Chapter 1 Introduction

1.1 Brief Overview

Traditional bioassay devices generally require large sample volumes and expensive setups for sample processing and measurement. A shift towards microfluidic systems has significantly reduced the need for high volumes of samples. Nonetheless, these systems still require an external power source for processing and measurement. The advent of open surface microfluidic platforms, such as those using porous paper surfaces, has completely eliminated the need for external power sources. In porous paper substrate, the spontaneous flow of fluids through porous media, either with or without external pumping, leading to the displacement of one immiscible fluid by another is known as imbibition. Wicking is the process where liquid moves due to the capillary action in porous materials, often against gravity whereas in imbibition liquid is absorbed by a solid or gel, causing it to swell. Imbibition is a type of diffusion where liquid moves into hydrophilic materials.

Wicking is a subset of imbibition, occurs when imbibition is driven by the inherent capillary pressure within the porous medium. In the context of paper substrates, wicking manifests through the micro pores present in the paper substrate, giving rise to the flow mechanism which is known as paper-based microfluidics. Paper-based microfluidic devices are capable of handling very small amount of liquid (1-100 μL) and compatible for sample and reagent storage. Paper-based microfluidics has gained significant popularity in research communities over the past few decades due to its diverse range of applications spanning from environmental monitoring, food testing [1], water testing [2], pesticide [3], heavy metal ions [4], to healthcare diagnostics [5].

Particularly in the development of point-of-care (POC) devices where minimally invasive techniques are applied for blood related pathological diagnostics, lab on a chip device based on paper-based microfluidic platforms are extensively employed. This is attributed to their ability to fulfil the criteria outlined by the World Health Organization (WHO), known as ASSURED: Affordable, Sensitive, Specific, User-friendly, Rapid and robust, Equipment-free, and Deliverable.

1.2 Motivation

Blood serves as a vital bodily fluid comprising approximately 45% cellular components and 55% plasma—a water-like liquid enriched with ions, minerals, and proteins. It acts as a primary indicator of human health, necessitating blood-based pathological diagnostics for a wide spectrum of conditions ranging from common febrile illnesses to cancer. These diagnostics can broadly be categorized into three types: 1) complete blood count (CBC) related tests, 2) quantification of protein concentrations in plasma, and 3) estimation of vitamins and minerals in plasma. For CBC, the separation of different cellular components is crucial, while the isolation of pure plasma is essential for the accurate assessment of proteins, vitamins, and minerals.

Traditionally, plasma separation from blood has been achieved using centrifugation, followed by the application of sophisticated analytical techniques to measure various analytes present in the plasma. However, these conventional methods face several challenges, including the need for large sample volumes, high operational costs, reliance on centrifugation equipment, and the requirement for skilled technicians. It has been reported that 70-80% of deaths in developing and underdeveloped nations are attributable to inadequate medical facilities and diagnostic infrastructure, particularly in rural and suburban areas [6]. Nearly 50% of the world's

population of 7.4 billion live in a state of poor access to primary and preventive healthcare. They either do not report illness or receive sub-optimum care, both of which result in the accumulation of disease burden. 100 million people have to choose between food and healthcare around the world every day. Even as there are complex social, cultural, economic, political, and geographical reasons and the bundle of reasons vary across geographies, one of the principal reasons for such deprivation includes high cost of diagnostic technologies.

The advent of paper-based microfluidic platforms has revolutionized the diagnosis of blood-related diseases by leveraging the inherent properties of paper substrates, such as self-pumping via capillary action, flexibility, ease of fabrication, and the ability to perform multiplexed assays for simultaneous detection of multiple diseases on a single platform. These platforms facilitate point-of-care (POC) diagnostics in resource-limited settings. The integration of such devices with smartphones or biosensors enables the simultaneous separation of blood plasma and disease detection on a single microfluidic device, providing rapid and accurate results. Moreover, these diagnostic methods require minimal training, thus also serving to create employment opportunities.

Porous filter paper, typically composed of cellulose fibres, is a key material in these platforms. The fibres are bonded to form a network of interconnected pores critical for its filtration capabilities. The physical properties of the filter paper, such as its fibrous texture and typically white or off-white colour, are important for its application in filtration. The porosity of the paper allows for the passage of liquids or gases while trapping larger particles or contaminants. Depending on the application, the chemical composition of the filter paper may be varied, with some variants treated or coated to enhance specific properties like chemical resistance or particle retention. For instance, Whatman grade 1 and LF1 filter papers have pore diameters of approximately 25 µm and 5 µm, respectively (**Figure *1.1***). The cellular components of a blood sample, which typically have a diameter of around 7-8 µm, are retained by the LF1 filter paper,

allowing only the plasma to pass through. The flexibility of the paper surface also permits further modifications for the on-strip measurement of pathological parameters, integrating technologies such as smartphone applications or biosensors.

