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PAPER MICROFLUIDICS: APPLICATIONS AND PERSPECTIVES

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Keywords: Paper microfluidics, biosensors, immunoassays, biodiagnostics, capillarity, cellulose fibers, wicking.

1 INTRODUCTION

The explosion of interest and development in paper microfluidics has so far been linked with a single application: integration in diagnostics for medical testing devices. Paper bio-diagnostics has the potential to revolutionize preventive health care in two ways. First, by providing developing nations and remote locations with the cheap, simple and robust tests they have not been able to afford or have access to; second, by developing a new generation of convenient, non-invasive and low cost tests for home and point of care testing. The latter category aims for the easy and early detection of illnesses, or monitoring a condition; early cancer detection, cholesterol and hemoglobin levels are three examples. Paper diagnostics are to be used directly by the patient or by untrained workers - sometimes in stress conditions. Faster, more reliable and accurate tests relying on modern instrumental analytical chemistry are already available. This market vision is essential to define the position and boundaries of paper diagnostics and their microfluidics component, in the spectral of biomedical analysis. Paper tests must be: 1) very cheap, 2) very easy to use, 3) robust and reliable, and 4) easily disposable. The paper-functional printing material-process combination offers one of the lowest cost and flexible manufacturing platforms; it is the keystone of the paper microfluidics/diagnostic success. From medical applications, paper microfluidics and diagnostics can then be adapted to more cost sensitive applications such as veterinary health test, food quality, and environmental monitoring.

A few excellent reviews of bioactive paper are already available. Pelton, from McMaster University, presented an authoritative and comprehensive analysis on bioactive paper as a low cost platform for diagnostics from a paper [1] and a health [2] perspective. Shen et al., from APPI-Monash University, published a perspective on paper-based microfluidics [3], patterned paper and alternative materials as substrates for microfluidics diagnostics [4]. Liana et al., CSIRO Australia, reviewed advances in paper-based sensors [5]. Whitesides, Martinez et al., from Harvard University, analysed microfluidic paper-based diagnostics [6] and electroanalytical devices [7] for the developing world. Then et al., also from APPI-Monash University [8] reviewed biodiagnostics for medical applications.

This review analyses the recent progress and development perspectives of paper microfluidics mainly as component of a low cost, easy to use and disposable paper bio-diagnostic. The usefulness of each development reviewed will be examined in terms of 4 issues: 1) Is it cheap? 2) Is it simple to use and practical? 3) Is it robust and sensitive? 4) Is it disposable or recyclable?

Paper microfluidics are usually small and disposable systems to transport an aqueous solution by relying on the natural capillary forces of paper. Paper can serves seven main functions: 1) to transport and measure samples and reactants, 2) to safely store biosamples and reagents, 3) to provide a substrate/surface for a reaction, 4) to separate, fractionate or concentrate a component, 5) to communicate the presence or concentration of an analyte, 6) to provide a permanent record of the analysis performed, 7) to safely capture and provide a media for the disposal of biosamples. Paper can be used as a substrate for patterning hydrophilic fluidic channels using printing technology. In the majority of applications, paper microfluidics are an integral component of a system used for fractionating or analyzing an analyte for health or environmental applications; very seldom are microfluidics system used independently. Most of the reports on microfluidic paper have been for low cost biodiagnostics, or instantaneous, rapid or stand-alone tests. Microfluidic systems are an integral part of most paper diagnostics. Paper biosensor, bioassays, immunoassay and diagnostics are used as synonyms for paper biosensors. These fluidic paper devices can be directly integrated with a colorimetric, chemiluminescence or an electrochemiluminescence detection mode to form a biosensor. The attraction toward paper microfluidics stems from their pump free fluid delivery mechanism based on wicking, their very low cost, and their integration in a functional system (diagnostics) that is easy to use and dispose of. The biocompatibility of paper with important biomolecules such as enzymes, antibodies, RNA and DNA is another advantage of paper which enables stable paper bio-diagnostics. Microfluidics systems can be printed on paper by various processes, and better, complete paper based biosensors are now designed to be manufactured completely by functional printing as part of an efficient roll-to-roll process which involves the sequential printing of the microfluidics and then the

chemical and biochemical reagent systems. This review aims at presenting a critical review of the current state of paper micofluidics systems and a perspective on their integration and applications into functional devices – current and future.

The 2007 article: "Patterned paper as a platform for inexpensive, low volume, portable bioassays" from Whitesides et al. at Harvard has revolutionized the field of low cost diagnostics [9]. The team of Whitesides demonstrated the modern concept of paper microfluidics by printing hydrophobic barriers into paper; they also deposited enzymes and reagents within the paper microfluidics system to highlight the potentials of integrating microfluidics into diagnostics. In the 6 years that have followed, hundreds of studies have been published and dozens of patents filed. Research groups in most countries are now investigating paper-based diagnostics integrating microfluidics systems; surprisingly, very few of these studies have come from traditional pulp and paper research teams. Paper microfluidics studies can be distinguished into two broad categories: microfluidics and biosensors development. This review paper analyses the recent development of paper microfluidics in the context of their application. In the first part, the mechanism for liquid transport in porous media in general, and paper in particular are reviewed; this includes wicking, wetting, diffusion and evaporation. Mechanisms of analyte/colloid separation by paper are then analyzed. In the second part of the study, the types of paper microfluidics and their specific applications are reviewed. The third part of the study presents a perspective of paper microfluidics applications, mainly as low cost test/biosensor. Alternative to paper diagnostics are also presented. It is the objective of this review to highlight the potential, limitations and future directions and development needs for paper microfluidics application.

2 TRANSPORT MECHANISMS

2.1 Why Paper Microfluidics?

As independent systems, paper microfluidics are not particularly efficient, polyvalent nor rapid systems to transport fluids. It is their combination of advantages for very particular applications – chemical and biochemical analysis relevant to rapid and simple to use diagnostics – that justifies their inherent low performance. Paper offers five main properties for diagnostics: 1) very cheap, widely available and easy to process material; 2) the capillarity wicking of aqueous solutions along the cellulosic fibers of paper provides a driving force to transport fluids without the need for external system/energy; 3) the porosity and biocompatibility of cellulose/paper allows reagents and biomolecules to be stored in the paper device; 4) paper combined with printing offer a very flexible and cheap material/ process combination to manufacture microfluidics and diagnostics; 5) paper is combustible, biodegradable and recyclable, offering many easy options of disposal after use [1, 6].

2.2 Mechanisms of liquid transport in paper

When a droplet of liquid contacts with paper, the competition of 4 main phenomena dictates its transport and distribution toward an equilibrium condition. First, the droplet wets the surface of paper; second, paper absorbs and wicks the droplet by capillary action, third evaporation proceeds, and fourth, diffusion occurs. The time frame of these phenomena varies with wetting occurring in seconds, absorption/wicking in seconds-minutes, evaporation and liquid diffusion in minutes-hours.

2.2.1 Wicking

Liquid transports in paper mostly by wicking through the inter-fibre spaces. The capillary driving force is caused by the difference in surface energy between the fluid and the solid. The resistances to movement are friction and viscous dissipation. Wicking can be simplistically described by the liquid flow in a capillary. The Lucas-Washburn equation states that a liquid of viscosity η and surface tension γ will flow in a capillary of radius r and length at a velocity V defined by [10]:

$$V = \frac{\gamma r \cos \theta_E}{\eta l} \tag{1}$$

here θ_E is the equilibrium contact angle formed by the liquid in the capillary. Liquid velocity can be increased by augmenting the driving force: increase surface tension of liquid (γ), improve wettability of fibers (lower θ_E), or by decreasing the resistance: bigger and shorter pores, decreasing the fluid viscosity.

Equation (1) is often expressed as:

$$l = \left[\frac{r\gamma t \cos \theta_E}{2}\right]^{\frac{1}{2}} \tag{2}$$

which better reflects the square root relationship between wicking distance and time. Wicking occurs only in dry paper – not in wet paper – as the driving force disappears once the pores are filled with a liquid. Wicking proceeds as long as there is a driving force: dry paper ($\cos \theta_E$) and a liquid reservoir. Wicking is counteracted by viscous dissipation and sometimes gravity – for vertical samples. While the choice of liquid is dictated by the application, paper surface treatment ($\cos \theta_E$) and the selection of paper (bigger and shorter pores) can improve wicking. Surfactants are detrimental (decrease γ).

2.2.2 Wetting

The contact angle of a static droplet resting at equilibrium on a surface with its vapour is expressed by the Young Equation:

$$\gamma_{\rm SV} = \gamma_{\rm LV} \cos \theta_E + \gamma_{\rm SL} \tag{3}$$

where γ_{SV} , γ_{LV} , γ_{SL} are respectively the solid-vapor, liquid vapour (surface tension) and solid-liquid interfacial energies. Equation 3 simply states that the equilibrium contact angle formed by a liquid droplet on a surface is dictated by the balance of three interfacial forces. While the choice of liquid is often set by the application, wetting can be favoured by surface treatment to increase the surface energy (γ_{SV}) and the use of surfactants to decease surface tension (γ_{LV}). For dynamic wetting, the instantaneous contact angle of a droplet wetting a smooth surface can be described by the Hoffman-Tanner equation [11, 12]:

$$\theta_E{}^3 - \theta_{(t)}{}^3 \propto \text{Ca} \tag{4}$$

Where Ca is the capillary number defined as:

$$Ca = \frac{\mu u}{\gamma} \tag{5}$$

and $\theta_{(t)}$ is the instantaneous contact angle formed by a fluid of viscosity μ and surface tension γ , moving at a velocity u, and θ_E is the equilibrium contact angle. Equation (4) states that the surface forces expressed by a function of the difference of the droplet equilibrium contact angle minus its instantaneous angle are balanced by viscous forces. A low equilibrium contact angle and fluid viscosity will promote wetting velocity.

Moderassi et al. investigated the effect of chemical and physical heterogeneity on the mechanism of wetting and absorption of water droplets over sized papers [13]. Wetting and absorption rates were calculated from the contact angle, volume, and contact line of the droplets on paper. Absorption was found to start only after the drop had wetted the surface to a certain extent. There was a time delay before absorption occurred. By the end of this delay, a pseudo-equilibrium contact angle was reached, a metastable contact angle function of chemical composition of the surface. Wetting on a partially hydrophobized porous surface follows a power law model with wetting rates slower than in hydrodynamic wetting by a factor H, a function of surface roughness. Surface roughness also affects the pseudo-equilibrium contact angle, as by entrapping air, it renders the surface more hydrophobic. The wetting dynamics was found to be independent of the chemical heterogeneity of the surface. The initial rate of wetting and wicking of water on paper are equivalent; however, at later stages, wetting slows down and decreases the driving force $(\theta_t^3 - \theta_E^3)$ while wicking proceeds at constant rate [13].

