# Chapter 5
# Paper Microfluidic Based Device for Blood/Plasma Separation


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**Abstract** With the advent of research advances in the field of microfluidic devices, medical science is also progressing its way through fast and efficient micro-fabricated medical diagnostics. As most of the medical diagnosis involves blood test as the preliminary or necessary step, a smart micro-diagnostic for blood analysis can be considered as an essential. Blood being the most vital fluid, governing all the hemostatic and physiological conditions by acting as a carrier of all the essential minerals and vitamins throughout the body, makes it a potential analyte for diagnosis. But blood being a complex mixture of hematocrit, white blood cells and plasma along with other essential proteins, the analysis of the same is difficult. Especially, the presence of red blood cells (RBCs) and its agglutination causes interference in the procedure of analysis. For example the presence red blood cells can be a potential interfering factor during colorimetric detection of an analyte in the blood. Similarly, the presence of agglutination of RBCs and its count can interfere during a coagulation diagnosis. Thus, separation of plasma/serum from the erythrocytes is an essential step during a blood analysis. Conventionally, centrifugation and sedimentation were used to separate plasma/serum from the whole blood before its analysis. With the progressing microfluidic technology the micro-devices have been using numerous techniques like bifurcation (Zweifach-Fung effect), geometric obstructions, membrane filtration, and acoustic techniques for plasma separation. Thus, there exists several polymer based lab on chip biomedical micro devices performing the blood separation. Although these devices are portable, fast and efficient with small turnaround time, its fabrication procedure is mostly complex simultaneously involving integration of micro valves. It also requires a syringe pump or a voltage power supply for controlling the blood flow through its micro channels. Considering the complexity of the fabrication the paper based micro devices are steeply gaining its way through as a potential medium for micro diagnostics. Micro pads also doesn't has requirement for any external driving

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source for guiding the fluid flow through. The porous nature of the same performs the capillary action, allowing passive fluid transport. Thus, the respective chapter emphasizes on the paper based micro-devices tailored by the researchers for blood plasma separation with a brief enunciation of their respective working mechanisms. It will also discuss the different fabrication methods intelligently employed by the porous media platform for efficiently performing the separation to further cater to the queries of future researchers in the respective area.

**Keywords** Plasma separation · Paper based diagnostics · Point of care (PoC) · Hematocrit · Microfluidic devices · Microfluidic paper-based analytical devices (mPADs)

## 5.1 Introduction

Blood being the most vital clinical analyte, the diagnosis of which can provide a detailed information about the present physiological condition of a candidate, its accurate characterization is the key to perform efficient pathological diagnosis. Plasma, erythrocytes, leucocytes, thrombocytes along with several proteins, vitamins and minerals are the main constituents of the blood. A simple clinical diagnosis of blood and its constituents often requires a preliminary step of plasma/serum separation from the whole blood. Either, if the diagnosis involves erythrocyte related information (e.g. hematocrit count) or a routine diagnosis concerned with that of the other blood constituents excluding the erythrocytes (e.g. glucose/uric acid tests), both require a necessary step of plasma separation from that of the whole blood in general. Several analytical methods like colorimetric, fluorescence and chemiluminescence based diagnostics often face challenges in detection methodology when using whole blood as an analyte. The transduced signal for the respective is optical in nature which is often impaired by the strong colorimetric interference by the RBCs. The presence of erythrocytes in the whole blood also interferes with that of the intricate biochemical reactions resulting the transduction of chemical signal to optical in-case of color/optical based sensing platforms (e.g. colorimetric, fluorescence and chemiluminescence). Similarly, electrochemical detection methodologies face similar challenges as the presence of erythrocytes affects the rheological dynamics of the blood. The presence of cellular components results the blood to act as a complex dielectric medium with challenging biochemical/electrochemical interactions in an electrochemical sensing platform. The analytical methods based on mechanical techniques are challenged by the biophysical characteristics of the RBCs in fluidic environment like rouleaux formation and agglutination caused by complex rheological dynamics of blood. Thus, the respective challenges mandate the efficient separation of cellular components from that of whole blood for efficient characterization of blood analytes. There exists several efficient conventional laboratory based methods like centrifugation and magnetic separation to separate plasma from whole blood (Basu and Kulkarni 2014; Svoboda 2000). These table top blood separating