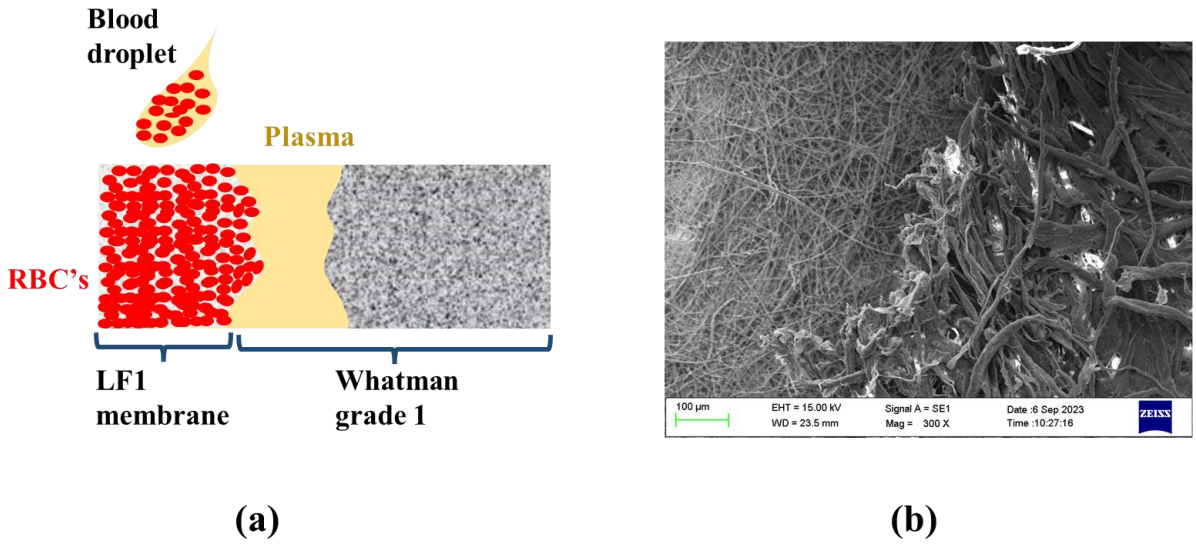

Figure 1.1 (a) Blood plasma separation through LF1 membrane filter paper (b) FESEM image of interface of varying pore diameter LF1 and Whatman grade 1 filter paper.

1.3 Liquid imbibition dynamics through porous filter paper: Overview

Liquid imbibition in porous substrate mediated by the suction capillary pressure on the walls of porous media due to the presence of dry and wet interface which leads to wicking action in paper matrix [7]. This capillary suction force is due to the mutual attraction between liquid molecules (cohesive force) and adhesion between the solid and liquid medium, domination of adhesion phenomenon over cohesion leads to wicking. Wicking is mainly responsible for liquid absorption in paper porous matrix. Traditionally two mathematical models are available to study the wicking phenomenon. The older one is governed by Lucas-Washburn (L-W) model

[8], which assume the porous medium as bundle of straight aligned cylindrical tubes of uniform radius and estimate the imbibition length where capillary force is counter balanced by viscous action. Apart from this there is another mathematical model based on Darcy's law which has been successfully used to derive wicking through porous matrix [7].

During the liquid flow through porous media inherent capillary force (self-pumping) allows the liquid to transport from a location to another. Initially, an experiment was conducted by Darcy (Darcy, 1856) to develop a model to measure the flow rate of liquid after passing through porous media. Darcy developed a model by considering a porous column of length L and liquid passed through it due to the pressure gradient developed which is due to the suction pressure at wet and dry section of the porous matrix. A schematic has been drawn to visualize the Darcy experimental set up as illustrated in

***Figure* 1.2**. The Darcy expression for flow rate measurement is represented in equation 1.3.1.

$$Q = -A\frac{Ka}{\mu}(\nabla P) \qquad 1.3.1$$

Here Q is the volume flow rate of liquid passing through porous surface (m^3/s), A is the cross-section area of the porous surface (m^2), Ka is the average permeability (m^2), L is the length through which liquid travel through porous structure due to the pressures gradient (∇P) at inlet and outlet section of the porous surface. Due to the high-level coupling of this model, the calculation of pressure gradient is quite complicated in wicking process. However, the time dependent solution of this model requires highly sophisticated computational facilities. In order to make the liquid transfer through porous surface easier we can assume the porous surface as bundle of parallel capillary tubes and fluid flow analysis can be carried out for a single capillary tube after balancing the inertia force, capillary force, and viscous force acting on the surface. In 1921 Lucas-Washburn (L-W) equation was modelled by two researchers to develop a

relation between capillary rise and time during the wicking process. They have considered the effect of inertia, capillary, and viscous force to develop the L-W model to estimate the liquid height along time. In L-W model following expression has been obtained after scaling liquid front height with time $h \sim t^{1/2}$.