The typical evolution of contact angle, baseline and volume of a water droplet $(5-13 \ \mu\text{L})$ deposited on sized paper is shown as a function of time in Figure 1 [13]. The effect of paper surface roughness on the dynamics of wetting and



Figure 1. Dynamics of a water droplet on sized paper highlighting 2 regimes: 1) governed by wetting; 2) dominated by absorption into paper. Evaporation was kept negligible. Figure 3 from ref [13] by Modaressi et al.



Figure 2. Effect of paper roughness on the apparent contact angle of water droplet. The average paper roughness achieved by calendaring is indicated. Figure 2 by Modaressi et al. [13].

absorption of water droplets on sized paper (ASA) calendered at two different intensities to create two levels of surface roughness at constant surface chemistry is shown in Figure 2 [13].

2.2.3 Diffusion

According to Fick's law, the Diffusion flux (J) is proportional to the negative gradient of concentration (c):

$$J = D \nabla c$$
 with $J_i = -D \frac{\partial c}{\partial x_i}$ (6)

with the diffusivity (D) as a proportionality constant. Diffusion is mostly used to describe the transport of gas systems. For an analyte in a liquid, diffusion is driven by a difference in concentration and also promoted by molecular movement. Diffusion becomes a transport mechanism for a solute once paper has been filled by the liquid and the driving force for liquid transport has vanished; it is however, significantly slower than wetting and wicking.

Nilsson et al. [14] investigated experimentally the diffusion of water vapour through pulp and papers. Two pulps and eleven grades of paper were studied. The diffusivities measured ranged from 2.1×10^{-8} m²/s to 5.4×10^{-6} m²/s. Effective water vapour diffusivity (D_{eff}) correlated with the density of the paper sheet (Figure 3), but was independent of relative humidity (Figure 4). The profile of

relative humidity across a stack of eight paper sheets is nearly linear, whereas the profile of the moisture content is non-linear (Figure 5) [14]. This means that if the relative humidity (water vapour partial pressure) in the gas phase is considered as the driving force, the effective vapour diffusivity remains nearly constant.



Figure 3. Effective vapour diffusivity as a function of paper sheet density. Figure 5 from Nilsson et al. [14].



Figure 4. Effect of Relative humidity across paper sheet on vapour effective diffusivity (D_{eff}). Figure 8 from Nilsson et al. [14].



Figure 5. Moisture content and relative humidity profiles across eight sheets of paper. Figure 9 from Nilsson et al. [14].

2.2.4 Evaporation

Evaporation provides a driving force for the transport of liquid in paper. Evaporation proceeds by heat and mass transfer from a combination of conduction, convection and radiation. The rate of evaporation of a given liquid from a surface is a function of the wetted surface area, temperature, relative humidity and the affinity liquid-surface; that is in absence of radiation and convection.

There are two modes of evaporation of liquid droplets on surfaces: one at constant contact area and one of constant contact angle [15]. The constant contact area mode is the dominating evaporation mode (variable contact angle). Erbil et al. analysed sessile droplet from surface under the constant angle mode [15]. Hu and Larson [16] investigated the evaporation of sessile droplets with a pinned contact line on a surface experimentally, analytically and by computation using the finite element method. The difference in water vapour concentration (1-H)c_v drives the evaporation of water into air according to the diffusion equation (7):

$$\frac{\partial c}{\partial t} = D \ \Delta c \tag{7}$$

Where c is the local water vapour mass concentration, D is the vapour diffusivity, H is the relative humidity of the ambient air and c_v is the vapour saturation concentration. At the air-liquid surface, the local evaporation flux J (r, t) is expressed by:

$$J(r,t) = D \nabla c \tag{8}$$

Hu et al. [16] approximated an expression for the droplet evaporation rate [m (t)] at any wetting contact angle $(0^{\circ} < \Theta < 90^{\circ})$ as:

$$-m(t) = \pi R D (1 - H) c_v (0.27 \ \theta^2 + 1.30)$$
(9)

At a given contact angle the evaporation rate is proportional to the contact line radius (R), the vapour concentration difference $(1-H)c_v$ and the diffusivity D and depends weakly on the contact angle Θ . Figure 6 compares the theoretical model with experimental results for the evaporation dynamics of a sessile water droplet on glass.

The additional effects of convection and radiation on free water evaporation were studied on lakes by Kohler et al. [17]; Uno provides evaporation formulas considering convection and temperature [18].

Swerin and Claesson investigated the evaporation of very small water droplets on paper and model surfaces in the context of inkjet printing [19]. Initial drying



Figure 6. Evaporation of sessile water droplets on glass. Comparison experiments– theory of the time-dependent weight of water droplets of radii 2.01, 2.53 and 2.93 mm on glass at T =22°C. Figure 14 from Hu and Larson [16].

rates of 0.4 to 0.5 pL/ms second were measured at 23°C and 45% relative humidity; typical 6-pL droplets evaporated in approximately 1 s [19]. The major role of the surface is to increase surface area of the droplet as the equilibrium contact angle between water and the surface decreases.

2.2.5 Liquid Transport in Porous Combined Materials and Imperfect Surfaces

Shen et al. [20] stylized the effect of groove geometry on wicking. Khan et al. [21] investigated the dynamic of wicking and wetting of droplets impinging a narrow V-groove on a quartz surface. The variables of interest were the impinging droplet velocity and the groove width; the surface energetic and droplet size were kept constant. Wetting and wicking velocities were measured by high speed image analysis. (Figure 7) The maximum wetting and wicking velocities were found to be equal (Figure 8). The wetting velocity reaches a maximum instant after it



Figure 7. Dynamics of a water droplet impinging, with and without initial velocity, a flat quartz surface or a V-groove ($w_0 = 0.5$ mm; quartz surface) on a quartz surface; images were taken at different magnifications; the inclined straight lines represent high contrast shadow of V-groove against white background. Khan [21].



Figure 8. Comparison of average liquid wetting and wicking distance on V-groove geometry: (a) $w_o = 0.5$ mm, (b) $w_o = 0.2$ mm. Khan [21].

touches the surface and decreases exponentially until the resting contact angle is reached, at which instant wetting stops. Groove wicking velocity has a similar behaviour. At steady state, wicking proceeds at constant velocity (0.05 m/s). The maximum wetting and wicking velocities both increase with the droplet impinging velocity.



Figure 9. Pictures of Coffee stain (a), dried colloid (b) and salt deposits (c); the bar represents 1cm. Figure 9 from Deegan et al [22].

Solids dispersed in a drying drop will migrate to the edge of the drop and form a solid ring [22] (Figure 9). In a classic manuscript, Deegan et al. investigated capillary flow as the cause of ring stains from dried liquid droplets [23]. This is the mechanism of the so-called coffee stain: when a spilled drop of coffee dries on a solid surface, it leaves a ring-like deposit around the perimeter. The coffee droplet, initially dispersed over the entire drop, becomes concentrated into a fraction of it, forming a ring at the drop perimeter. The characteristic pattern of colloid/dye deposition was ascribed to a capillary flow in which pinning of the contact line of the drying drop ensures that liquid evaporating from the edge is replenished by liquid from the interior. The full mathematical characterization of the coffee ring formation by an evaporating drop is provided in ref [22]. Deegan et al. predict the flow velocity, the rate of growth of the ring and the distribution of solute within the drop (Figure 10).

Plant scientists describe transpiration as the motion of water from the soil through a vascular plant and into the air. Transpiration has long been linked to "passive wicking"; however wicking cannot transport water across dozens of meters to nourish the highest trees. Wheeler and Stroock [24] recently described this mechanism by a cohesion-tension mechanism theory by which the loss of water by evaporation reduces the pressure of the liquid water within the leaf relative to atmospheric pressure. This reduced pressure pulls liquid water out of the soil and up the xylem to hydrate the tree (Figure 11). The absolute pressure within the capillaries of the xylem is negative such that the liquid is under tension. Quantitatively, the difference in pressure generated in the capillaries of plants to drive flow can be two orders of magnitude higher than those generated by wicking [24].

2.2.6 Water Transport in Paper

Schilling et al. [25] measured capillary wicking in fully enclosed paper channels (Figure 12). The channels were 8 cm long and 2 mm wide. The channels were



Figure 10. Distribution of colloids as a function of time in an evaporating droplet. The temporal and special coordinates are normalised as t/tf and r/R, respectively. Fluorescent latex was dispersed in evaporating drops and the intensity is expressed as a function of time and distance. The solid lines of a) represents the theoretical model. Figure 10 from Deegan et al. [22].

enclosed with zero (0 toner), four (4 toner), or six (6 toner) layers of toner (Figure 12 B–E). Images of the wicking experiments were taken after dipping the bottom of the device into a reservoir of aqueous blue dye for 5 and 25 min. The experiments were carried out at two levels of relative humidity: 53% and 100%. Up to 5 min, all channels wicking showed almost identical results. After 25 min, large



Figure 11. Schematic representation of the transport of water through the tree and transpiration of water at negative pressure. Figure 11 from Wheeler and Stroock [24].

differences in the distance that each fluid wicked appeared, showing the effect of evaporation. Wicking distance was plotted as a function of time and square root of time [25] (Figure 12F).

2.3 Separation of solutions in paper

Paper can not only transport liquid, it can also separate the components from analyte mixtures. A few separation mechanisms are possible: filtration/size exclusion, chromatography/selective adsorption, ion-exchange and electrophoresis. Research and development on paper for separation culminated in the 50s and 60s [26].

2.3.1 Paper Filtration – Size Exclusion

Paper is commonly used in air filters for domestic and industrial applications and liquid filters: oil filters for vehicles and water filters. Filter papers are extensively used for laboratory application and Whatman paper filter #1 has become by default the basesheet for paper microfluidics and biosensor prototypes. Few studies have quantified the role of the paper basesheet structure and filter paper pore size on filtration and sample separation. Su et al. investigated the effect of



Figure 12. Capillary wicking in channels fully enclosed by toner coating. A) experimental representation of paper coated with various layers of toner coting and different humidity; F) wicking dynamics as a function of thickness of coating and relative humidity. Figure from Schilling et al. from ref [25].

paper filter pore size, basesheet basis weight and type of fibers on the efficiency to separate blood aggregates from stable red blood cells (RBC) in blood typing paper sensors [27]. Filtration and size exclusion was the basis of separation to segregate RBC-agglutinated by a specific antigen from the stable red blood cells in paper based blood typing biosensors [28, 29].

