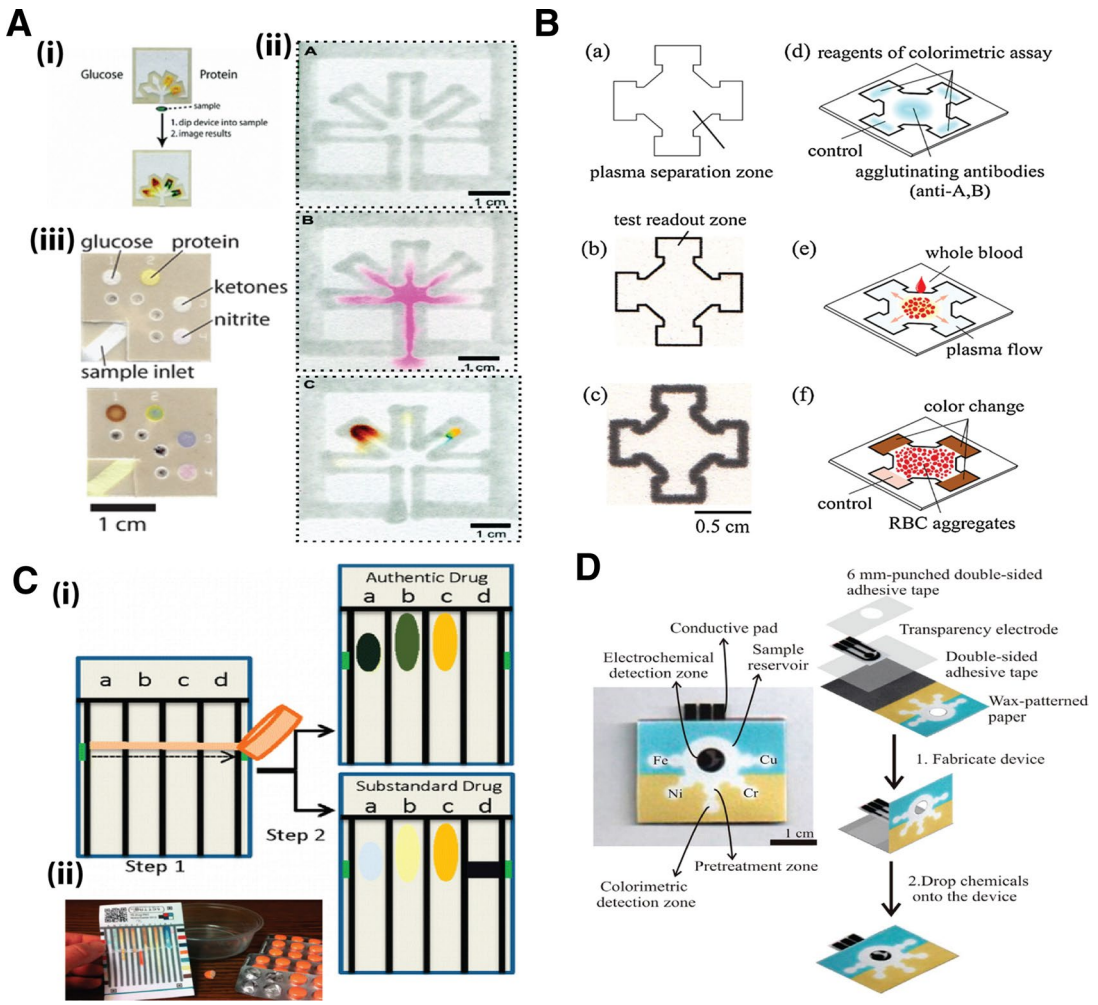

Figure 6: Multiplexed colorimetric detection. **a**, **b** Applications of μPADs for clinical diagnostics separation of various components of blood–blood cells and plasma, followed by detection of glucose in plasma. **c** Application of μPADs in pharmaceutical field (i) schematic depicting the protocol for testing quality of drugs, (ii) device showing twelve lanes for detection of multiples drugs. **d** A hybrid μPAD containing both electrochemical and colorimetric detection of six different analytes **a** (i) the first μPAD designed for the colorimetric detection of glucose and protein, reprinted with permission from ref [45] Copyright [2013] American Chemical Society. (ii) Modified version of the μPAD for the detection of glucose, BSA and pH sensing in artificial urine samples, reprinted with permission from ref [66]. Copyright [2013] American Chemical Society. (iii) Multiplexed sensing of glucose protein, ketones and nitrites, reproduced from ref [44] with permission from The Royal Society of Chemistry. **b** reproduced from ref [94] with permission from The Royal Society of Chemistry. **c** reprinted with permission from ref [99]. Copyright [2013] American Chemical Society. **d** reprinted with permission from ref [108]. Copyright [2014] American Chemical Society.

proteins)[93]. Conventional blood analysis requires centrifugation and involves expensive instruments. Paper could potentially be used to replace these. Towards this goal, Khan et al. developed a paper device to separate the components of blood through agglutination of blood cells followed by flow of plasma into multiple test zones, where color change reactions were conducted[94] (Fig. 6b). Yang et al. developed a simple paper device to separate blood cells from plasma along with measurement of glucose levels[95]. Use of μPADs for clinical diagnostics is an extremely rapidly growing area of research and recently well-reviewed by Sher et al.[96].