The process of liquid wicking in porous media can be described through a comprehensive equation that accounts for various forces such as inertial force, capillary force, and viscous resistance, as discussed by Masoodi and Pillai (2012)[8]. This can be expressed by equation 1.3.2.

$$-\rho \frac{d(h\dot{h})}{dt} = -\frac{2\gamma cos\theta}{R_c} + \frac{8\mu h\dot{h}}{R_c^2} \qquad 1.3.2$$

Where ρ is the density of liquid, h is the capillary height, $\dot{h}$ is the rate of capillary rise, t is the travel time, γ is the surface tension of the liquid, R_c is the capillary radius of the tube, θ is the static contact angle, μ is the viscosity of the liquid. This equation is derived from a force balance analysis, where each term corresponds to a specific force: inertia force, surface tension force, viscous resistance force. In the Lucas-Washburn (L-W) model to simplify the analysis, only viscous force and surface tension force are considered significant in determining the capillary rise of liquid through porous media.

$$\frac{2\gamma cos\theta}{R_c} = \frac{8\mu h\dot{h}}{R_c^2} \qquad 1.3.3$$

or,

$$h = \sqrt{\frac{\gamma R_c\, Cos\theta}{R_c^2}}\ \sqrt[2]{t} \qquad 1.3.4$$

This simplification allows for a representation of the capillary rise in terms of porous media properties, where Darcy's velocity can be utilized to further describe the process i.e,

$$\frac{dh}{dt} = \frac{u}{\varphi_a} = -\frac{K_a}{\varphi_a}\frac{dp}{dh} \qquad 1.3.5$$

or,

$$h = \sqrt{\frac{2K_a P_c}{\varphi_a \mu} t} \qquad 1.3.6$$

Where, P_c is the capillary pressure, and φ_a is the average porosity.

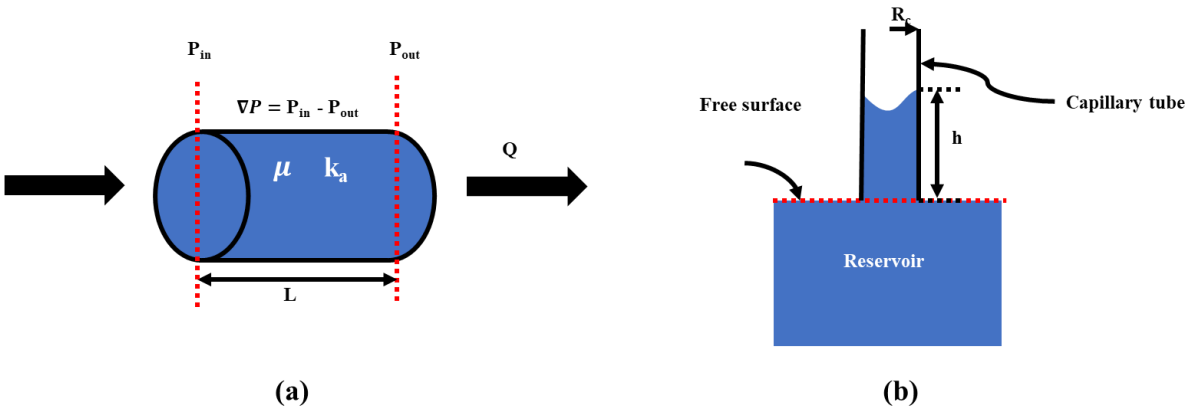

Figure 1.2 (a) Darcy model for liquid transport through porous structure (b) L-W equation for liquid transfer through porous structure.

These equations illustrate the complex interplay of forces within the porous matrix, enabling a deeper understanding of the wicking process essential for various applications.

1.4 Factors Influencing Paper Substrate Selection in microfluidic devices

In the fabrication of filter paper, cellulose fibers are consolidated while preserving interstitial voids to form a porous matrix. The dynamics of fluid flow within this matrix are governed by

a range of chemical and physical factors. These include the quality of the pulp material, the characteristics and distribution of voids, the chemical composition of the surface, pore radius, permeability, as well as the surface tension and viscosity of the liquid. A detailed examination of the influence of several of these factors is presented in the subsequent section.

Capillary Flow Time and Flow Rate: The selection of paper substrates in microfluidic devices is critically influenced by characteristics such as capillary flow time, which is defined as the time required for a liquid sample to laterally traverse the pore structure of the substrate. This parameter is inversely proportional to the capillary flow rate, typically expressed in units of seconds per centimetre (s cm^{-1}). Accurate estimation of capillary flow time is essential for precise positioning of test and control lines on the membrane, facilitating optimal device functionality.

Paper Thickness: The thickness of the paper substrate is another pivotal parameter in the design of paper-based microfluidic devices. The required sample volume is contingent upon the pore volume of the paper; thicker membranes necessitate larger sample volumes but offer enhanced mechanical strength, while thinner membranes may compromise structural integrity. Commercially available membranes typically range from 100 to 500 µm in thickness, including products such as Whatman and LF1.