2.3.2 Paper Chromatography and Paper Thin Layer Chromatography

Paper chromatography was first studied by Runge et al. in the period 1850–1910 [26]. Zweig et al. reviewed the advances of paper and thin layer chromatography mostly for biological research and separation of inorganic compounds [30]; a complete list of references is provided. A comprehensive manual on paper chromatography is also offered [26]. For optimum chromatographic separation three rules are suggested. First, to keep solvent composition and temperature constant; second, to move solvent at a slow rate (2–3 cm/h); third, the choice of solvent should be one in which the components to be soluble have a small but definite solubility. If adsorption and ion exchange are neglected, the movement of a substance in paper chromatogram is a function of its solubility in the developing solvent [26]. In their book, Block et al. provided many practical considerations to optimize 1D and 2D paper chromatographic separation [26] and presented many applications and paper chromatography separation systems, including amino acids and peptides.

The resolution of mixture of solute on filter paper depends on surface adsorption, ion exchange and partition between solvents [26]. However, the predominant factor is the partition between two immiscible phases. Excellent amino acid separation was achieved with solvents that were only partially miscible with water. The theory of chromatography is often based on an analogy with distillation using fractionating columns. A chromatographic column is divided into successive layers of thickness such as the solution issuing from each is in equilibrium; the thickness of the layer is termed the height equivalent to one theoretical plate (H.E.T.P.) [26].

2.3.3 Paper Electrophoresis

Konig published the first report on paper electrophoresis in 1937 [31]. Electrophoresis is the movement of charged particles in solution under the influence of an external electrical field [32]. Paper electrophoresis typically employs filter paper strips soaked in buffer solutions. Paper electrophoresis is the technique used for the separation of small charged molecules, especially amino acids and small proteins. Continuous flow electrophoresis has been found very useful in blood separation application. Block et al. also published a comprehensive manual of paper electrophoresis in 1958 [26].

3 PAPER MICROFLUIDICS AND DIAGNOSTIC DESIGN

Paper microfluidics aim at transporting, measuring, mixing, reacting, separating and storing liquid solutions. Paper systems rely on the porous paper structure to achieve these functions. The most important role of paper is capillary wicking which provides the driving force for liquid transportation without the need of external system or energy. The initial development phase of paper microfluidics has concentrated on the development of hydrophilic channels defined within hydrophobic barriers formed on paper [3, 9, 33–35]. The development of paper microfluidics has then been driven by low cost paper diagnostic application for easy and rapid analysis.

Paper diagnostic design currently focuses on the perceived limitation of performing multiplex assays using micro paper pads (μ PADs), optimizing methods for both structure and fabrication. In many circumstances, single step assays are invaluable, however the simplicity is unsuitable for tests requiring multiple step processes. The design research has hence expanded from single paper sheets to 2D networks and 3D designs, which have demonstrated a degree of multi-test ability. However, this increases the complexity of the design, necessitating a balance between simplicity and functionality. This section analyses the different types of PAD design.

Device designs can be simply differentiated by the directional flow of fluid. For instance, when the flow of liquid is in a single direction it is cited as 1D. 2D describes lateral flow in multiple directions on the horizontal plane (eg. the spread of liquid from a single corner to multiple detection zones) and 3D designs add a vertical component (Figure 13).





3.1 1D Paper Diagnostics

Dipstick assay tests are the commonly known 1D lateral flow paper diagnostics that have been used for decades, and were first used to detect glucose levels in urine [36, 37]. The dipstick test is used by dipping one end of the stiff paper strip into the liquid sample to be analyzed (Figure 14). The fluids are then passively transported to a reagent zone through the cellulose fibres [38]. Testing evolved during the early 1960s, creating a triple analyte test with the ability to also detect protein albumin and pH levels of urine. Since then, dipstick tests have expanded to a 10-type multi-analyte test with the ability to detect a wide range of biomarkers, namely leukocytes, nitrite, ketones, bilirubin and urobilirubin [39]. Some assays are also able to measure and report the specific gravity of the sample [39].

Further development during the 1980's led to the added ability of immunorecognition using dipsticks. Antibodies were spotted and immobilized on nitrocellulose creating a wider market range for PADs. Take-home drug and pregnancy tests are a prime example of urinalysis tests available. Other applications include immuno-based PADs that test blood analytes for cholesterol levels, diabetes, autoimmune screening and pathological diseases such as hepatitis C and human immunodeficiency virus type 1 (HIV-1) [40–42]. In 1989, dipstick technology integrated capillary-driven lateral fluid transport, thereby eliminating the need for incubation and wash steps for certain applications. The result was an improved lower limit of detection (LOD), achieved by amplifying the total number of captured and detected bioanalytes [43, 44].

Typical dipstick assays have the analytes dried and stored in the fleece sections of the assay during fabrication. This allows for different detection zones to be present on a single stick. The dried reagents are dissolved by the fluid sample solutions, hence allowing the reaction to occur. A typical dipstick assay is illustrated in Figure 14. The flow of liquid from one end to the other of the stick is one dimensional, thereby forming a 1D paper diagnostic. These tests are cheap, reliable and easy to use. Despite the success of 1D paper diagnostics, they are limited to single step tests and are unable to perform multiple-step diagnostic assays which are often required for techniques such as enzyme-linked immunosorbent assay (ELISA).



Figure 14. An example of a multiple-analyte 1D paper diagnostic on a dipstick.

3.2 2D Paper Diagnostics

3.2.1 Simple 2D PADs

Multidirectional flow in paper diagnostics is enabled by the ability to pattern paper with microchannel designs. In 2007, Martinez et al. [9] patterned 3 detection zones onto paper, creating a simple analytical device (Figure 15). It had the ability to detect glucose and protein, as well as a control zone. Hydrophobic boundaries were printed using photolithography, forming channel 'walls' which directed fluid into the 3 separate zones without cross-contamination. This added a spatial advantage compared to the conventional 1D- μ PAD.

Patterning methods for diagnostic design have been summarized in previous reviews. Such methods include: photolithography, plotting, inkjet or plasma etching, cutting and wax printing [6]. A summary of the patterning techniques analysis is presented in Table 1. Shen [3] highlighted 10 processes by which microfluidic channels have been patterned on paper. Of those, there are 6 printing processes: 1) photolithography, 2) ink jet printing, 3) inkjet etching, 4) wax





Table 1. Analysis of μ PAD fabrication by functional printing

Paper Patterning Techniques				
Photolithography	Description: Patterned using chromatography paper soaked in SU-8 photo resist polymer solution before being selectively exposed to ultraviolet (UV) radiation using a patterned mask to shield desired pathways. Shielded regions remain hydrophilic and the unreacted SU-8 is washed away. Unshielded regions become hydrophobic after undergoing polymerization.			
	Advantages: Convenient, useful; Disadvantages: Expensive chemicals and equipment, multiple steps, time consuming, reduced paper flexibility; Examples: Martinez et al. (2007)[9], (2008)[48]			
"FLASH" printing	Description: Fast Lithographic Activation of Sheets (FLASH); Much like photolithography, except the paper is laminated between a transparent film and a black paper sheet. A standard ink-jet printer is then used to print a black ink mask onto the film. After polymerization the black paper and film is removed.			
	Advantages: Faster, customized masks; Disadvantages: Expensive, multiple steps, reduced paper flexibility; Examples: Martinez et al. (2008)[48]			
Etch Printing	Description: Completely hydrophobised paper, using a polystyrene toluene solution, is "etched" using a toluene solvent printed on the surface which dissolves the solution to allow for the hydrophilic channels to form.			
	Advantages: Custom designs, faster; Disadvantages: Corrosive/ flammable, chemicals; Examples: Abe et al. (2008)[34] (2010) [33]			
PDMS Printing	Description: PDMS is dissolved in hexane and printed onto filter paper using a modified x-y plotter. To form the hydrophobic barriers, the PDMS solution penetrates through the paper thickness.			
	Advantages: Enhanced flexibility; Disadvantages: Reduced channel resolution due to "creeping" solution; Examples: Bruzewicz et al (2008)[49]			
Plasma Printing	Description: Paper previously hydrophobised using the cellulose reactive compound, alkyl-ketene-dimer (AKD) is patterned using metal masks that are clamped to the paper before being placed in a plasma asher. The AKD hydrocarbon chains are then oxidised by the plasma, leaving the hydrophilic channels.			

	Advantages: Flexible; Disadvantages: Expensive, slow manufacturing rate; Examples: Li et al. (2008)[47], (2010)[50], (2010)[45],
Wax Printing	Description: Multiple techniques. The simplest involves pattering both sides of filter paper with a wax crayon before heating it. He was then melts into the substrate to form hydrophobic barriers. Also can be extended to inkjet printing for more complicated designs with higher resolution, but at an increased cost.
	Advantages: Good in resource limited settings; Disadvantages: Low resolution; Examples: Lu et al (2009)[51], Carrilho et al. (2009)[52]
Laser Cutting	Description: Uses a computer-controlled x-y knife plotter to cut the paper into the desired design with very high detail. Does not utilize imbibing techniques.
	Advantages: Cheaper fabrication costs, detailed design, clear labelling, can be fully or partially enclosed; Disadvantages: experimental technique, heating; Examples: Fenton et al. (2008) [39]
Inkjet	Description: Office or specialized inkjet printer receive hydrophobic and bioactive ink cartidges. Resolution of 20 μm or better determined by the diameter of the ink droplet. Advantages : low cost and flexibility of digital printing, established technology. Disadvantages : interaction ink-paper critical, plugging of nozzles. Examples: Li (2010)[45].
Lithography, Flexography, Silk Screening	Description: contact printing techniques. Advantages: fast, cheap, established technology. Disadvantages : require a mold, blanket or negative

printing, 5) flexo printing and 6) screen printing; and 3 radiation/paper processes: 1) plasma treatment, 2) paper cutting and 3) laser abrasion. The design concept of 2D- μ PADs is to create hydrophobic barriers onto or within the paper structure, relying on the capillarity of the substrate for liquid flow. Original techniques created rigid and brittle barriers, however, technology has progressed to allow channels as narrow as 250 μ m width to be created [45]. Multi-step assays are still unable to be performed using common 2D-PAD designs. They are also prone to contamination and fluid evaporation. Despite this, 2D-PADs are very cheap, easy to use, versatile and robust.