8.1.2 Pharmaceutical Analysis

Supply of low quality pharmaceutical products can occur because of several reasons such as

counterfeit drugs, supply of substandard products, damage due to poor storage conditions, etc. This is a serious global health concern. Development of paper-based devices for screening large number of samples for substandard drugs presents an exciting opportunity. A vast majority of the published literature in this field is focused on screening pharmaceutical drugs for quality control, whereas there is some literature applied to screening of pharmaceutical residues in food, environmental samples, and biological fluids[97]. In one of the first publications, Shimora et al. developed a method to check the level of 4-aminophenol in paracetamol-containing medicines using Whatman filter paper[98]. The two compounds were separated and detected separately. In another well-known colorimetry-based detection method, the Lieberman group used paper-based devices to screen dosage forms containing the beta lactam antibiotics or combinations of the four first-line antituberculosis drugs[99]. The device contained a panel of 12 strips having different colorimetric indicators (Fig. 6c). The steps of using this device are briefly demonstrated in Fig. 6c (i). The user must simply rub the powdered drug onto different lanes, and then dip the bottom of the device in water. Color change reactions in all lanes report for active drugs and common fillers found in counterfeit drugs. The Lieberman group subsequently extended this idea to develop BioPADs—biologically based paper analytical devices (BioPADs) to detect antibiotics of the tetracycline family, which could be used to test antibiotic contamination in liquids[100]. In another article, detection of three commercially available drugs was performed using a paper-based kit and colorimetric detection using an iPhone[101]. Craig et al. used wavelength-modulated Raman spectroscopy (WMRS) to develop a sensitive paper-based device for the detection of paracetamol and ibuprofen[102]. Murphy et al.[103] developed μPADs for the detection of ascorbic acid (AA) and dopamine (DA) in biological fluids. Electrochemical paper-based microfluidic devices were also used for in situ screening of anticancer drugs in a multiplexed manner[104]. Gomes et al. demonstrated a colorimetric assay on paper strips to detect oxytetracycline in spiked environmental water, a widely used antibiotic in aquaculture[105]. For further information in this area, readers are referred to the following focused reviews on the applications of PADs for in pharmaceutical analysis[97, 106].

8.1.3 Environmental Analysis

μPADs for environmental applications are comparatively less popular than those in the field of biomedical assays for diagnostics[107]. In one of the first articles, Nie et al. designed a device for multiplexed detection of Ni, Fe, Cu, Cr, Pb and Cd—common metal contaminants in soil, water, air and food[108]. They designed a hybrid three-dimensional μPAD (Fig. 6d) combining both colorimetry and electrochemical detection techniques in separate layers owing to the fact that the concentrations of the environmental contaminants vary in orders of magnitude in space and time[108]. The sample was passed laterally to four segregated channels enabling colorimetric detection of Cu, Ni, Fe and Cr (which are present at comparatively higher concentrations), and the sample also travelled vertically to the bottom layer for electrochemical detection of Pb and Cd. In another design, Fe, Cu and Ni from an air filter were detected simultaneously in a μPAD. The air filter having particulate matter was placed in the device and upon addition of water, the sample got transported to detection zones where colored complexes were formed[109]. Jayawardane et al. developed several devices for detection of contaminants in waste water[110–112]. For further details on various devices developed for environmental applications, we direct the readers to some focused review articles[88, 113].

Apart from the above-mentioned applications, μPADs have also found application in the food industry in detection of pesticides in food and beverages[114, 115], neurotransmitters in biological samples[116], and in wine quality analysis[117].

8.2 Signal-Enhanced Immunoassays

Another important application area of paper-based microfluidic devices has been conducting immunoassays that can surpass the sensitivity of traditional LFIAs. This has primarily been enabled by clever engineering to make new shapes and structures from paper that enable conducting multi-step fluidic operations, e.g., sample delivery, washing, delivery of substrates for a color change reaction, etc. Three fundamentally different device designs have been used to accomplish this: (i) 2DPNs, (ii) P-ELISA, and (iii) sliding devices. We will now review the development in each of these categories.