Pore Size and Particle Retention: In porous paper substrates, an extensive network of fibers forms interconnected microcapillaries that facilitate smooth fluid flow. The pore size within this network is a critical parameter for predicting the particle retention capabilities of the substrate. The bubble point method is commonly employed to measure this pore size. In this method, the porous paper substrate is first fully saturated, followed by the passage of air through it. The emergence of the first bubble indicates the largest pore diameter. However, since the pore sizes are not uniform across the paper surface, a pore size distribution is utilized

for more accurate calculation. According to the bubble point method, the pore radius is directly proportional to the amount of pressure required to produce the first bubble.

$$d = \frac{4\gamma cos\theta}{P} \qquad 1.4.1$$

Here d is the average pore diameter, γ is the surface tension of liquid, p is the pressure applied to move the air, and θ is the contact angle between liquid and paper surface.

Porosity and Permeability: Porosity, a measure of the void spaces within the paper substrate, is typically quantified by assessing the volume of liquid the paper can absorb. It is a critical factor influencing the fluidic properties of paper-based devices. Permeability, which describes the substrate's resistance to fluid flow, is inherently linked to porosity but also depends on other structural features of the paper. Porosity can be expressed as:

$$\varphi = \frac{V_o}{V_T} \qquad 1.4.2$$

Here V_o is the volume of voids or pores, and V_T is the total volume of the substrate. Permeability indicates the level of resistance offered by a porous structure for liquid passes through narrow porous passage. In case of porous paper substrate fibres are arranged in random manner, an empirical formula that incorporates the average fibre radius(r_f) can be used to estimate air permeability, as detailed by Van der Westhuizen and Du Plessis (1996)[9]:

$$k = r_f^2 \frac{\pi\varphi\left(1 - \sqrt{1-\varphi}\right)^2}{24\,(1-\varphi)^{1.5}} \qquad 1.4.3$$

k is the permeability of a fluid for porous substrate, φ is the porosity of the porous substrate. Nabovati et al. (2009) [10] have established a correlation that links permeability with porosity for randomly arranged fibre network in porous substrate. This relationship includes the critical porosity (φ_c) and factors C_1 and C_2, which describe the network's geometric characteristics.

$$k = C_1 r_f^2 \left(\sqrt{\frac{1-\varphi_c}{1-\varphi}} - 1 \right)^{C_2} \qquad 1.4.4$$

Furthermore, for isotropic granular substances like nitrocellulose membranes, the Kozeny-Carman equation offers a method to predict permeability.

$$k = \frac{d^2 \varphi^3}{180(1-\varphi)^2} \qquad 1.4.5$$

This approach, which has been reinforced by research from Choi et al. (2016) [11], uses the material structure and properties to effectively predict the resistance to fluid flow.

These parameters are vital for understanding and optimizing the fluid dynamics within paper-based microfluidic systems, aiding in the precise control and manipulation of fluid flow for various diagnostic applications. Incorporating these characteristics into the design process ensures that the microfluidic devices are both effective and efficient in sample handling and analysis.

1.5 Paper based microfluidics: an overview

Recently, the micro- and nano- scales modified platform have obtained significant popularity due to automation, versatility, and ease of integration in different domains such as micro-electro-mechanical systems (MEMS), targeted drug delivery, as well as microfluidic systems related to healthcare diagnostics. Among these platforms, the microfluidic paper-based analytical devices (μPAD) have emerged as an attractive option for applications in medical diagnostics, environmental monitoring, water testing, food safety analysis, and many more. The μPAD have several distinct advantages including cost-effectiveness, environmental friendliness, low operational volumes, high surface-to-volume ratios, adaptability, moldability,

and biocompatibility, making them highly adaptable and suitable for a diverse range of scientific and industrial applications. It is also worthy to mention that fluid flow takes place through these porous paper substrates without the requirement of any external pump.

Microfluidic devices have revolutionized the analysis of biological samples by enabling precise control and manipulation of small volumes of fluids. However, measuring pathological parameters using general microfluidic devices presents several challenges:

1. Complex Fabrication Processes: Microfluidic devices typically require advanced fabrication techniques such as photolithography, soft lithography, and micromachining. These processes can be expensive, time-consuming, and require specialized equipment and expertise, limiting accessibility and scalability.

2. Sample Loss and Contamination: Handling very small fluid volumes increases the risk of sample loss and contamination. Ensuring sample integrity throughout the microfluidic channels and reaction chambers is crucial for obtaining accurate and reliable results.

3. Limited Reagent and Sample Compatibility: The materials used in fabricating microfluidic devices, such as polydimethylsiloxane (PDMS), glass, and silicon, may have limited chemical compatibility with certain reagents and biological samples. This limitation can affect the performance and reproducibility of assays.

4. Flow Control and Channel Clogging: Achieving precise control over fluid flow within microfluidic channels can be challenging. Variations in pressure, channel dimensions, and surface properties can lead to inconsistent flow rates. Additionally, microfluidic channels are prone to clogging by particulate matter or biofouling, which can disrupt device functionality.