Of interest is the work of Citterio et al. [33–35], from Keio University, Japan, with inkjet printing. Well-defined 3D hydrophilic microfluidic patterns were

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created on paper by inkjet etching. Filter paper was soaked in a polystyrene solution, dried and flow channels and sensing area were etched by inkjet printing toluene [33, 34]. A direct inkjet patterning method for microfluidic paper devices was also developed by Citterio by printing a hydrophobic UV-curable ink [35]. Shen et al. developed microfluidic systems by inkjet printing a reactive hydrophobic ink made of sizing agent (AKD) [45, 46]. Microfluidic systems were also achieved by plasma treating heavily sized paper; the pattern was defined by the shape of a mask [47].

3.2.2 Partially and Fully Enclosed PADs

The basic 2D- μ PAD design is usually exposed, thus leading to the possibility of contamination and evaporation of reagents and sample. This is due to both sides of the device being uncovered. Also, contact with the support can potentially result in loss of reagent and sample fluids. Early investigations attempted to avoid such loss and contamination by adding samples and solutions while the device was held in mid-air. This is simply impractical for testing purposes [25].

A method proposed by Fenton et al. (2008) [39] avoided the imbibing of hydrophobic/hydrophilic patterns on paper. Paper was alternatively shaped into 2D designs using a computer-controlled x-y knife plotter. Three designs were proposed: 1) a single sheet of paper cut into the desired shape; 2) paper was mated with one layer of polyester cover tap before being cut; and 3) pre-cut paper was sandwiched between two layers of cover tape. The latter was the first example of a fully enclosed μ PAD. Decreased rates of operator error were reported for partially or fully enclosed PADs; however, this may due to less room for errors in interpretation when paper is cut into clearly labeled sections [39]. This design is reportedly cheaper to fabricate and is impervious to external contaminants.

A partially enclosed device was reported by Olkkonen et al. (2010) [53] The design involved flexographically printing polystyrene xylene/toluene ink onto the back of the device, while the microfluidic channel design was printed on the front. A protective layer was provided by the hydrophobic back layer, preventing fluid from escaping via contact with the underlying support. It also protects against contamination from the support. Due to the direct roll-to-roll production, this method is well suited for high throughput manufacture. Full penetration of the hydrophobic barrier was achieved by the enclosed back, protecting from contaminants and loss of fluids.

Schilling et al. (2012) [25] recently investigated using printing toner to produce fully enclosed μ PADs. A protective layer was printed by thermally bonding a thin plastic onto the paper. The laser printers and toner are similar to commonly purchased office printers, which have been extensively used for μ PAD fabrication. This method is cheap and convenient for μ PAD manufacture. Since the

toner reportedly did not diffuse into paper, it does not affect the microfluidic channels, nor does it come off when wetted. A fully enclosed μPAD can be achieved with four printed layers and faster wicking rates were reported.

Since the biological reagents are affixed to the paper prior to printing so to be enclosed beneath the layer of toner, the heat of 180°C required for this laser printing method could potentially have a negative effect on the functionality of the reagents. Proteins undergo denaturation at extreme heats, losing the ability to bind to other molecules and thus becoming inactive. Schilling et al. reported an enzymatic function decrease of 90% when using glucose oxidase as an example [25]. A negligible effect to the sensitivity was reported when testing for glucose concentration, demonstrating a limit of detection (LOD) of 1mM. However, the 90% decrease in enzyme activity raises concerns regarding the optimization and economics of this method. Schilling also reported an alternative method where the surface was designed with a 1mm diameter hole. The hole was to act as a reagent addition port, allowing reagent delivery after enclosing the μ PAD. Wicking would transport the reagent to the storage zone (Figure 16). With the exception of the port area, no loss of reagent or enzyme function was reported as the reagents were now protected from the surrounding environment.



Figure 16. Example of a fully enclosed 2D paper diagnostic; showing separate testing zones (T) connected to a single sample zone (S). (Redrawn from Schilling et al. (2012) [25]).

3.2.3 PAD Networks

'Traditional' single sheet µPADs are limited to single-step processes. While ideal for the end-user, single-step designs restrict the applicability as most laboratorybased diagnostic assays are actually multi-step processes. Assays, such as the ELISA, have signal amplification and washing steps to improve sensitivity and specificity. Multi-step PADs were attempted using two-dimensional paper networks (2D-PNs). While using a single activation step to retain simplicity and affordability of the single-step µPADs, the 2D-PNs expanded to involve multiple processes within the network. A 2D-PN was developed by Fu et al. (2010) [54]. The design retains the autonomous nature of µPADs with a single activation step, but allows multiple reagents to be delivered sequentially to the detection zone (Figure 17) [54, 55]. The first design reported three methods that allowed the simultaneous convergence of multiple inlets to a single point [54]. The focus was varying the delivery time of the fluid. This was achieved by 1) varying paper length, 2) varying paper width or 3) the implementation of a dissolvable barrier. consisting of trehalose, with the function to slow the liquid in its tracks. Paper composition, pore size and surface chemistry were other contributing factors that could potentially affect fluid flow rate. However, these were not explored. PAD fabrication involved using a laser cutter, before being supported on a glass substrate using double-sided tape [39, 54]. The inlets held absorbent pads for the application of reagents, relying on wicking for transportation to the detection zone. The 2D-PN design used dye and pH as examples to show the staggered delivery of individual components to the convergence site. The same design was later used to demonstrate chemical signal amplification [54, 56].



Figure 17. Example of a 2D paper network used for the sequential testing of multi-step analysis with a single activation point. (Redrawn from Fu et al. (2012) [55]).

While the transport mechanisms of fluid through the paper networks were explored, the effects of paper composition, pore size and surface chemistry were not addressed[57]. Despite the 2D-PN design allowing multiple-step processes, the added complexity also increases the possibility of errors.

Alternatively, a method using a single fluid source was explored, removing the individual sources for each of the reagents [58]. The device was 'programmed' to sequentially disconnect each reagent. A single source well was used, while each reagent section had a varying length of paper in the 2D-PN. A plastic casing of poly (methyl methacrylate) (PMMA) was used to house the plastic strips. The purpose was to reduce evoparation, as well as provice a support to mount the device into the source well. The casing and well design allows a paper cartridge/strip to be inserted by the user. Paper strips are immersed into the fluid well at different depths, depleting as fluid wicks into paper. As the level of fluid in the source well depletes, it 'disconnects' strips. Disconnection is controlled by 1) the rate of fluid source. Disconnection also relies on a large and thick regulator strip made of paper; without it, the lengths of paper varied only slightly. Fluid flow is managed using basic principles of wicking, allowing the 2D-PNs to be programmed. This series of experiments used colored dyes, previously dried onto paper.

3.2.4 Microfluidics to Control Liquid Flow

Certain applications require mixing, reacting or sequencial addition of reactants. This has been achieved with paper microfluidic systems. Shen et al. designed valves and reactors on paper to control the addition of fluids, their retention time and extent of reaction [3, 35, 46, 59].

3.3 3D Paper Diagnostics

3D paper devices developed from the desire to increase the number of microfluidic channels available, allowing for the design of more complex systems. This in turn would lead to the ability to test more analytes simultaneously, and thus create a multiplex 3D- μ PAD. Several prototypes have been reported, each consisting of multiple 2D paper layers. While 3D- μ PADs offer certain advantages, the increased functionality sacrifices the design's simplicity, cost and practicality.

The first 3D-µPAD design alternated layers of patterned paper and perforated double-side tape, each stacked upon the other. The fluid was then allowed to flow vertically through the device in addition to the lateral flow shown in 1D and 2D devices. In order to connect each layer, the perforated holes were filled with cellulose powder, thus providing the 'vertical micro-channels'. Interweaving pathways could be created to allow the analyte to reach several detection zones while



Figure 18. Side-view representation of an example 3D paper diagnostic. Redrawn from Martinez et al. (2010) [61].

avoiding cross contamination. An initial design by Martinez et al. (2008) [60] demonstrated a 4 channel device. Each channel had 8 connecting channels that allowed fluid to travel from the top layer of the device to the bottom, where the detection zones were located. Each layer could hold a varying purpose, including fluid distribution, filtration or combination with other reagents (Figure 18).

The fabrication process usually predetermines the function a device test. A modified $3D-\mu PAD$ design demonstrated a single device which could be used for specific applications [61]. Multiple analytes could be configured on-site using a single device, enabling some specificity depending on the patient's need by choosing the channels required and 'activating' each specific test. Activation is achieved by leaving certain perforations in the tape without cellulose powder. These are labeled 'on-buttons'. One need only press to the button down to connect it with the desired channel, and thus turn the test 'on'. Unpushed buttons would remain disconnected, only allowing flow to the required tests. This could prove particularly useful when the sample quantity is limited. The single platform could be hailed as a 'universal device' to test a wide range of samples, eliminating the need for separate designs for each test.

However, once a button is pressed, it cannot be turned 'off'. The paper compresses when pushed and deforms inelastically. The simplicity, ease of use and reliability decrease with test complexity, especially in times of stress or illness. These tests are getting too complex to be used directly by the patient.

3.3.1 Origami

Origami is the art of paper folding. 3D-µPADs by Liu and Crooks (2011)[62] report a design method using a single sheet of folded paper (Figure 19). Chromatography paper is patterned with micro-channels using a single photolithographic step. A metal frame then provides the template for the paper folding to ensure the alignment is correct. A particular sequence is followed during the folding process, allowing micro-channels to be matched, and thus flow in both lateral and vertical directions. This design also calls for an aluminum clamp, where the four corners

of the folded paper are trimmed in order to fit. The clamp holds four holes drilled on top, access ports allowing samples and solutions to be injected into the device. Origami-PADs (*o*-PADs) allow for the elimination of double-sided tape, which reportedly diffuse into the paper after long time periods, thus decreasing the micro-channels' hydrophilicity. It also removes required assembly tools such as laser cutters. Despite these advantages, origami PADs still require an aluminum clamp to ensure proper alignment. This increases costs of the device, complexity of use and decreases disposability.

Another 3D- μ PAD which utilizes the origami principles was reported by Govindarajan et al. (2012) (Figure 19) [63, 64]. However, the origami steps do not simplify fabrication, but are instead integrated as part of the function. By folding the layers in a certain sequence, the origami steps allows for complex processing. This was demonstrated to prepare collected DNA samples for molecular diagnosis. Stacked lays of cellulose paper and double-sided tape were used to assemble the device. Additionally, single-sided tape, a repositionable adhesive layer and paper card were used and folded. During fabrication, a lazed cutter is



Figure 19. Redrawn examples of origami microfluidic paper diagnostic for multi-step analysis designed by a) Govindarajan et al. (2012) [63, 64]. b) Liu and Crooks (2011) [62].

used after stacking to pattern the fluidic channels. When the adhesive layer is peeled away, full and partial cuts on the device leave fluidic channels on the substrate.