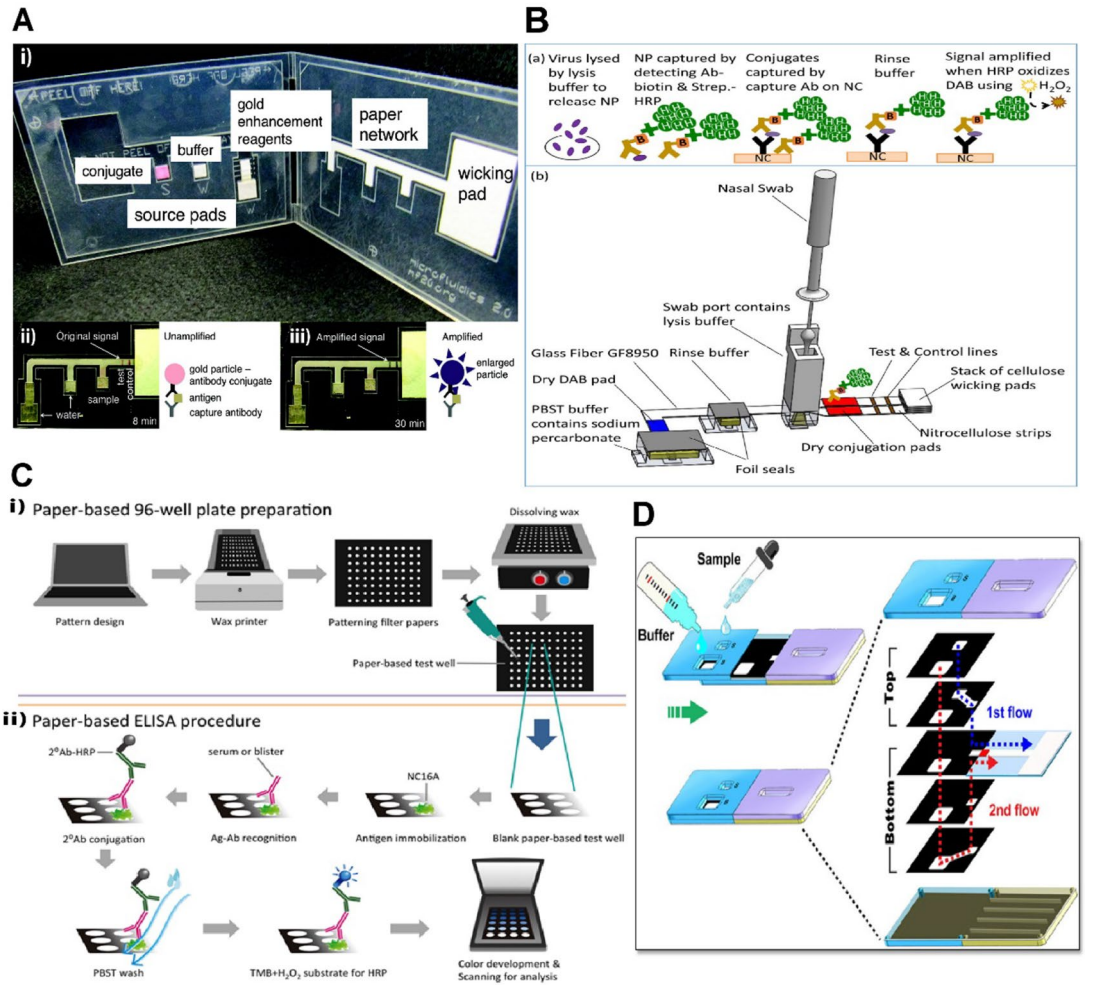

Figure 7: Signal-enhanced immunoassays. **a**, **b** 2DPNs for conducting sandwich immunoassays. **a** (i) 2DPN design. (ii) and (iii) 2DPN assay results for a sandwich immunoassay using gold as label without signal amplification (ii) and with signal amplification (iii). **b** Automated single step 2DPN activation device for influenza detection. **c** (i) Schematic of fabrication of P-ELISA devices. (ii) schematic of operation steps in P-ELISA devices. **d** Schematic representation and operating principle of a 3D slip-PAD **a** reprinted with permission from ref [11]. Copyright [2012] American Chemical Society. **b** reprinted with permission from ref [122]. Copyright [2017] American Chemical Society. **c** reproduced from ref [123] with permission from The Royal Society of Chemistry. **d** reprinted from ref [134].

8.2.1 Two-Dimensional Paper Networks (2DPNs)

2DPNs, defined in Sect. 3 (Fig. 3d) of this review, automate sequential delivery of multiple fluids to a test zone. In a typical 2DPN, the sample (along with a conjugated secondary antibody) enters from one leg, a wash buffer enters from a second leg, and a signal amplification reagent enters from a third leg (Fig. 7a). The improvement in the limit of detection (LOD) of various biomarkers of clinical importance such as hCG (human chorionic gonadotropin) and PfHRP2 (malarial antigen) has been demonstrated in the 2DPN assay format[118, 119]. In one of the first demonstrations, an ~ 4× improvement in LOD for detection of hCG was demonstrated[120]. Commercially available gold enhancement reagents were used for signal amplification. In a similar subsequent study, Fu et al. demonstrated a 4×-improvement in the LOD for the detection of PfHRP2[11]. Traditional ELISA assays typically use an enzyme-linked secondary antibody and a colorimetric substrate like diaminobenzidine (DAB), which is converted into a colored product, catalyzed by the enzyme. This strategy can improve LODs substantially and has also been successfully implemented in 2DPNs. Ramachandran et al. reported an automated 2DPN card for performing a sandwich ELISA using a horseradish peroxidase (HRP)-DAB

enzyme–substrate system[121]. This study also demonstrated long-term dry preservation of reagents for performing ELISA. Activity of HRP-labeled antibodies and DAB was retained even after storage at 45 °C for 5 months, making the device ideal for POC testing at low-resource settings. Huang et al. have demonstrated a fully integrated disposable device based on 2DPNs for performing a sandwich immunoassay to detect influenza A and B with a simple single activation step of inserting a nasal swab and closing the device (Fig. 7b)[122]. The total test time for the device from activation to result was approximately 35 min. Performance of the prototype device was tested in a hospital yielding a success rate of 92%.

8.2.2 P-ELISA

P-ELISA devices, defined in Sect. 3 of this review (Fig. 3c), are essentially paper-based well-plates with wicking pads to absorb fluids placed under the paper surface. A schematic of a typical procedure for fabrication and operation of a P-ELISA device is shown in Fig. 7c[123]. Cheng et al. demonstrated significantly reduced assay time, reagent volumes, and cost per assay compared to ELISA using a 96 micro-zone P-ELISA, but the sensitivity was 10× -lower compared to conventional well plate-based ELISA[46]. Several studies have been conducted using 96 micro-zoned paper plates to improve the sensitivity, quantification, accuracy and choice of test cut-off values[8]–[126]. Le et al. described a pen type pH meter-based portable device to increase the sensitivity of P-ELISA making it comparable to conventional ELISA[127]. In a novel use of the detection method, Chen et al. developed a paper-based SERS immunoassay for mouse IgG as a model analyte[128]. Polymerization-based signal enhancement was another technical innovation in which eosin, a photo-initiator, was used as a label and coupled to the detection antibody[129]. Sample was added to the test region containing the capture antibody, followed by addition of eosin-labeled detection antibody. The sandwich immunocomplex formed was then exposed to monomers that got polymerized, catalyzed by eosin, which is a photo-initiator. The resulting hydrogel formed on the positive test zone produced a bright pink color on addition of NaOH, which was used for colorimetric detection. Lathwal et al. compared enzymatic-based, nanoparticle-based, and polymerization-based signal amplification and demonstrated that each colorimetric test has a unique set of optimal conditions[130]. In a recent article, Ortega et al. demonstrated a paper-based magnetic ELISA for detection of dengue that can achieve 700× lower LOD compared to traditional ELISA[131].