5. Integration of Detection Methods: Incorporating detection methods such as optical, electrochemical, or fluorescence-based sensors into microfluidic devices often requires complex alignment and integration strategies. Ensuring the compatibility and efficiency of

these detection systems within the microfluidic platform can be challenging and add to the overall complexity and cost.

Advantages of Paper-Based Microdevices in overcoming these Challenges

Paper-based microfluidic devices, also known as microfluidic paper-based analytical devices (μPADs), offer several advantages that address the challenges associated with general microfluidic devices:

1. Simple and Cost-Effective Fabrication: Paper-based microdevices can be manufactured using straightforward and inexpensive techniques such as wax printing, inkjet printing, and screen printing. These methods do not require the sophisticated and costly infrastructure needed for traditional microfabrication, making paper-based devices more accessible and cost-effective.

2. Reduced Sample Loss and Contamination: The inherent porosity of paper enables capillary-driven fluid flow, reducing the risk of sample loss and contamination. The absorbent nature of paper helps localize samples in predefined regions, thereby maintaining sample integrity and improving the accuracy of measurements.

3. Enhanced Reagent and Sample Compatibility: Paper-based devices are highly compatible with a wide range of reagents and biological samples. The versatility of paper substrates allows for the integration of various chemical and biological assays, making them suitable for diverse applications in pathological parameter measurement.

4. Reliable Flow Control and Resistance to Clogging: The open and porous structure of paper-based devices ensure consistent fluid flow driven by capillary action, eliminating the need for external pumps or pressure control systems. This design also reduces the likelihood of channel clogging by particulate matter or biofouling, ensuring reliable and continuous operation.

5. Easy Integration of Detection Methods: Paper-based microdevices can easily incorporate various detection methods. Colorimetric assays can be directly visualized on paper substrates without the need for complex instrumentation. Additionally, conductive inks can be printed onto paper to create electrochemical sensors, facilitating the development of low-cost, portable, and user-friendly diagnostic tools.

General microfluidic devices face challenges related to complex fabrication processes, sample loss and contamination, limited reagent and sample compatibility, flow control issues, and the integration of detection methods. Paper-based microfluidic devices overcome these challenges through simple and cost-effective fabrication, reduced sample loss and contamination, enhanced reagent and sample compatibility, reliable flow control, resistance to clogging, and easy integration of detection methods. Consequently, paper-based microdevices represent a promising alternative for the measurement of pathological parameters, particularly in resource-limited settings where simplicity, affordability, and reliability are crucial.

In 2007, Whitesides et al.[12] pioneered the use of paper as a microfluidic device, introducing new fabrication processes and bioassay identification techniques specific to paper based microfluidic devices (Bruzewicz et al., 2008[13]; Martinez et al., 2008[14], Cheng et al., 2010[15]). Liquid manipulation plays a crucial role in creating paper-based devices, with control facilitated by patterning (hydrophilic-hydrophobic) on the porous substrate surfaces (Sen et al., 2018[16]). Chatterjee et al. (2018)[17] conducted experimental studies showcasing lateral and transverse liquid transport on paper-based substrates using wettability patterning. Leveraging liquid manipulation through patterned surfaces and colorimetric reactions, paper-based microfluidic devices have been deployed for detecting analytes present in blood such as haematocrit, haemoglobin, blood-glucose, and so on. This section delves into the diverse fabrication techniques, structural configurations, chemical and physical properties, and detection methodologies of paper-based microfluidic devices.

Paper-based device fabrication method:

Whitesides et al.[12] initially demonstrated the utility of paper-based microfluidic devices for identifying glucose and protein in urine samples (Martinez et al., 2007[18]). They employed SU-8 2010 coating on chromatography paper, followed by UV exposure to create hydrophilic channels. However, this photo-lithographic technique was deemed complex and costly. Subsequently, numerous researchers explored alternative methods for fabricating hydrophobic barriers in paper-based microfluidic devices, including PDMS coating for enhanced flexibility (Bruzewicz et al., 2008[13]), photo-lithography (Martinez et al., 2008[19], [20]; Dungchai et al., 2009[21]), cutting and stacking techniques (Thuo et al., 2014[22]), and inkjet printing to enable mass production (Abe et al., 2008[23]; Li et al., 2010[24]; Delaney et al., 2011[25]).

PDMS coating having limitation with hydrophobicity, bonding difficulties, deformation, and solvent-induced swelling. Photolithography encounters issues with achieving high-resolution patterns, managing complex multilayer structures, material compatibility, and environmental sensitivity. Cutting and stacking techniques face precision in cutting, accurate layer alignment, handling fragile materials, and ensuring strong adhesion between layers. Inkjet printing is limited by droplet size resolution, ink formulation, substrate compatibility, nozzle clogging, and effective curing and drying processes. Overcoming these challenges is essential for producing reliable and high-performance microfluidic devices.