instruments often produced accurate results due to its intricate instrumentation which made the instrument costlier and often required trained personnel to operate the same. In addition to its installation, operating and maintenance charges, it was bulkier and required a proper clinical or laboratory setup environment for its efficient performance. Apart from the above listed shortcomings, the vital drawback was its higher turnaround time which necessitated the research advances in micro technology for blood analyzers or separators. Thus, the plasma separation using paper as a substrate provides an ideal platform for designing an inexpensive and efficient PoC diagnostic. Despite the presence of paper platform for several immunoassays, whole blood characterization is not preferred due to the optical and dielectric interference caused by the erythrocytes during PoC diagnosis. As PoC is a demanding assay methodology for emergency surgical/peri-operative situation or remote/developing area (out-patient) scenarios, the obvious analyte will be whole blood rather than plasma/serum. Thus, an embedded blood plasma separation unit is a must for any kind of PoC diagnostics. Some of the applicative areas of PoC diagnostics include hematoccrit testing, coagulation analyzer (PT, APTT etc.), blood metabolites analyzer (uric acid, glucose, cholesterol etc.), cancer biomarkers tester, pathogen analyzers (malaria/dengue diagnostics) and blood type analyzers. As evident the plasma separation is a necessary step for PoC diagnostics, several plasma separation membrane (PSM)/paper has been tailored by the researchers to cater the process of plasma separation in PoC immunoassays. These PSM types of paper include (i.e. Whatman LF1, MF1, VF1 and VF2) that are designed specifically to separate plasma from sample of whole blood (Songjaroen et al. 2012; Gong et al. 2014; Noiphung et al. 2013). The downside of these specially tailored membranes is that they are comparatively expensive (100 times of What man no. 1 and no. 4 filter papers), so there is good reason for more research to be done to find cheaper methods for plasma separation. Thus, it is evident that, it is important to engineer a low-cost, yet effective platform, which will have an integrated setup for separating RBCs and plasma from the whole blood and will accordingly detect the analytes of interest. In effort to obtain separation using low cost traditional filter paper (cellulose paper/chromatography paper), several microfluidic assays have been developed using agglutination, aggregation and physical separation methods. The detailing of the following mechanisms and its application with their separation strategies are detailed later in the following sections. A detailed enunciation of the several paper based plasma separation assembly using physiochemical mechanisms (aggregation/agglutination) and capillary driven flow dynamics in porous media is made to provide a clear idea for the respective domain. Thus, following chapter, discusses the mechanism of plasma separation in physiological domain along with that of its evolution of respective biomimetic concepts used in paper based microfluidic platform. Secondly, it discusses the recent advances made by the researchers in plasma separation methodology used by paper based substrates and its composites with pre designed polymeric plasma separating membranes along with is process of fabrication. The applicative areas of the designed devices are also simultaneously discussed. Lastly, a concluding section with future prospects in the following area is included.

## 5.2 Physiological Hemodynamics and Porous Media Hemodynamics

Blood is a circulating fluid with a complex combination of cellular components, proteins, ions, minerals and extracellular fluid, primarily responsible for transporting nutrients and waste from one part of the body to another (Silverthorn and Johnson 2010; Marieb and Hoehn 2007). It is largely responsible for several physiological conditions such as regulating body temperature, proper osmotic pressure, and pH of body fluids ($\sim$7.4), carrying the cells for defense against the invasion of pathogens, regulating clotting mechanisms etc. The main constituents of blood include erythrocytes (RBCs), leucocytes (WBCs), thrombocytes, and plasma/serum (which comprises the fluidic part of the blood along with several proteins and minerals). While performing clinical diagnosis either whole blood is used or preferably plasma/serum part is extracted out before the assaying. Serum is the fluidic component of the blood excluding the cellular hematocrit and clotting factors and plasma is serum along with clotting factors. Blood is generally characterized either for the presence of essential metabolites/bio-analytes (uric acid, glucose, cholesterol etc.) or hematocrit content. Excluding the cellular components of blood plasma/serum behaves as Newtonian fluid. But the presence of hematocrit make the flow dynamics complex. The normal range (Billett 1990) of hematocrit found in adult men is 40–54% and 36–48% for adult women. Prior to several clinical attempts to characterize blood, several efforts were made to understand the nature and flow dynamics of blood in actual in vivo physiological conditions. In the process, several biophysical phenomenon like Fahraeus effect and Fahraeus-Lindqvist effect were discovered. According to Fahraeus effect, an obvious decrease of hematocrit content can be noted if a flow occurs from a large vessel to small diameter vessel (Fåhræus 1929). Simultaneously a decrease in apparent viscosity was bound to occur in the smaller vascular network which was a consequence of Fahraeus-Lindqvist effect (Fåhræus and Lindqvist 1931). Upon further research, in the field of hemodynamics researchers observed that the RBCs share a tendency to migrate away from the micro-capillary walls caused by hydrodynamic interactions mediated by typical deformabilities of the RBCs. These type of cellular interactions leads to formation of plasma layer near the vessel walls. In attempt to mimic the biophysical environment and micro-capillary/vessel networking in on chip microfluidic technology to obtain plasma separation several other microfluidic phenomenon like electro-osmotic flow, (Fournier 2012), bifurcation (Zweifach-Fung effect), (Toksvang and Berg 2013; Secomb and Pries 2013) geometric obstructions, (Haynes 1960; Pries et al. 1992) were discovered. With the help of several aforementioned phenomenon polymer based microfluidic technology was able to develop potential blood analysis and separators which could produce efficient results at low volumes, low turnaround times at very low cost. However, the major challenges for respective devices were the need for an external force such as syringe pump or high voltage power supply to control fluid flow in the micro-channels. Additionally, it required complicated fabrication methodologies for integration of