8.2.3 Sliding Devices

Sliding devices are an innovation that enables eliminating pipetting steps by replacing them with sliding operations, so they can be conducted by minimally trained users, making them ideal for POC use. A portable paper-based sliding device for performing an ELISA for rabbit IgG as a model analyte was designed to minimize operator steps[132]. The movable strip containing the test zone was moved through a series of zones where one reagent was delivered at a time. Though the assay was faster compared to conventional ELISA, the limit of detection was 5× higher. In an extension of this device, a sliding strip device in which reagents were dry stored at different positions was developed for detection of C-reactive protein, a marker of neonatal sepsis[133]. The sensing zone could be positioned to be under one of the dried reagent reservoirs, and water could be added to deliver that reagent to the sensing zone. The total assay time was 90 min which was significantly less than conventional well-plate ELISA (>3 h). Han et al. developed a more user-friendly 3D slip-PAD device (Fig. 7d) enabling sequential delivery of multiple reagents with a single user activation step[134]. Sample and buffer solution were added to respective inlet ports and the top section was slid over the bottom section initiating the assay. Different paper path lengths for different reagents resulted in automatic timed delivery of reagents to the test zone.

8.3 Nucleic Acid Amplification Tests

The genetic material of any organism is its unique identifier and nucleic acid amplification tests (NAATs) exploit this principle for the detection of various biological species. NAATs have widely been used for identification of pathological species like bacteria and viruses which cause infectious diseases[135]. They are also used for detecting genetic variants to identify vulnerability towards genetically inherited diseases[136], detection of mutated strains of pathogens [137], and for DNA manipulation in genetic engineeringtechniques[138, 139]. As the name suggests, NAATs amplify the existing genetic material (DNA or RNA) in a sample, producing large copy numbers that are compatible with standard detection mechanisms based on fluorescence, colorimetry, pH or electrical measurements.

NAATs provide a platform for development of extremely specific and sensitive assays with limits of detections going down to a few copies of the pathogen.

Currently, the polymerase chain reaction (PCR) is the gold standard for NAATs and is being widely used in clinical laboratories for diagnosis. However, PCR involves rapid and continuous temperature cycling, which requires an extremely sophisticated instrument with efficient temperature control, often prohibitively expensice[140]. A new class of isothermal NAATs is becoming increasingly popular over the past few years as these could potentially overcome some of the limitations associated with PCR. Instead of temperature cycling, isothermal NAATs operate at a single temperature. There exist a wide variety of isothermal NAAT chemistries, e.g., loop mediated isothermal amplification (LAMP), helicase dependent amplification (HDA), recombinase polymerase amplification (RPA), rolling circle amplification (RCA), strand displacement amplification (SDA), which have been extensively discussed in numerous reviews[141, 142].

Since NAATs are highly sensitive and specific, there existed a need of pairing them with a platform that is affordable, equipment-free and deliverable to the end users to make them amenable to POC use. Paper devices have found an important role in achieving this target[90, 143]. An initial analysis of suitability of paper matrices for NAATs was carried out by Rohrman et al.[144], wherein glass fiber (GFCP203000, Millipore), cellulose (CFSP223000, Millipore), GF/DVA, MF1, VF2, and Fusion 5 (Whatman) were compared for compatibility with RPA. These materials had different physical properties in terms of membrane thicknesses, matrix compositions, porosity, and fluidic absorbance and these characteristics played a vital role in selection of membranes for various purposes. For instance, in this study the sample wick and absorbent pad were composed of cellulose while the reaction pad was composed of glass fiber. Another significant study has been carried out by Linnes et al.[145] for comparing the suitability of five different paper substrates as platforms for DNA/RNA amplification. DNA/RNA amplification reactions were carried out in cellulose paper, glass fiber, nitrocellulose, polyethersulphone (PES) and polycarbonate using techniques of LAMP, HDA and PCR. It was found that PES proved to be the most suitable membrane for LAMP and HDA, while none of the membranes supported PCR. There have been multiple proof-of-concept studies in the literature where paper-based amplification has been successfully demonstrated using isothermal techniques like LAMP[145–154], RPA[144, 155, 156],HDA[145, 157–159],RCA[160] and iSDA[161]. Clearly, LAMP has been the isothermal nucleic acid amplification technique used by most number of studies followed by RPA and HDA. The paper matrices used as amplification platforms in these studies have varied widely and have included Whatman #1 chromatography paper[151, 154, 156, 157, 160, 162], glass fiber[144, 147, 148, 153, 159, 163], PES[145, 152, 163], FTA Membranes[149–151], and 3MM CHR chromatography paper[158]. Since NAATs require sample processing to obtain purified DNA/RNA for the amplification step, the FTA card, which is a commercially available paper membrane containing a DNA stabilization matrix, has been the matrix of choice for multiple studies that have demonstrated DNA/RNA extraction followed by amplification.