3.4 Detection Principles in Paper Diagnostics

The detection of diseases using PADs is a two-fold process that relies on the ability to report a specific recognition event. Various applications require different reporting and recognition methods. This field is currently undergoing a very fast development as witnessed by the proliferation of publication and patents. Such methods are discussed below.

3.4.1 Methods of Reporting

Half the challenge of accurate and successful diagnosis is detecting the presence of an analyte. It also requires a reliable and simple method of relaying the information to the end-user. Most PADs visually report results. Additionally, some devices have attempted to integrate electronic reporting processes. A brief overview of the development and issues of the main technologies is provided in this section.

3.4.1.1 Colorimetry and Visual Signals

The visual change within the detection zone of a device is the most used reporting method for PAD analysis. The most common technique described in the literature is colorimetric reporting. A visual color change occurs in the presence of a desired target once a sample has been tested. A prime example is the ELISA, where a biosensor is immobilized onto the surface of a substrate. Once the desired analyte is captured, a second reporting enzyme is bound and induces a color change, announcing its presence.

Colorimetric analysis on PADs is simple. Also, it is applicable to point of care (POC) situations. Analytical instrumentation for accurate interpretation is often required for other visual reporting methods, such as florescence or absorbance. In its simplest form, a qualitative binary result can be provided by colorimetric analysis, i.e. whether or not a color change is observed. The white background makes paper an ideal substrate for colorimetric analysis because it provides an excellent contrast. However, it can also interfere with fluorescence and absorbance reading methods, further supporting the use of colorimetric reporting. Lignin residues, dyes and UV brighteners sometimes used in paper can interfere with fluorescence measurements.

However, colorimetric analysis does have some disadvantages. Interpretation may require trained personnel or analytical instruments, especially for quantita-

tive analysis. In the modern age, telemedicine can be used to communicate, interpret and/or transmit results for analysis [6, 65].

Alternatively, another concept of printing symbols or text has been demonstrated for qualitative results to contour user-barriers. The "writing" technique was first reported by Bodenhamer [66], describing a displacement assay for transparent packaging. Immobilized antigens were printed in a pattern upon the substrate and saturated by a corresponding dye-coupled antibody. When interacting with the antigenic target, the dye is then released resulting in disappearance of the printed pattern, and thus indicating the presence of the target. In this case the target was a pathogenic antigen, resulting from undesired exposure. Li et al. printed directly the results of blood typing analysis by controlling blood-antigen coagulation in microfluidic letter patterns formed on paper [67].

3.4.1.2 Reporting with Electronics

Stand-alone paper diagnostics are ideal. However, many reporting methods rely heavily on colorimetric results which require some interpretation by trained medical personnel. A combination of mobile phones with camera capabilities, and μ PADs could allow for rapid and accurate interpretation of results. This is known as telemedicine [65]. The image of a PAD can be taken using a scanner or a camera, before sending the image via satellite to an expert for analysis. This would eliminate the need for an on-site expert during interpretation. The test interpretation would then only take as long as the time needed for the results to reach the intended expert. Another possibility would be to rely on smart phone applications for direct analysis.

Electrochemical reporting methods have also been explored [68]. Electrochemical analysis provides simplicity, reduced costs and portability to current analytical methods. Integration with paper-based microfluidic devices could be an ideal solution for analyses that utilize electrochemistry reporting methods [69]. An example is reported by Carvalhal et al. (2010) [69] describing a paper-based separation device that electrochemically detects the presence of ascorbic acids (AA) and uric acids (UA). Patterned strips of paper can be printed with thin-layer electrodes for coupling to an instrument. The paper acts like a chromatography column, allowing for separation. The electrode can detect the presence of electroactive compounds when the solvent dissolves the AA or UA samples, allowing for quantitative concentrations to be detected chromo-ampero-graphically. Liu and Crooks (2011) [68] also reported a battery-powered electrochemical sensing platform that electro-chromically displays results. Nie et al. (2010) [70] described electrochemical paper-based microfluidic devices (µPEDs), fabricated by screen printing electrodes using conducting inks (e.g. carbon, or Ag/AgCl). Furthermore, research also combined uPEDs with commercial electrochemical readers, such as

glucometers, for analyzing numerous biomarkers in blood or urine [71]. Such biomarkers include glucose, cholesterol, lactate and alcohol. Paper-based electrochemical ELISA devices were also explored to detect rabbit IgG [72].

Another electro-based detection method which has been gaining interest in recent years is electrochemiluminesence (ECL) [73-76]. Microfluidic paper devices can be coupled with a chemiluminescence reaction, which is initiated and controlled by the application of an electrochemical potential. ECL is reported to combine the advantages of luminescence and electrochemistry, but also added selectivity to the device. Delaney et al. (2011) [73] report using an ECL reagent, $Tris(2,2'-bipyridyl)ruthenium(II),(Ru(bpy)_{3}^{2+})$ to detect co-reactants, such as nicotinamide adenine dinucleotide (NADH). The reaction between ECL and its co-reactant generates light upon oxidization. Mobile phone cameras are then employed as luminescence detectors for ECL emissions. The device is still reported to maintain simplicity and low-costs as the fluidic channel detection zones are printed with ECL using an inkjet printer, similar to other reported methods of µPAD fabrication. Yu et al. (2011) [74] successfully demonstrated the ability to detect UA in artificial urine using electrochemiluminescence. Subsequently, a paper-based ELISA using chemiluminescence detection was explored using biomarkers such as ∞-fetoprotein, cancer antigens and carcinoembryonic antigens as examples [75].

3.4.2 Biorecognition in Paper Diagnostics

Accurate diagnosis can rely on the ability to detect the presence of specific biomolecules selectively. This is known as biorecognition. There is an abundance of distinct analytes within the human body. Each could potentially be used for the diagnosis of disease, both pathological and physiological. When the correct biomolecular target is identified, a detection method can subsequently be developed. Nucleic acids, enzyme proteins and antibodies are prime examples of biomolecules which can be immobilized on paper. They can reportedly be dried onto paper or the substrate's surface without denaturation, which allows for storage and use in remote areas lacking access to laboratory facilities.

However, to achieve biorecognition, the correct target is required to detect a specific marker in the body. The greatest challenge is utilizing this ability to unequivocally detect the presence or absence of a disease. Current diagnostic methods often provide the basic outline of detection principles. However, biomolecular behavior on paper sometimes varies from the behaviour exhibited in solution, when using established laboratory techniques.

Examples of selective biorecognition on paper devices include applications for diseases such as diabetes, malaria, HIV-1 and cancer; and non-disease related analyses such as liver function and blood typing. Each example detects a different

type of biomarker. Diabetes-related devices are able to detect and quantify the concentration of glucose in blood or urine [9, 34, 39]. A cancer diagnostic has been reported with the ability to detect a specific miRNA sequence indicative of lung cancer [77]. Alternatively, other biomarkers could include proteins such as alkaline phosphatase (ALP) and aspartate aminotransferase (AST) enzymes which are used for liver function analysis [78]; or *Plasmodium falciparum* histidine rich protein 2 (*Pf*HRP2) used to detect a malaria infection [42, 55]. The specificity of antigen-antibody interactions can also be utilized for biodiagnostics on paper. Prime examples are its use in blood typing [28, 29, 67], HIV-1 detection [79], and Hepatitis B & C detection [80].

The potential of biorecognition on paper is limitless. Success is not dictated by the use of one specific type of biomolecule over another, but is a result of the detection methods available. As can be seen, each application is different, using samples from a range of sources (e.g. urine, blood and saliva), and thus requires separate examination and methodologies for the paper analytical device (PAD) to succeed.

4 PERSPECTIVES

Four important aspects of paper microfluidics remain to be analyzed in this review. The first is the effect of paper structure on microfluidics; the second are alternatives to paper for low cost microfluidic systems able to be integrated in a diagnostic; the third is an overview of the manufacturing process; the last is an outlook of challenges and future directions of paper microfluidics.

4.1 Paper structure

Very few studies have analyzed the effect paper structure has on microfluidics performance. Paper structure represents the 3 dimensional distribution of fibers within the paper matrix; its negative is the structure of the void space. Two observations are of importance. The first is that only the porous void fraction of paper, bordered and driven by the hydrophilic fibers, transports liquids in paper by wicking. The second is that wicking of a liquid in paper only occurs in dry paper; the driving force disappears once a liquid has filled the paper's pores. Critical variables are the diameter, orientation and length of pores, which are defined by fiber orientation and spatial distribution.

Paper offers a three dimensional (3D) flow in the design of microfluidics as liquid can be transported and fractionated not only in the plane of the surface (X-Y), but also through the thickness of paper (Z direction). The fiber type and spatial distribution dictate the size, connectivity and directionality of the

capillaries through which a liquid transports and the analytes distribute. Of special interest are the type of fibers, paper density and thickness (basis weight), and the fiber orientation. The important papermaking process variables to engineer the basesheet for microfluidics paper include the jet to wire speed differential, the choice of chemical additives (filler, sizing agents), calendering and surface treatments such as surface sizing and coating.

X-ray microtomography is a powerful technique to characterize the 3D microstructure of porous media; for paper, it allows the visualization and digitalization of fibers and pores. Paper thickness-porosity profiles, microtomograph segmentations and 3D visualization can then be derived. Bloch et al. have combined X-ray synchrotron microtomography with transport modeling of a fluid within the digitalized paper structure [81–83]. Flow rate, filtration size cut-off and separation efficiency can then be simulated. The model derived can also be used to design the optimal paper structure for a specific application.

4.2 Alternatives to paper microfluidics

Paper combined with functional printing provides a very cheap and flexible material/process combination for manufacturing microfluidic devices to transport liquids. Currently, the primary application of these microfluidics is in low cost diagnostics for medical applications. There are at least three material/process alternatives to the paper technology that can serve this function. These are: 1) threads, 2) hydrophilic and porous polymeric films and 3) grooves on hydrophilic surfaces.

Multifilament cotton and cellulosic threads have been investigated by a few groups including Shen [84–86], Whitesides [87] and Juncker [88] as alternative to paper for low cost microfluidic systems and biodiagnostics. Capillarity drives the flow of a fluid along the thread. While thread and paper are chemically similar, many properties of the material differ. The main differences include the length of the fiber, the interfiber bonding and the porous channel structure [86]. Shen et al. also devised mechanisms to control the liquid flow in thread based systems [86]. Figure 20 illustrates the effect of thread structure (with one, two and three threads) on the capillarity flow rate [86] for vertical devices. The fluid penetration length varies with the square of time according to equation 2, until gravity and evaporation become significant phenomena. Similar to paper, thread is supported by a very established, robust, widely available and low cost technology that can be used to manufacture diagnostic devices.