Plasma treatment was also utilized to create filters and switches within paper devices (Li et al., 2008[26]). In 2009, Whitesides introduced a rapid wax printing method (Carrilho et al. 2009[27],; Lu et al., 2009[28], [29]), although this approach faced challenges such as low resolution and printer availability. Laser treatment techniques were later explored for modifying hydrophobic paper surfaces using CO_2 lasers (Chitnis et al., 2011[30]). More recent innovations include wax printing and reflow using a diode laser (Zhang et al., 2019[31]) and

chemical deposition techniques such as dip coating, spray coating, and CVD methods (Wang et al., 2016[32]; Baidya et al., 2017[33]; Lam et al., 2017[34]; Kwong and Gupta, 2012[35]; Chatterjee et al., 2018[17]; Pascual et al., 2019[36]). Additionally, novel methods like AKD deposition without a wax printer (Hamidon et al., 2018[37]), using eyeliner and correction pens (Mako and Levine, 2019[38]; Mani et al., 2019[39]), and laser printer toner ink as a hydrophobic agent (Ghosh et al., 2019[40]) have been explored. Techniques such as paper cutting and laser cutting have also been utilized for precise fabrication (Fenton et al., 2009[41]; Evans et al., 2014[42]), albeit compromisation for cost and skill of operator. Despite advancements over the past decade, ongoing research continues to introduce new techniques and applications in the field of paper-based microfluidic devices.

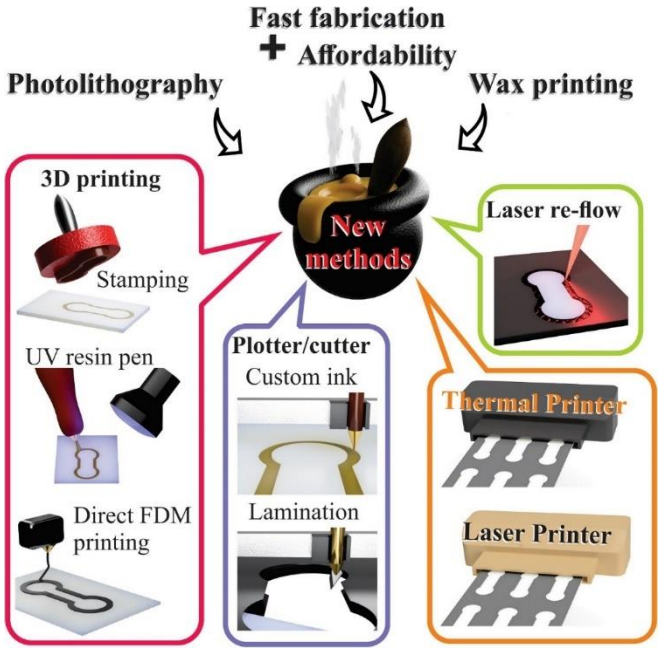

Figure 1.3 Recent advancements in the fabrication techniques for microfluidic paper-based devices build upon the foundational principles set by initial methodologies, incorporating modern technologies and innovative approaches. These advancements are directed towards enhancing the efficiency of the manufacturing process, substantially reducing both the production time and costs associated with these devices[43].

Physical and chemical characteristics of paper substrates:

Paper comprises natural wood fibres from which cellulose is extracted using the pulping method, involving lignin dissolution. In paper manufacturing, various chemicals are employed to enhance characteristics like brightness, strength, color, and water resistance. Papers are categorized into grades based on manufacturing processes. Intermolecular hydrogen bonds link cellulose fibres, and chemical modifications are conducted on paper surfaces to enhance H-bond strength, making it additive or repellent to specific substrates. Paper surface physical properties such as topography, stiffness, roughness, porosity, permeability, wettability, and capillarity, are tailored for diverse applications. Paper grades differ in pore size, thickness,

mechanical strength, and flow rate, owing to their heterogeneous porous structure. Anisotropic pore distribution influences liquid flow differently vertically and laterally; larger pores enhance flow rate and optical transparency. Porous papers with high porosity exhibit greater flow rates and lower opacity. Colorimetric reactions on paper substrates depend on both thickness as well as color of the paper. Strength and stiffness are crucial for handling, stacking, de-stacking, folding, and unfolding paper. These properties vary with humidity and moisture content of papers. Physicochemical attributes like topography, roughness, and surface energy are vital for different applications and are modified to maintain strength and modulus in wet conditions. Understanding these chemical and physical properties of paper substrates are essential for selecting suitable papers for specific applications.