An ideal sample-in-to-answer-out POC diagnostic device would be one that takes in a crude biological sample and provides a simple readout without user intervention. Till date, there have been two studies in the literature that have accomplished this level of systems integration. The first study from the Paul Yager group[161] presented a prototype named MAD NAAT (Multiplexable autonomous disposable nucleic acid amplification test; Fig. 8a). The device was designed to detect *Staphylococcus aureus* (*S. aureus*) starting with patient nasal swab samples. Two different gene targets were chosen to detect methicillin-resistant strains (*ldh1* gene for *S. aureus* and *mecA* for methicillin resistance). On introduction of the nasal swab sample and pressing of the actuation button, the entire diagnosis mechanism was automated. All the rehydration buffers and dried reaction reagents were pre-stored in the device. Achromopeptidase (ACP) enzyme mix was used for chemical lysis of the cells followed by heating at 95 °C for 10 min to denature ACP and fragment the DNA. The sample was then branched into two standard 17 glass fiber membranes, each of which contained dried amplification reagents, sequence-specific probes, and gold nanoparticles for lateral flow detection (LFD). After completion of the amplification step, paper-to-paper valves released the amplification products into nitrocellulose strips for LFD. This study also looked at the stability of lyophilized reagents at 23 and 40 °C at 2–3% relative humidity. A strikingly significant feature of this device was the incorporation of internal amplification controls that proved very useful in ruling out false-negative amplification results.

The second fully integrated device was presented by Tang et al.[159] and was designed to

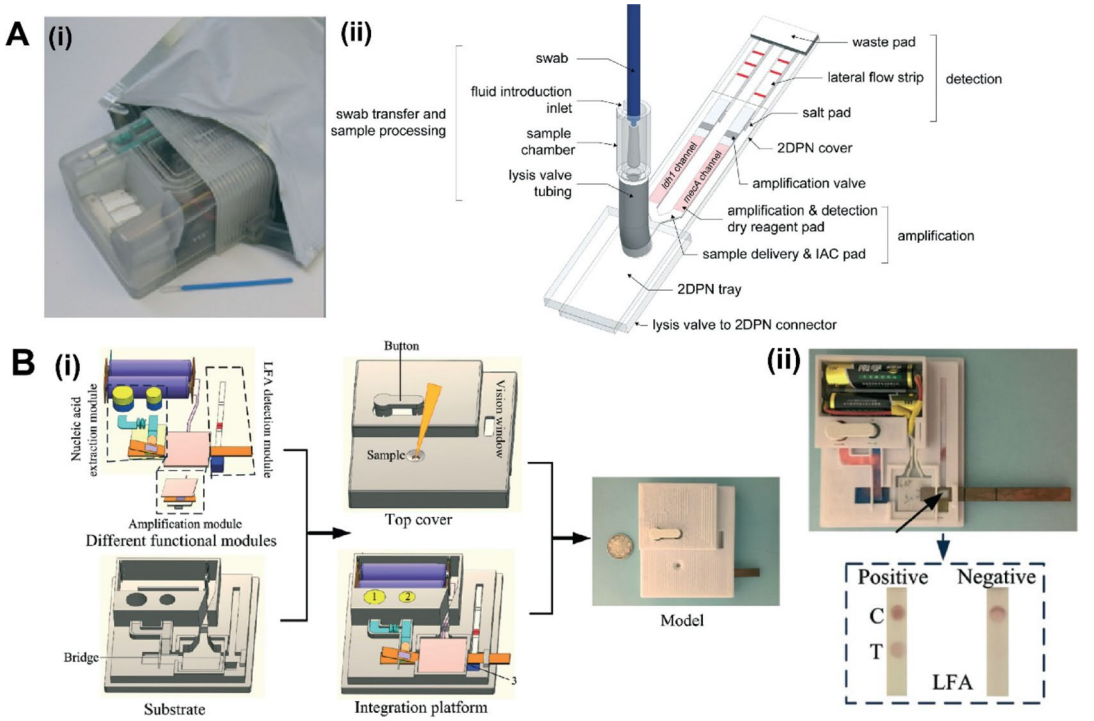

Figure 8: Systems integration for conducting NAATs **a** (i) Prototype for MAD NAAT showing the device body and swab for sample collection. (ii) Detailed description of the device design. **b** (i) Schematic of the integrated device demonstrating the different device modules (ii) The prototype for the device with lateral flow results **a** Reproduced from ref [161] with permission from the Royal Society of Chemistry. **b** Reproduced from ref [159] with permission from the Royal Society of Chemistry.

detect *Salmonella typhimurium* from a wide range of samples like wastewater, milk, juice and egg (Fig. 8b). The device was designed to have separate modules for nucleic acid extraction, amplification and detection. Sample was introduced into the Fusion 5 capture disk, which was followed by sequential introduction of the lysis buffer and wash buffer that were pre-stored in two separate sponge-based reservoirs. Excess fluid volumes were wicked into a waste absorption pad made of filter paper. The Fusion 5 disk containing the captured DNA was then moved to the amplification module and brought into contact with the glass fiber pad containing the dried HDA amplification reagents. Amplification was carried out at 65 °C, wherein a positive temperature coefficient ultrathin ceramic heating tablet with a temperature control switch was used with an in-built battery-support to maintain the required reaction conditions. A running buffer pre-stored in a sponge reservoir was then used to elute the amplicons to the LFD strip. The results could be read by naked eye by observing the colorimetric lateral flow signals.

Our group recently published an extensive review on the use of paper-based devices for conducting NAATs, focusing on the different workflows that may be used for device integration[164]. For detailed information about the field of paper-based NAATs, we direct the reader to this review.

8.4 Blood Plasma Separation

Many diagnostic tests performed in conventional laboratory settings involve blood specimens, more specifically, blood plasma. Changes in the composition of blood plasma reflect the status of pathological processes affecting organs and tissues throughout the body. The separation of plasma from whole blood is a critical step in these tests. Red blood cells and white blood cells interfere with several assay chemistries and the intense red color of blood interferes with color change readouts. Traditionally, plasma has been separated from blood using centrifugation or magnetic separation. However, new types of paper membranes have now found applications in separating plasma from blood, thus obviating the need to use bulky instruments like centrifuges. While this area has been well reviewed by

Songjaroen et al.[165], we present the highlights of two notable techniques below.