Hydrophilic and porous polymeric films represent another alternative to paper, which can, however, use the same process technology. Cellulosic materials, and especially nitrocellulose films, have long been used in low cost diagnostics. Yager et al. [89], from the University of Washington in Seattle, reviewed the evolution



Figure 20. Effect of the thread structure on the capillary flow of an aqueous dye solution penetrating vertically suspended threads with an end immersed in a reservoir solution. Figure 3 from Shen et al. [86].

of nitrocellulose as a material to engineer bioassays [89]. Biomolecules (RNA-DNA complexes) were immobilized on porous nitrocellulose membranes in the 1960s [89, 90]. After 25 years of development, porous nitrocellulose membrane has become a prominent material for lateral flow (LF) diagnostics; it provides liquid capillary flow and preserves the functionality of immobilized biomolecules [89]. Nitrocellulose has a unique compatibility with three of the most important classes of biomolecules: proteins (enzyme, antibody), DNA and RNA [89, 90]. Yager et al. have developed complex and elegant diagnostics including dissolvable fluidic time delays for programming multisteps assays with nitrocellulose membranes [91].

Shen et al. have investigated V-grooves on quartz surfaces as microfluidic systems for fluid delivery and diagnostic applications [92]. For a given (hydrophilic) surface, the wicking velocity of the liquid increases as the angle of the grove decreases but increases with the grove width.

4.3 Manufacturing Paper Microfluidics and Diagnostic Devices

The combination of roll-to-roll process combined with paper and polymeric films as materials offer a very efficient, low cost and flexible process/material combination to manufacture paper microfluidics and paper diagnostics on a large scale. The manufacturing process consists of a sequence of unwinders, printers, IR/UV dryers and rewinders. An example is the large-scale production of paper tests to measure the level of glucose in blood; this can be a 2D PAD involving a microfluidic system and an enzyme for the detection. A roll of paper is unwound and fed through a first inkjet printer to pattern the microfluidic system (AKD solution or UV sensitive hydrophobic ink) and then into a UV/IR drver to cure the hydrophobic barriers. Next, patterns of the enzyme/reactants are printed on paper within the detection zones of the microfluidic system using a second inkjet printer. The roll of microfluidic/enzyme paper diagnostic is then sandwiched between two films of polymer wrapping, fused and cut, using the standard equipment for diaper manufacturing. Such a manufacturing line operating at a velocity of 800 m/min with a paper roll 1m wide, and producing diagnostics $10 \text{cm} \times 10 \text{cm}$, will process 100 million diagnostics/day. Assuming the international market for diabetes type II to be 300 million (5% population) needing a daily test, the world's needs can be supplied with only 3 such functional printing manufacturing lines. Being first to market and having proper intellectual property then become critical for product protection.

4.4 Challenges and Future Directions

Two types of paper microfluidics development are needed to make paper diagnostics a commercial reality. The first is a simplification of the microfluidic/diagnostic systems to further decrease manufacturing cost and enable commercial viability of products for price sensitive markets (such as water quality testing). The second is an increase in the performance of the paper diagnostics for specialized health applications that might require separation of the sample components on paper, more sensitive mechanisms of detection, and easier and better mechanisms for the communication of results.

Implementation of new technology often first proceeds with the high value products and then rolls down toward commodities, as a preferred technology is selected, processes are optimized and manufacturing price decrease. Following paper tests for human health diagnostics, applications in veterinary health diagnostics, food quality (antibiotics in milk, proscribed pesticide on fruits, hormones in meat, fish freshness) and environmental diagnostics (heavy metals in water, bacteria in restaurants kitchens) are expected. The level of microfluidics sophistication is foreseen to decrease in this order as the product value is projected to decrease. When large volumes of the test liquid sample are available, a simple dipstick suffices; simpler modes of diagnostics analysis with a basic yes/no binary logic are needed. To ensure paper diagnostics provide a reliable analysis, a first challenge is to control the threshold analyte concentration at which a positive is reported. A second challenge is to better control false positive and false negative tests and to err on the side of caution for health safety.

The very low cost of paper microfluidic systems offers opportunities for routine indicators for personal care devices and packaging [93]. Microfluidic systems can indicate wetness in diapers, or be printed as integrity indicator on paper packaging.

Developing paper as tool for advanced and sensitive diagnostics has a few challenges. The first is the sensor's ability to analyse complex samples with separation capabilities integrated into paper. A second challenge is to design and develop label-free analytical platforms. A third challenge is to significantly decrease the detection limit beyond nanomolar or part per billion (PPB) [94]. The last few years have witnessed the integration of microfluidic paper analytical devices with optical and electrically active nanomaterials [94]. Ngo et al. reviewed applications and issues with nanoparticles treated paper [95]. Ngo et al. also investigated the effect of gold nanoparticles (AuNP) treated paper on the signal enhancement to detect very low concentrations of chemical and biochemical analytes using Surface Enhanced Raman Spectroscopy (SERS) technology [96–99]. The effect of AuNP concentration, aggregation state and paper 3D distribution on the detection selectivity, sensitivity and reproducibility of SERS analysis were studied. However the use of nanoparticles on paper for signal enhancement drastically increases the cost of the diagnostics and requires the use of analytical instrumentation.

5 CONCLUSION

Paper microfluidics are not the most efficient or useful systems to transport liquids when taken as independent systems; neither are bioactive papers particularly sensitive or accurate for medical diagnostics. However, combining microfluidics with bioactive paper enables the creation of invaluable diagnostics for health care applications. This is the result of the very low price, great design flexibility, and simplicity of use given by paper-based diagnostics manufactured by functional printing and roll-to-roll process. Paper can serve many complementary functions for a diagnostic. The capillarity wicking along the cellulosic fibers of paper provides a driving force to transport fluids without the need for external system/energy. The porosity and biocompatibility of paper enable reagents and biomolecules to be stored. Paper offers the separation of analyte constituents by filtration and chromatography. Finally, paper is a combustible, biodegradable and recyclable material

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offering many easy options of disposal after use. Surprisingly, very little attention has been given to optimizing paper structure for microfluidics and diagnostics application. This offers a great opportunity to further push the boundaries of microfluidic paper and engineer new generations of low cost diagnostics.

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6 REFERENCES

- 1. Pelton, R., *Bioactive paper provides a low-cost platform for diagnostics*. Trends in Analytical Chemistry, 2009. **28**(8): p. 925–942.
- 2. Pelton, R. *Bioactive paper A paper science perspective*. in *Proceedings of the 14th Fundamental Research Symposium*. 2009. Oxford.
- 3. Li, X., D. Ballerini, and W. Shen, *A perspective on paper-based microfluidics: Current status and future trends*. Biomicrofluidics, 2012. **6**(1): p. 011301.
- Ballerini, D., X. Li, and W. Shen, Patterned paper and alternative materials as substrates for low-cost microfluidic diagnostics. Microfluidics and Nanofluidics, 2012. 13(5): p. 769–787.
- Liana, D.D., et al., Recent advances in paper-based sensors. Sensors, 2012. 12(9): p. 11505–26.
- 6. Martinez, A.W., et al., *Diagnostics for the Developing World: Microfluidic Paper-Based Analytical Devices*. Analytical Chemistry, 2010. **82**(1): p. 3–10.
- Maxwell, E.J., A.D. Mazzeo, and G.M. Whitesides, *Paper-based electroanalytical devices for accessible diagnostic testing*. MRS bulletin, 2013. 38(04): p. 309–314.
- 8. Then, W. and G. Garnier, *Paper Diagnostics in Biomedicine*. Reviews in Analytical Chemistry, 2013: p. Submitted.
- Martinez, A.W., et al., *Patterned Paper as a Platform for Inexpensive, Low-Volume, Portable Bioassays.* Angewandte Chemie International Edition, 2007. 46(8): p. 1318–1320.
- Washburn, E.W., *The Dynamics of Capillary Flow*. Physical Review, 1921. 17(3): p. 273–283.
- Basu, S., K. Nandakumar, and J.H. Masliyah, A Study of Oil Displacement on Model Surfaces. Journal of Colloid and Interface Science, 1996. 182(1): p. 82–94.
- 12. Seaver, A.E. and J.C. Berg, *Spreading of a droplet on a solid surface*. Journal of Applied Polymer Science, 1994. **52**(3): p. 431–435.

- Modaressi, H. and G. Garnier, Mechanism of Wetting and Absorption of Water Droplets on Sized Paper: Effects of Chemical and Physical Heterogeneity. Langmuir, 2002. 18(3): p. 642–649.
- 14. Nilsson, L., B. Wilhelmsson, and S. Stenstrom, *The diffusion of water vapour through pulp and paper*. Drying Technology, 1993. **11**(6): p. 1205–1225.
- 15. Erbil, H.Y., G. McHale, and M. Newton, *Drop evaporation on solid surfaces: constant contact angle mode*. Langmuir, 2002. **18**(7): p. 2636–2641.
- 16. Hu, H. and R.G. Larson, *Evaporation of a sessile droplet on a substrate*. The Journal of Physical Chemistry B, 2002. **106**(6): p. 1334–1344.
- 17. Kohler, M. and L. Parmele, *Generalized estimates of free-water evaporation*. Water Resources Research, 1967. **3**(4): p. 997–1005.
- Uno, P.J., *Plastic shrinkage cracking and evaporation formulas*. ACI Materials Journal, 1998. 95: p. 365–375.
- Oko, A., P. Claesson, and A. Swerin, *Imbibition and evaporation of water droplets on paper and solid substrates*. Journal of Imaging Science and Technology, 2011. 55(1): p. 10201–1.
- 20. Khan, M.S., et al., *Effect of liquid droplet impact velocity on liquid wicking kinetics in surface V-grooves*. Chemical Engineering Science, 2011. **66**(23): p. 6120–6127.
- 21. Khan, M.S., *Bioactive Papers: Printing, Activity and Stability*, in *Chemical Engineering* 2009, Monash University Clayton, Melbourne. p. 292.
- 22. Deegan, R.D., et al., *Contact line deposits in an evaporating drop*. Physical Review E, 2000. **62**(1): p. 756.
- 23. Deegan, R.D., et al., *Capillary flow as the cause of ring stains from dried liquid drops*. Nature 1997. **389**: p. 827–829.
- 24. Wheeler, T.D. and A.D. Stroock, *The transpiration of water at negative pressures in a synthetic tree*. Nature, 2008. **455**(7210): p. 208–212.
- Schilling, K.M., et al., Fully Enclosed Microfluidic Paper-Based Analytical Devices. Analytical Chemistry, 2012. 84(3): p. 1579–1585.
- 26. Block, R.J., E.L. Durrum, and G. Zweig, *A manual of paper chromatography and paper electrophoresis*. 2nd Edition ed1958, New York: Academic Press.
- 27. Su, J., M. Al-Tamimi, and G. Garnier, *Engineering paper as a substrate for blood typing bio-diagnostics*. Cellulose, 2012. **19**(5): p. 1749–1758.
- Khan, M.S., et al., Paper Diagnostic for Instantaneous Blood Typing. Analytical Chemistry, 2010. 82(10): p. 4158–4164.
- 29. Al-Tamimi, M., et al., Validation of Paper-Based Assay for Rapid Blood Typing. Analytical Chemistry, 2011. 84(3): p. 1661–1668.
- Zweig, G. and J. Sherma, *Paper and thin-layer chromatography*. Analytical Chemistry, 1976. 48(5): p. 66R-83r.
- 31. Konig, P. Employment of electrophoresis in chemical experiments with small quantities. in Acta e terceiro Congresso sud-americano de chimica Rio de Janeiro e Sao Paulo. 1937.
- 32. Lubran, M., *Paper electrophoresis*. JAMA: The Journal of the American Medical Association, 1966. **197**(5): p. 360–361.
- Abe, K., et al., *Inkjet-printed paperfluidic immuno-chemical sensing device*. Analytical and Bioanalytical Chemistry, 2010. **398**(2): p. 885–893.