Different detection techniques:

Various laboratory-based detection techniques are employed in paper-based microfluidics to detect different substances efficiently. These techniques aim for fast, simple, and cost-effective detection, making them ideal for paper-based devices. Colorimetric detection is commonly used for qualitative analysis, offering easy interpretation for users, especially in scenarios like pregnancy tests (Cardoso et al., 2017[44]). Quantification in colorimetric detection is achieved through calibration curves using initial color intensity data, captured and processed with image analysis software (Duangdeewong et al., 2020[45]). For enhanced sensitivity as well as specificity and accurate quantitative analysis, electrochemical techniques are favoured. Electrodes can be fabricated on paper through methods like screen printing of carbon ink or graphite drawing, typically forming a circular region for electrode junctions (Ortone et al., 2021[46]). Electrochemical reactions at these junctions are quantified via current generation or voltage changes measured using an amperometry and voltammetry device. Optical readout techniques such as electrochemiluminescence, chemiluminescence, and fluorescence offer visible light emission for easy detection, especially useful in low reagent volume tests and

sensitive analyses (Wang et al., 2019[47]). While these techniques are widely used, researchers are exploring integrated methods for easier quantification. Paper-based microfluidic devices can easily be integrated with these above-mentioned sensors making these integrated devices much deserving candidates for extensive medical diagnostics with low cost yet with uncompromised accuracy ***(Figure 1.4)***. Paper-based microfluidic devices have emerged as powerful tools for various applications in healthcare [48], environmental monitoring [49], and analytical chemistry [50]. These devices offer portability, cost-effectiveness, and ease of fabrication compared to traditional microfluidic systems. First step towards paper device fabrication process is to create a flow path along hydrophilic paper surface.

The thesis focuses on the study of fluid flow on porous paper substrate, imbibition dynamics followed by practical application of paper-based platforms for blood related pathological diagnostics. State of the art, research gap, and chapter organisation are described in detail in the next section.