There have been several attempts by various groups to separate RBCs from whole blood samples using agglutination reagents and electrochemical methods. Yang et al.[166] demonstrated a simple method for separating plasma from whole blood directly in paper. A circular device with a central plasma separation zone and peripheral test zones was fabricated. The central plasma separation zone was functionalized by spotting agglutinating anti-A, B antibodies. A drop of whole human blood (~7 μL) was introduced into the central zone where RBCs agglutinated and only plasma reached the test zones at the periphery, where colorimetric glucose detection assays were carried out. The method showed effective results for separation of blood plasma from whole blood, but suffered from the limitation that it involved the use of expensive antibodies. Kar et al.[167] came up with a rather inexpensive method for the separation of blood plasma involving capillary-driven diffusion of blood samples in an H-filter cut on standard laboratory-grade filter paper using a knife cutter. Equal volumes of whole blood and PBS buffer were introduced into the two legs of the H-filter and the smaller molecules comprising plasma diffused rapidly into the PBS stream, enriching it with plasma components. This plasma-enriched buffer was used for detection of glucose content in the blood. The plasma separation efficiency was found to be 75.4 ± 9.1% for a device of 5 mm width and 3 cm length. Such a plasma separation device is desirable within the purview of low-cost POC devices. The integration of plasma separation and subsequent colorimetric detection of analytes in plasma in paper-based analytical devices is rapidly gaining popularity and it is expected to be an integral part of future POC serodiagnostics.

9 New and Emerging Applications of Paper Networks

9.1 Tissue Engineering and Cancer Diagnostics

Paper has recently found important use in tissue engineering. The field of tissue engineering aims at combining principles of engineering and life sciences towards development of synthetic tissues by growing cells on artificial substrates commonly known as scaffolds[168]. These scaffold matrices provide structural support for cells to form tissues. Cells, when seeded in scaffolds under controlled biochemical environments, produce mature tissues. An ideal scaffold must be biocompatible (allowing cells to attach and function normally), biodegradable (allowing cells to reconstruct and produce its own matrix)[169], and mechanically strong (providing rigidity for the cells to grow)[170].

Paper can be morphologically defined as a bundle of cellulose microfibers that inherently form a microfibrous porous 3D architecture. Given that there are a large number of different types of commercially available paper products, e.g., filter paper, weighing paper, nitrocellulose etc., paper also offers great diversity in surface topography, internal microstructure, and mechanical properties for 3D scaffold fabrication[171]. Paper produced from natural sources is biocompatible. Further, because it can be manufactured in large quantities due to well-established extremely low-cost fabrication processes, paper is a potential substrate for development of alternate platforms for cell culture and tissue engineering.

In vitro cell culture has widely been used to understand the behavior of cells. The standard practice involves propagation of cells in 2D (petri dish), which maintains the cells in a uniform nutrient and physiological environment. There are significant ways in which 2D cell cultures differ from 3D tissue cultures. The spatial distribution of cells influences the biomolecules in signaling pathways[172, 173]. Cell behavior and morphology depend on the mechanical property of the adherent surface; hence, 2D culture fails to mimic 3D tissues[174]. A gradient of diffusion of solutes, nutrients and oxygen is observed in 3D culture whereas monolayer cell culture experiences a homogeneous distribution[175–177]. Although a significant amount of drug efficacy testing has been conducted in 2D cell cultures, the 2D model fails to replicate results from the in vivo systems[178]. As a result, 3D tissue cultures have rapidly gained popularity as promising models for drug screening. The development of new scaffolds for tissue engineering has now become a large and very active area of research[179]. The most recent trend in this area is direct 3D printing of scaffold-free tissues, but that area is outside the scope of this review[180].

Paper-based 3D cell culture systems have physiological conditions relevant for oxygen diffusion and nutrient gradients similar to native conditions[181, 182]. Stacking of multiple paper layers to produce 3D tissues facilitates on-demand isolation of cells from a particular layer for analysis by de-stacking the layers[183] (Fig. 9a). The