- Abe, K., K. Suzuki, and D. Citterio, *Inkjet-Printed Microfluidic Multianalyte Chem*ical Sensing Paper. Analytical Chemistry, 2008. 80(18): p. 6928–6934.
- Citterio, D., K. Maejima, and K. Suzuki. VOC-free inkjet patterning method for the fabrication of "paperfluidic" sensing devices. in 15th International Conference on Miniaturized Systems for Chemistry and Life Sciences, Seattle, Washington, USA. 2011.
- Free, A.H., et al., Simple specific test for urine glucose. Clinical Chemistry, 1957. 3(3): p. 163–8.
- Comer, J.P., Semiquantitative Specific Test Paper for Glucose in Urine. Analytical Chemistry, 1956. 28(11): p. 1748–1750.
- Haeberle, S. and R. Zengerle, *Microfluidic platforms for lab-on-a-chip applications*. Lab on a Chip, 2007. 7(9): p. 1094–110.
- 39. Fenton, E.M., et al., *Multiplex Lateral-Flow Test Strips Fabricated by Two-Dimensional Shaping*. ACS Applied Materials & Interfaces, 2008. 1(1): p. 124–129.
- Binder, S.R., Autoantibody detection using multiplex technologies. Lupus, 2006. 15(7): p. 412–21.
- 41. Hawkes, R., E. Niday, and J. Gordon, *A dot-immunobinding assay for monoclonal and other antibodies*. Analytical Biochemistry, 1982. **119**(1): p. 142–7.
- 42. Dineva, M.A., et al., *Simultaneous visual detection of multiple viral amplicons by dipstick assay.* Journal of Clinical Microbiology 2005. **43**(8): p. 4015–21.
- 43. Rosenstein, R.W. and T.G. Bloomster, *Solid phase assay employing capillary flow*, US Patent 4, 240, Editor 1989.
- 44. Gordon, J., M.E. McMahon, and S. Ching, *Chromatographic Test Strip for Determining Ligands or Receptors*, US Patent 4, 691 Editor 1990.
- 45. Li, X., et al., *Fabrication of paper-based microfluidic sensors by printing*. Colloids and Surfaces B: Biointerfaces, 2010. **76**(2): p. 564–570.
- Li, X., J. Tian, and W. Shen, Progress in patterned paper sizing for fabrication of paper-based microfluidic sensors. Cellulose, 2010. 17(3): p. 649–659.
- 47. Li, X., et al., *Paper-Based Microfluidic Devices by Plasma Treatment*. Analytical Chemistry, 2008. **80**(23): p. 9131–9134.
- 48. Martinez, A.W., et al., *FLASH: A rapid method for prototyping paper-based microfluidic devices.* Lab on a Chip, 2008. **8**(12): p. 2146–2150.
- Bruzewicz, D.A., M. Reches, and G.M. Whitesides, Low-Cost Printing of Poly(dimethylsiloxane) Barriers To Define Microchannels in Paper. Analytical Chemistry, 2008. 80(9): p. 3387–3392.
- Li, X., J. Tian, and W. Shen, *Quantitative biomarker assay with microfluidic paper-based analytical devices*. Analytical and Bioanalytical Chemistry, 2010. **396**(1): p. 495–501.
- Lu, Y., et al., Fabrication and Characterization of Paper-Based Microfluidics Prepared in Nitrocellulose Membrane By Wax Printing. Analytical Chemistry, 2009. 82(1): p. 329–335.
- Carrilho, E., A.W. Martinez, and G.M. Whitesides, Understanding Wax Printing: A Simple Micropatterning Process for Paper-Based Microfluidics. Analytical Chemistry, 2009. 81(16): p. 7091–7095.

- Olkkonen, J., K. Lehtinen, and T. Erho, *Flexographically Printed Fluidic Structures in Paper*. Analytical Chemistry, 2010. 82(24): p. 10246–10250.
- 54. Fu, E., et al., *Chemical signal amplification in two-dimensional paper networks*. Sensors and Actuators B: Chemical, 2010. **149**(1): p. 325–328.
- 55. Fu, E., et al., *Two-Dimensional Paper Network Format That Enables Simple Multistep Assays for Use in Low-Resource Settings in the Context of Malaria Antigen Detection.* Analytical Chemistry, 2012. **84**(10): p. 4574–4579.
- 56. Fu, E., et al., *Enhanced Sensitivity of Lateral Flow Tests Using a Two-Dimensional Paper Network Format.* Analytical Chemistry, 2011. **83**(20): p. 7941–7946.
- 57. Fu, E., et al., *Transport in two-dimensional paper networks*. Microfluidics and Nanofluidics, 2011. **10**(1): p. 29–35.
- Lutz, B.R., et al., Two-dimensional paper networks: programmable fluidic disconnects for multi-step processes in shaped paper. Lab on a Chip, 2011. 11(24): p. 4274–4278.
- 59. SHEN, W., et al., Switches for Microfluidic Systems, 2010, WO Patent 2,010,017,578.
- Martinez, A.W., S.T. Phillips, and G.M. Whitesides, *Three-dimensional microfluidic devices fabricated in layered paper and tape*. Proceedings of the National Academy of Sciences, 2008. **105**(50): p. 19606–19611.
- 61. Martinez, A.W., et al., *Programmable diagnostic devices made from paper and tape*. Lab on a Chip, 2010. **10**(19): p. 2499–2504.
- Liu, H. and R.M. Crooks, *Three-Dimensional Paper Microfluidic Devices Assembled* Using the Principles of Origami. Journal of the American Chemical Society, 2011. 133(44): p. 17564–17566.
- Govindarajan, A.V., et al., A low cost point-of-care viscous sample preparation device for molecular diagnosis in the developing world; an example of microfluidic origami. Lab on a Chip, 2012. 12(1): p. 174–181.
- 64. Govindarajan, A.V., et al. *Microfluidic origami for point-of-care extraction of nucleic acids from viscous samples.* in *Micro Electro Mechanical Systems (MEMS), 2011 IEEE 24th International Conference on.* 2011.
- 65. Martinez, A.W., et al., *Simple Telemedicine for Developing Regions: Camera Phones and Paper-Based Microfluidic Devices for Real-Time, Off-Site Diagnosis.* Analytical Chemistry, 2008. **80**(10): p. 3699–3707.
- 66. Bodenhamer, W., G. Jackowski, and E. Davies, *Method and apparatus for selective biological material detection*, 2000.
- Li, M., et al., Paper-Based Blood Typing Device That Reports Patient's Blood Type "in Writing". Angewandte Chemie International Edition, 2012. 51(22): p. 5497–5501.
- Liu, H. and R.M. Crooks, Paper-Based Electrochemical Sensing Platform with Integral Battery and Electrochromic Read-Out. Analytical Chemistry, 2012. 84(5): p. 2528–2532.
- 69. Carvalhal, R.F., et al., *Electrochemical Detection in a Paper-Based Separation Device*. Analytical Chemistry, 2010. **82**(3): p. 1162–1165.
- 70. Nie, Z., et al., *Electrochemical sensing in paper-based microfluidic devices*. Lab on a Chip, 2010. **10**(4): p. 477–483.

- 71. Nie, Z., et al., Integration of paper-based microfluidic devices with commercial electrochemical readers. Lab on a Chip, 2010. **10**(22): p. 3163–3169.
- 72. Li, X.J., et al., *Paper-Based Electrochemical ELISA*, in *Miniturized Systems for Chemictry and Life Sciences*, 14th International Conference on2010: Graningen, The Netherlands. p. 1487–1489.
- 73. Delaney, J.L., et al., *Electrogenerated Chemiluminescence Detection in Paper-Based Microfluidic Sensors*. Analytical Chemistry, 2011. **83**(4): p. 1300–1306.
- Yu, J., et al., A novel chemiluminescence paper microfluidic biosensor based on enzymatic reaction for uric acid determination. Biosensors and Bioelectronics, 2011. 26(7): p. 3284–3289.
- 75. Wang, S., et al., *Paper-based chemiluminescence ELISA: Lab-on-paper based on chitosan modified paper device and wax-screen-printing.* Biosensors and Bioelectronics, 2012. **31**(1): p. 212–218.
- Yan, J., et al., Paper-Based Electrochemiluminescent 3D Immunodevice for Lab-on-Paper, Specific, and Sensitive Point-of-Care Testing. Chemistry – A European Journal, 2012. 18(16): p. 4938–4945.
- Yildiz, U.H., P. Alagappan, and B. Liedberg, *Naked Eye Detection of Lung Cancer* Associated miRNA by Paper Based Biosensing Platform. Analytical Chemistry, 2012. 85(2): p. 820–824.
- Vella, S.J., et al., Measuring Markers of Liver Function Using a Micropatterned Paper Device Designed for Blood from a Fingerstick. Analytical Chemistry, 2012. 84(6): p. 2883–2891.
- Cheng, C.-M., et al., *Paper-Based ELISA*. Angewandte Chemie International Edition, 2010. 49(28): p. 4771–4774.
- Kendel, F., et al., Effect of Pre-coat and Substrate Porosity on Conventional Barrier Coatings Performance, in Appita Conference and Exhibition (62nd: 2008: Rotorua, N.Z.)2008, Appita Inc.: Carlton, Vic. p. 229–232.
- Idris, Z., et al., *Microstructural effects on the flow law of power-law fluids through fibrous media*. Modelling and Simulation in Materials Science and Engineering, 2004. 12(5): p. 995.
- Viguié, J., et al., Analysis of the hygroexpansion of a lignocellulosic fibrous material by digital correlation of images obtained by X-ray synchrotron microtomography: application to a folding box board. Journal of materials science, 2011. 46(14): p. 4756–4769.
- 83. Koivu, V., et al., *Transport properties of heterogeneous materials. Combining computerised X-ray micro-tomography and direct numerical simulations.* International Journal of Computational Fluid Dynamics, 2009. **23**(10): p. 713–721.
- 84. Ballerini, D.R., X. Li, and W. Shen, *Flow control concepts for thread-based microfluidic devices*. Biomicrofluidics, 2011. **5**: p. 014105.
- Ballerini, D.R., X. Li, and W. Shen, *An inexpensive thread-based system for simple and rapid blood grouping*. Analytical and Bioanalytical Chemistry, 2011. **399**(5): p. 1869–1875.
- Li, X., J. Tian, and W. Shen, *Thread as a Versatile Material for Low-Cost Microfluidic Diagnostics*. ACS Applied Materials & Interfaces, 2009. 2(1): p. 1–6.