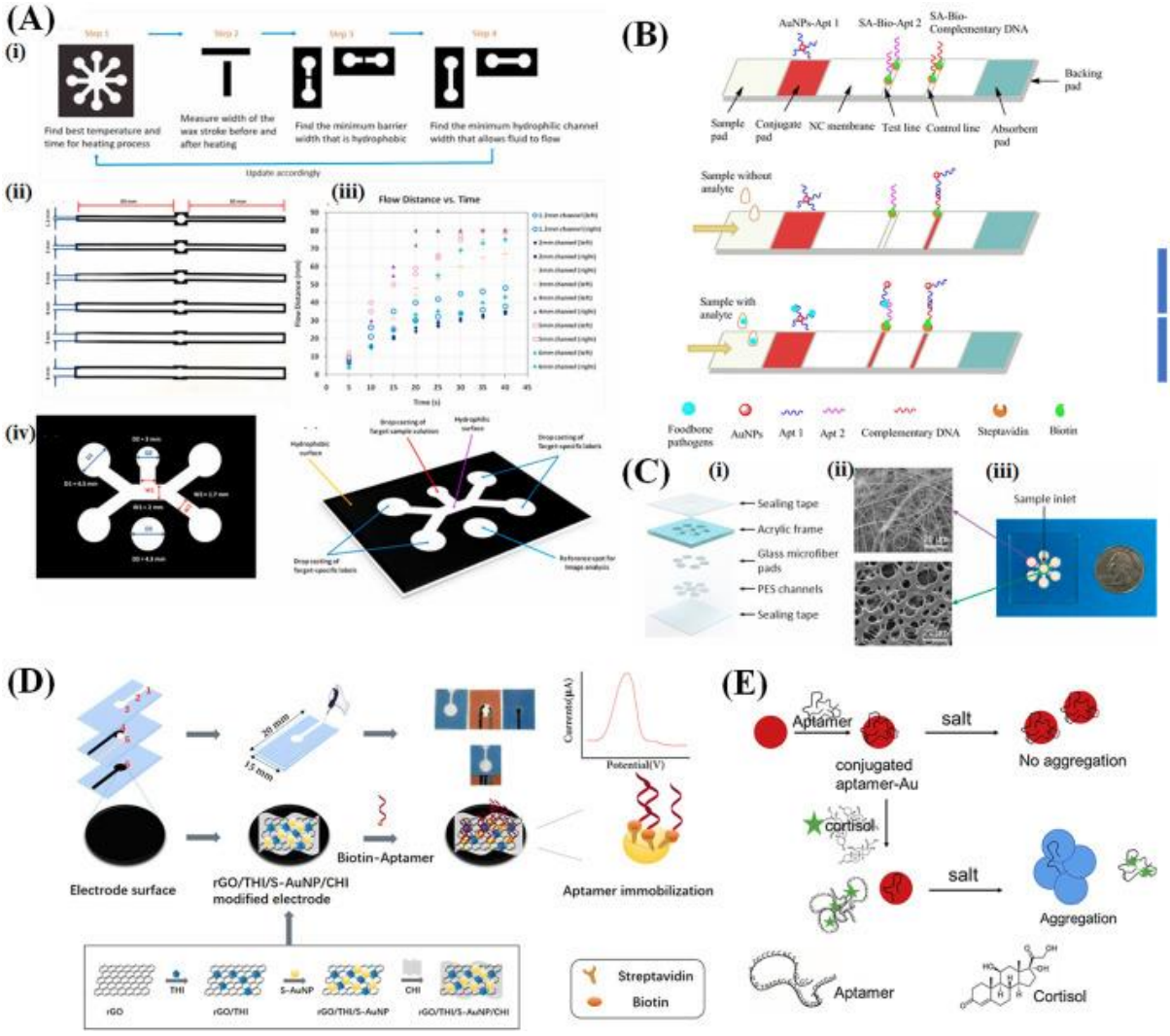

Figure 1.4 (A) Experimental phases of the wax device printing process included: (i) the session dedicated to the creation of the device, (ii) optimization of flow rates by varying channel widths, (iii) analysis of flow rate versus time across different channel dimensions, and (iv) the final device configuration. (B) Schematic representation of the detection device based on aptamer-functionalized lateral flow assays (LFAs). (C) Design and development of the paper-based sensor encompassing: (i) steps for constructing the sensor featuring one inlet and six reaction chambers, (ii) scanning electron microscopy (SEM) images displaying the structural details of glass microfiber (top panel) and PES membrane (bottom panel), and (iii) the completed device version. (D) Assembly and modification of electronic paper-based analytical devices (ePADs) integrated within microfluidic channels for biotin detection. (E) Development

process of an aptamer-based sensor for cortisol detection. These descriptions and methodologies are used with permission from references [51], [52], [53], [54], [55].

1.6 Research Gap

The literature review reveals that while numerous microfluidic devices exist for measuring various analytes in blood samples, such as plasma viscosity, plasma creatinine, and plasma ascorbic acid, practical application in remote locations remains unfeasible. The shortcomings of the current measurement methodologies are outlined as follows:

1) Studies on liquid imbibition dynamics in porous paper surfaces primarily focus on factors like ambient conditions, sample volume, channel geometry, and other experimental conditions that influence fluid flow rate fluctuations. However, among different fabrication processes of porous paper substrate there is a significant variation of flow mechanism, which has rarely been studied in the available literature. The effect of fabrication process on the paper substrate has been studied experimentally for both edge cutting and whole surface involvement during cutting process has been studied. This information will help to manipulate the flow rate of newly paper based device for fabricated device and

2) Measurement of plasma viscosity often requires large sample volumes and complex instrumentation. Additionally, devices may necessitate supplementary information, including liquid density, surface tension, and reference fluids, to accurately measure viscosity. Despite these complexities, existing devices have not been adequately validated with real clinical samples, raising concerns about their practical applicability.

3) For plasma creatinine measurement, the separation of plasma from blood samples typically requires a centrifugal device, and separate mobile equipment such as smartphones, scanners,

or spectrophotometers is needed for data acquisition. In order to develop a genuine point-of-care (POC) device, an integrated system that encompasses all diagnostic steps from separation to results-display via single step is essential.

4) The measurement of ascorbic acid (AA), although crucial for monitoring human health, is often hindered by expensive settings and complicated sample preparation methods. These barriers limit the widespread adoption of AA measurement techniques in clinical sectors.

Overall, there is a critical need for the development of integrated, simple, and cost-effective microfluidic devices that can overcome these challenges and facilitate reliable analyte measurement in remote and resource-limited settings with uncompromised accuracy involving minimally trained personnel.

1.7 Outline of thesis

Following thesis is divided into six different chapters. Chapter one provides the detail insight about paper based porous structure and its application in blood related pathological parameter measurement.

In chapter two an experimental analysis has been carried out to understand the variation in liquid flow rate over the paper microchannels which were fabricated using different protocols. The details of experimental methods and obtained results were discussed further in the same chapter. Further, in this chapter water is used as to perform the flow analysis leading to understanding of flow imbibition through porous paper substrate due to variation of fabrication processes.

In chapter three, four, and five we are going to study blood-plasma imbibition through paper-based microfluidic platforms in which plasma, on the one hand is similar to water specially

while considering imbibition dynamics through porous media and on the other hand the same has rheological complexity due to the presence of many analytes such as proteins, vitamins, etc.

Accordingly, chapter three is dedicated to develop a miniaturized paper strip for determining plasma viscosity.

Subsequently, chapter four focuses creatine estimation from whole blood sample in a porous paper substrate integrated with RGB sensors and mediated by spontaneous blood-plasma separation confirming sample-to-answer integration via single step.

Chapter five highlights ascorbic acid (vitamin C) estimation directly from whole human blood sample in which micro-paper based analytical device has been integrated with screen printed electrode where nanoparticle has been used as catalyst leading to the development of a portable vitamin C estimation test kit engaging minimal infrastructure and resources.

Chapter six finally provided the concluding remark and future scope of work where we can further envision the 3D printed device fabrication method and use of AI/ML in healthcare leading to the development of all-in-one diagnostic test kit deployable to resource constraint settings as extreme point-of-care devices.