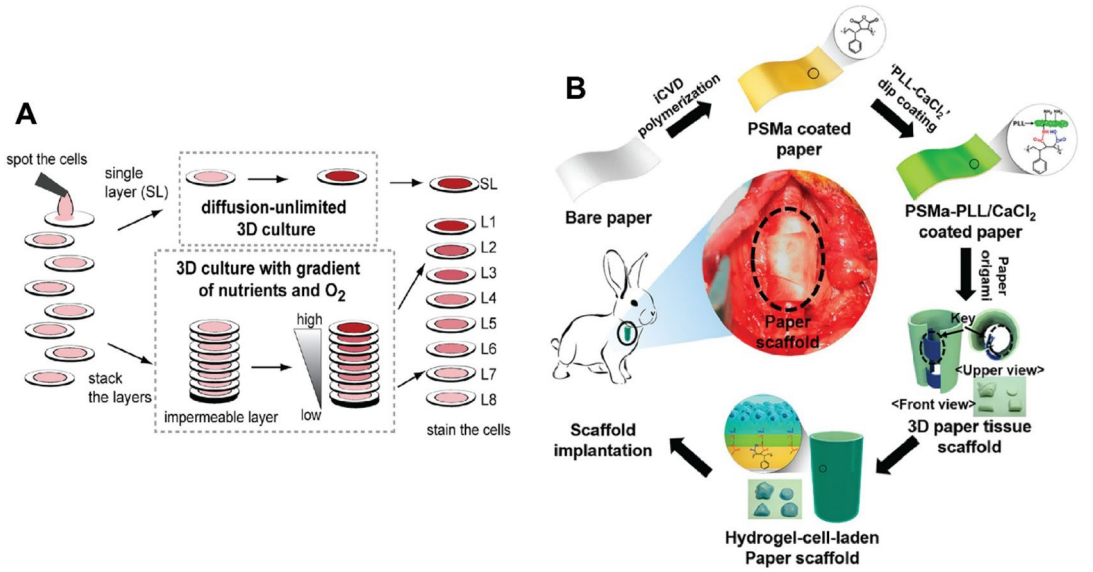

Figure 9: Applications of paper in tissue engineering **a** Schematic representation of multilayer stacking of paper forming a 3D tissue, reproduced with permission from Ref [183]. **b** Schematic representation of fabrication of hydrogel-cell-laden paper, reproduced with permission Ref [185].

3D structure of cellulose-based paper scaffolds provides appropriate rigidity and porosity that facilitates transport of nutrients for tissue engineering[183, 184]. Various strategies for successful development of paper-based tissue engineering have been reported. Kim et al.[185] modified paper surface with poly (styrene-co-maleic anhydride) layer via chemical vapor deposition (iCVD) followed by immobilization of poly-L-lysine and deposition of Ca^{+2} forming an alginate hydrogel (Fig. 9b). The hydrogel-laden paper was folded by simple origami approach making a cylindrical 3D scaffold, which, when seeded with chondrocytes, developed mature trachea. Cells behaved different depending on surface topographies and mechanical properties of the paper.

Paper scaffolds made of commercially available weighing paper enhanced osteogenic differentiation and in vivo bone generation of human adipose-derived stem cells (hADSCs)[184]. Also, stacking of paper with osteogenically differentiated hADSCs and human endothelial cells led to vascularized bone formation in vivo. Wang et al.[186] have successfully induced human pluripotent stem cells into functionally beating cardiac tissues leading to a "beating heart on a paper", exhibiting stable functional property of 40–70 beats per minute for up to 3 months, demonstrating the durability of paper as a substrate.

Coating of paper with hydrophobic materials like wax, PDMS or Teflon inhibits cell attachment and creates barriers, constraining cell growth to desired areas of paper[187]–[189]. This strategy was used by Derda et al. to create a 96 micro-zone paper device containing 96 tissues comprising cells laden in hydrogels[183]. Hydrophobic PDMS barriers separated the 96 tissues and the device was used for high-throughput analysis of cell migration behavior in 3D tissues. This cell culture platform is called cell-in-gel-in-paper (CiGiP)[181, 190]. 3D stacking of paper printed with wax seeded with aortic valve cells and collagen mixture developed a synthetic aortic valve. The customized stacking of paper permitted adjusting culture thickness and cell density and the tissues survived for 14 days[182].

Early detection of cancer cells is important and fundamental for diagnostics and therapeutics. Traditional cyto-screening is widely used due to its high selectivity and accuracy. Developing rapid low-cost cancer cell detection technologies is a necessity and a challenge. An attempt to overcome the drawbacks of traditional techniques includes paper with plasmonic nanorods as a 3D scaffold for cancer detection[191]. Another high-sensitivity, noninvasive and rapid cancer screening platform uses SERS of adsorbed plasmonic gold rods on filter paper for cancer screening[192, 193]. Wu et al.[194] introduced paper-based electrochemiluminescence device consisting of aptamers-modified Au-paper electrodes (working electrodes) to screen multiple cell types for cancer.

The emergence of paper as a potential substrate for developing 2D and 3D cell culture platform is promising, yet some challenges remain. Mechanical properties of paper decline when immersed in cell culture medium because of the breakage of hydrogen bonds which hold cellulose together. The slow degradation of paper also limits its applicability for studying tissue transplant. Commercially available papers contain additives (clay and calcium carbonate) which may be released during culture and interfere with cell growth. These shortcomings must be overcome to realize the full potential of paper as a substrate for tissue engineering.

9.2 Energy Generation and Storage

Another interesting, almost unexpected application of paper, has been in the field of paper-based energy storage devices. One of the primary motivations for these efforts has been that conventional batteries are often used in association with paper-based devices to power operations like fluorescent or electrochemical readouts, among others. However, batteries pose difficulties in terms of their toxicity, weight, and disposal. Paper has, therefore, been looked upon as an alternative substrate to replace bulky batteries with a goal to integrate energy generation/storage directly into integrated paper-based devices[195]. Several researchers have now developed paper-based batteries (fuel cells and lithium ion or alkaline methods to produce power) and energy storage devices (supercapacitors). A recent review by Sharifi et al.[195] provides a good review of the field. We will, however, highlight a few important developments below.

Paper can be made highly conductive by printing conductive material onto it by using simple solution method of conformal coating of single-walled carbon nanotubes (CNT) and silver nanowires films. CNTs provide high stability, electrical conductivity, flexibility and surface area. These conductive papers can be used as an alternative to metallic current collector in lithium ion batteries[196]. Fuel cells are a promising means for energy generation as the power generated is highly efficient and has minimal negative environmental impact. They have been employed as power sources for microfluidic devices by various research groups. Esquivel et al.[197] developed microfluidic fuel cells as paper-based power sources in a standard lateral flow test format. The need of external pumps was eliminated because reactants flowed by capillary forces; the device delivered power densities in the range of 1–5 mW cm^{-2} using solutions of methanol and potassium hydroxide.