- Reches, M., et al., *Thread as a Matrix for Biomedical Assays*. ACS Applied Materials & Interfaces, 2010. 2(6): p. 1722–1728.
- Safavieh, R., et al. in Proceedings of MicroTAS 2009, The 13th International Conference on Miniaturized Systems for Chemistry and Life Sciences. 2009. ICC Jeju, Jeju, South Korea.
- 89. Fridley, G.E., et al., *The evolution of nitrocellulose as a material for bioassays*. MRS bulletin, 2013. **38**(04): p. 326–330.
- 90. Tonkinson, J.L. and B.A. Stillman, *Nitrocellulose: a tried and true polymer finds utility as a post-genomic substrate.* Frontiers in bioscience: a journal and virtual library, 2002. 7: p. c1.
- 91. Lutz, B., et al., *Dissolvable fluidic time delays for programming multi-step assays in paper diagnostics*. Lab Chip, 2013.
- 92. Tian, J., et al., *Capillary driven low-cost V-groove microfluidic device with high sample transport efficiency*. Lab on a Chip, 2010. **10**(17): p. 2258–2264.
- 93. Shen, W., et al., *Method of fabricating paper-based microfluidic systems by printing*, 2012.
- Abbas, A., et al., Multifunctional Analytical Platform on a Paper Strip: Separation, Preconcentration, and Subattomolar Detection. Analytical Chemistry, 2013. 85(8): p. 3977–3983.
- 95. Ngo, Y.H., et al., *Paper surfaces functionalized by nanoparticles*. Advances in Colloid and Interface Science, 2011. **163**(1): p. 23–38.
- Ngo, Y.H., et al., *Effect of cationic polyacrylamide dissolution on the adsorption state of gold nanoparticles on paper and their Surface Enhanced Raman Scattering properties*. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 2013. **420**(0): p. 46–52.
- Ngo, Y.H., et al., Effect of cationic polyacrylamides on the aggregation and SERS performance of gold nanoparticles-treated paper. Journal of Colloid and Interface Science, 2013. 392(0): p. 237–246.
- Ngo, Y.H., et al., Gold Nanoparticle–Paper as a Three-Dimensional Surface Enhanced Raman Scattering Substrate. Langmuir, 2012. 28(23): p. 8782–8790.
- 99. Ngo, Y.H., et al., *Gold nanoparticles paper as a SERS Bio-diagnostic Platform.* Journal of Colloid and Interface Science, 2013. **Submitted**.

Transcription of Discussion

PAPER MICROFLUIDICS: APPLICATIONS AND PERSPECTIVES

Gil Garnier and Whui Lyn Then

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Wolfgang Bauer Graz University of Technology

The paper industry is always looking for new applications; can you make an estimate of how many kilos or hundreds of kilos, or tons of paper you might need for those systems if they have worldwide application?

Gil Garnier

The paper mentality is that you sell tons. What I am talking about is the profitability of the paper mill. Whether you do that with 1000 tons a day or 1 ton does not matter. Imagine that we make one ton of paper on this machine but we add gold nanoparticles and make 0.5 tons and we could sell it at the same price. So what we are talking about is value, and we are talking about engineering, and that is something the paper-makers do not see. We need to work on the paper-makers and modifiers so that there is a change of paradigm. So I'd rather not talk about volume, but the value is pretty impressive because the size of the paper needed for the tests is small.

Wolfgang Bauer

But, isn't it true that the value will be achieved by the persons selling the biosensor and not by the supplier of the paper?

Gil Garnier

Why can't we do it rather than letting Harvard chemists do it? I agree with you,

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but the pulp and paper, you know newsprint paper for communication, is, let's face it, in the past. Packaging, personal care, yes I think it is our job: I think we stay too much behind our concept of technology. We refine, we make paper; no we do not do that, we engineer properties. That has been the advantage of our colleagues who have no idea of how to make paper, they were making diagnostics. Forget paper, use the science, make diagnostics, and maybe it would be paper and maybe another company. One of our plans was to use an old tissue machine for diagnostics and to forget mass production.

Wolfgang Bauer

It is probably a better philosophy to just become independent, start your own company and buy a small 50 cm paper machine and then you can really manufacture a lot of devices.

Gil Garnier

Yes. That is what I want to see and basically here, we talk too much technology. No, the volume is not there, but the value is there. Forget about the number of tons. That is my vision.

Jean-Francis Bloch University of Grenoble

I have one comment and then one question. The first comment is: do not worry if Harvard has introduced science into paper, their students are happy and do good work. I just want to underline one point, what we need is people who are doing some multi-physics. I want to take also the example of Bob Pelton. You cannot do this research if you are only a chemist, or if you are only good in physics. You have to have a cooperation, a cross-link between different scientists; that is where innovations come from, and that is what they are trying to do at Harvard.

Gil Garnier

I wish we could bring together half paper-makers and half biomedical people and bring the communities together; that would be so powerful. Harvard is so strong because they broke the boundary of technology and it just liberated the mind and that is incredibly powerful.

Jean-Francis Bloch

I agree it is not only about science but also costs; it has to be cheap. So, it is

important, there are some economical questions. Who is going to make the paper? Who is going to make the device and where? Because it can be prepared with either huge or small local printers. Are 'they' going to prepare the devices, for example, in Africa, in a small village? Will they take Whatman Filter Paper and try to develop something that will decrease the cost? Or a huge company will make a global device for low cost diagnostics and then sell it worldwide? In other words, who is going to make the diagnostic devices based on paper?

Gil Garnier

Who is going to sign the cheque for the Whatman No. 1? It is very expensive, 100 dollars a box.

Jean-Francis Bloch

But the aim is absolutely not to do that. In the US, they know what the problem is. At the end, what they want to do is to take any piece of paper and build their own device. But if they want to understand something in this complex field, they are using this 'simple' Whatman paper because it is/was present in their chemistry and biology labs and they knew about it. But the final objective is to consider any paper, even with fillers for example. We all know that some problems occur due to the variations of paper properties or structures and so even if it works once, will it work hundreds of times? Scientists have to demonstrate that it works. So I think you may take it as a joke to say they are working only with Whatman No. 1 paper, but it is in order to understand the complex phenomena, to make a development and then work on many different kinds of paper including all the properties and all the chemical modifications, taking into account the variations and so on. But once again, a question for you; who is going to make the paper? Where and who is going to apply the needed technology to transform a piece of paper into a useful diagnostic device?

Gil Garnier

In just a short answer, I do not know.

Bob Pelton McMaster University

A nice review Gil, this is incredibly rapid moving field and it is brave even to try to do what you have done. I would like to make a comment on the question about tons of paper. I cannot see the diagnostic business transforming the paper industry, but I do see opportunities for paper support of microfluidics and related technologies, and also completely different applications. If you can come up with a reason

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why a farmer might coat his fields with a sheet of paper – that it is giving out nutrients or whatever – or if you can see a method of building intelligence into the packaging material, I think that some of the techniques originally developed for the biomedical applications could be incorporated into applications involving many, many tons of paper.

Gil Garnier

I would like to reinforce Bob's comment. I think we are too shy. Let us try to make better paper with better material for other fields. I think that is one good way to help our industry, and agriculture, in particular, is a big, big field, especially in Australia where we have a lot of weeds and it is dry. We can functionalise, we can detect problems. We have wonderful science and a wonderful process and let's try to make paper for use in other fields, biomedical or otherwise. There are many possible applications in agriculture and elsewhere.

Li Yang Innventia

As we heard yesterday, paper is intrinsically variable and non-uniform and that can be important for wicking because of the importance of time for test measurements. So, my question is: what what kind of paper structure is preferable for this kind of test instrument, be it 2D or 3D?

Gil Garnier

Basically the paper is designed for the application. Wei Shen will make a presentation about blood. He has tried to separate according to coagulation, so therefore we want to think of controlled size. He worked a lot with tissue papers; the paper hand towels of our university was one of the best papers. So that is one application, but we might want to separate according to speed of wicking and this would require a different base sheet. Or if you require chromatography effects another one will be needed. You might be able to have a normal base sheet and to functionalise the surface, or perhaps buy an older paper machine when what you need is few rolls. I think it is a speciality application and we have to use speciality engineering in each case.

Tetsu Uesaka Mid Sweden University

I have a technical question. No market issue at all! You mentioned microfluidics and micro-fluids. Are you really using the special effects of microfluidics? Because microfluidics means that we are talking about a very small volume of fluid with interfaces, surfaces and so on so that there are complex surface and interference effects at the very, very small scale, which normally deviate from macroscopic descriptions of fluid behaviour. This obviously gives us a lot of opportunity for designing things. I fully agree with that idea. But what you mentioned here today seems to be, essentially, the usual paper wicking phenomena, not really microfluidics. What do you think?

Gil Garnier

You are certainly right. Basically, it is not really microfluidics, it is macrofluidics, but micro is more sexy, especially in the context of nanotechnology. It is difficult to publish in the specialist analytical chemistry and biomedical journals but if you put the word "microfluidic" in the paper, it works well. I would say it is still "meso" for the low cost film, and certainly "macro" for the paper. We have talked about the length scale but there is also a timing scale; because for the antibody and antigen interactions, sometime we have to wait for half an hour. So low flow, basically, if you activate it and wait a few minutes, this is very compatible.