There is an ever-increasing demand for efficient, thin, and flexible energy storage devices. Pushparaj et al.[198] developed a device by integrating distinct electrochemical and interfacial characteristics into a single device, made out of nanoporous cellulose paper embedded with carbon nanotube electrodes and electrolytes. They used room temperature ionic liquid (RTIL) to overcome the insolubility of cellulose in most solvents. The resulting supercapacitor demonstrated good electrochemical performance across diverse range of temperatures, electrolytes and mechanical deformations.

The integration of paper networks capable of generating energy or storing energy with paper networks for conducting diagnostic assays is an extremely nascent and promising area. Paper has certain fundamental advantages as a substrate for energy generation/storage, i.e., high porosity, high solvent absorption, and strong binding with nanomaterials. This could lead to new developments in advanced energy storage and conversion applications, yet this potential has only barely been tapped yet.

10 Concluding Remarks

After the original microfluidics revolution of the early 1990s, the development of paper-based microfluidics appears to be the next 'mini-revolution' of sorts in the field. Several factors have contributed to this revolution including (i) a worldwide interest in conducting research in global health, (ii) a general increasing interest in 'frugal engineering', (iii) a push from funding agencies to conduct research that 'gives back to society', and (iv) involvement of some major research groups that initiated the microfluidics revolution in the 1990s, developing paper-based microfluidics, e.g., the Whitesides group from Harvard University. Just like any other new technology, paper-based microfluidics has created a wave of excitement and the expectations from the technology are currently very high. This is apparent from its use in diverse fields of application as reviewed in this article. The real test for the technology, however, will be in its translation to commercial products. Paper-based microfluidics could have a competitive advantage here because the LFIA market is already mature (estimated at ~ $5 billion in 2016)[199]. In certain application areas such as the development of signal-enhanced immunoassays, if researchers can develop devices

that are only slight modifications of the LFIAs, but that can provide significant improvement in sensitivities, the chances for successful commercialization could be high. Similarly, commercialization potential will remain high in areas like use of electrochemical detection of analytes on paper because of the existing blood glucose monitoring device market.

In a recent review published by our group[164], we have marked the current position of paper microfluidics technology on the Gartner hype cycle[6] and we argue that the technology is currently at the peak of inflated expectations. According to the hype cycle, this phase is typically followed by a trough of disillusionment as the real challenges of the technology surface. We anticipate that the trough will be shallower for paper microfluidics as it is building upon a couple of decades of experience from traditional microfluidics. Yet, no commercial product based on the multidimensional paper networks discussed in this review exists. While immense functionality for medical diagnostics using such devices has been demonstrated, some of the challenges yet to be overcome are achieving sensitivities comparable to state-of-the art instruments available in central labs, robust and reproducible performance, and long shelf lives. To move towards commercialization, these aspects of paper microfluidic devices should be the focus of further research.

From an academic perspective, it is anticipated that the next 5 years will continue to see a rise in the number of publications on paper-based devices and that new application areas will continue to emerge. There continues to be an interest in incorporating new valving and flow control techniques in paper devices. While many different methods have been demonstrated, their integration into functional paper devices that can conduct multi-step assays remains limited. This should be considered a thrust area for further development. Many methods have now been demonstrated for fabrication of paper-based devices; this appears to be a solved problem at this point. It should be noted that the shapes of paper membranes that are being used for various applications are often designed by trial and error. Appropriate mathematical models to predict flow rates through different shapes of paper could be used as effective design tools, but they seem to be lacking in the field. The field would benefit tremendously if such models were developed and provided to the community.

Some application areas of paper-based microfluidics are far from commercialization but extremely promising, e.g., nucleic acid amplification tests. The idea of replacing expensive PCR machines with paper-based devices that can conduct isothermal amplification of nucleic acids is ground-breaking in many ways. As such, new research in this niche area is expected to emerge rapidly. Multiplexed colorimetric detection based on simple color change chemistries using μPADs, while elegant, may not be able to achieve the sensitivities required for detecting many analytes. However, for a select few applications, these devices may be the right app. Extension of these devices for conducting ELISA's, i.e., the P-ELISA format and the newer sliding designs for conducting ELISA appear promising because they could reach detection sensitivities required for real world applications. Another highly promising approach to conducting ELISA's is the 2DPN design, which requires only initial user actuation step/s. Because the user need not perform multiple timed steps to operate these devices, these designs are more suitable for POC diagnosis conducted by untrained users. The use of paper in tissue engineering is another exciting area but given the complexities of biological tissues and the need to maintain controlled conditions over several days/weeks, it is possible that paper will only find limited utility in niche applications in this area.

In conclusion, like in any other 'hot' area of research, innovative ideas and concepts are rapidly emanating through new literature on paper-based microfluidics. However, the community must not lose sight of the goal, which is to develop simple low-cost devices that could aid in conducting medical diagnostics in low resource settings. Whenever possible, collaborations must be sought with hospitals to test devices with clinical samples. If possible, devices must be deployed into the field for operation by minimally trained users. If a collaborative community of researchers, clinicians, and healthcare workers get involved from an early stage, paper-based microfluidic devices have the potential to generate game-changing apps, especially in the field of POC diagnostics.

Acknowledgements

This work was supported by the Department of Science and Technology (DST) India in the form of an Extramural Research Grant (EMR) to BT

and a National Postdoctoral Fellowship (NPDF) to DR; by the Department of Biotechnology (DBT) India in the form of an Innovative Young Biotechnologist Award (IYBA) to BT; and by the Indian Institute of Science in the form of a generous start-up grant to BT.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no competing interests.

Received: 18 March 2018 Accepted: 10 May 2018
Published online: 30 May 2018

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