valves and pumps. Whereas, microfluidic paper-based analytical devices (mPADs) doesn't require any external driving sources for capillary flow. The porous nature of the same results in self assisted passive flow of fluid media through the substrate. Thus, the extra costing of integration of pumps and complex fabrication is eliminated and the fabrication methods implemented for designing mPADs like ink-jet printing, wax-printing etc. are comparatively simpler but efficient and is capable of producing the device at large scale is very low cost. Thus, it provides a huge scope for exploration of different designs or geometries by the researchers to explore an efficient plasma separation platform in paper based assays. Apart from exploration in geometrical variation in shapes of the micro-capillaries or the flow channels the researchers have also tried reagent assisted methods. Separation by agglutination and aggregation are two main reagent assisted methods used in paper based platform. Hemagglutination is a biochemical phenomenon triggered by antigen specific antibody bonding of RBCs. It causes an agglutination of RBCs in presence of its matching antibody (Mitra et al. 2014; Mujahid and Dickert 2016). Hemagglutination triggers clumping of RBCs, thus resulting in an elevation of viscosity which can impede the flow of blood through the porous platform. This phenomenon is used by the researchers to create a differential flow dynamics between RBCs and plasma to wick out plasma from the whole blood. Similarly, clustering of RBC is achieved by another phenomenon known as erythrocyte aggregation. Erythrocyte aggregation is a resultant of face-to face stacking of RBCs which gets dispersed in an environment with high shear and reforms at low shear environment. Erythrocyte aggregation is primarily influenced by the composition of plasma proteins, the surface properties of RBCs, and the magnitude of shear force being experienced (Baskurt and Meiselman 2003). Thus, the following section will present a detailed enunciation of the advances in paper based plasma separation devices employing several working mechanisms along with its applicative area.

## 5.3 Recent Advances in Paper Based Blood Plasma Separation Devices

With the advent of growing research advances in PoC diagnostics, paper can be regarded as the most preferred and economic choice as a diagnostic platform in following domain. Several characteristics of paper like biocompatibility, easy disposability, low cost and easy availability are responsible for its increasing popularity as a substrate for the PoC diagnostics. Paper being naturally hydrophilic in nature also provides an easy actuation of flow of bio-analytes through its micro-channels (Sher et al. 2017). Thus, unlike other polymer based electromechanical micro-devices the paper based devices doesn't require any additional external pumps or sources to drive the flow. Capillary driven fluid flow in paper is governed by the intermolecular force between the fluid and the porous cellulose matrix (IET 2017). The hyrophyllic characteristic of paper makes it a viable choice

for an appropriate substrate for several micro-fabrication methods. The porous nature of the paper aids in efficient adsorbance of ink and adherence of any other functionalizing materials like nanoparticles. Thus, paper is regarded as one of the best choices for substrate for flexible diagnostics fabricated using screen printing, ink-jet printing or wax printing. In addition, to conventional cellulose/filter paper, there exists some paper like materials which are used alone or in combination with conventional cellulose paper in designing the PoC medical diagnostics which includes nitrocellulose, polysulfone, polyvinylidene difluoride, nylon and fiber glass. Apart from conventional cellulose paper, polysulfone and fibre glass are the most popular substrate used for plasma separation application. As discussed earlier, plasma separation is a mandate in characterization of blood especially in PoC diagnostics to overcome the interference caused by the cellular components during signal transduction. Plasma being the informative fluid rich in proteins and organic molecules containing the essential metabolites and biomarkers essential in easy and rapid diagnosis of internal pathological condition of a patient, its efficient separation is necessary. The following section discusses several paper based point of care plasma separators in chronological order which efficiently overcome the interference of hemoglobin from RBCs as well as the release of nucleic acids (Mielczarek et al. 2016) and other cellular components from blood cells to provide a secondary level of efficient characterization of plasma fluid for PoC diagnosis. It also includes enunciation of several geometrical patterns of microfluidic channels which assist the capillary driven flow via diffusion or filtration with or without the assistance of other reagents. The survey mainly emphasis on the separation of plasma from whole blood using chromatography or cellulose/filter paper as a separation platform following the separation assisted via agglutination, aggregation or physical separation. Physiologically, separation of erythrocytes from that of its counter medium plasma occurs either due to flow rate differences caused due to Fahraeus-Lindqvist effect inside the vascular network or due to RBC clumping inside the vascular system due to agglutination, aggregation or clot formation. Yang et al. reported (Yang et al. 2012) a hemagglutination based plasma separation in paper substrate. Although separation of RBCs can also be performed using paper substrates with pore size smaller than 2.5 um, agglutination is preferred methodology for separation. Reduction in pore size reduces the flow rate $Q$ of separating plasma as scales with the forth power of the pore diameter ($Q \propto d^4$) (Beebe et al. 2002) and additionally the filtered RBC packs tends to block the flow plasma leading to complete cessation of plasma flow through the membrane as shown in Fig. 5.1c. Thus, hemagglutination is preferred over simple cross filtration or diffusion based separation mechanisms. In other words, the agglutination mechanism performs simple filtration where the effective size of RBC is increased using agglutination (Ludewig and Chanutin 1949; Zuk et al. 1985) which is further easily filtered using filter paper of much larger pore size consequently resulting in a significant increased rate of volumetric flow of filtered plasma as described in Fig. 5.1.

The paper based assay for plasma separation proposed by Yang et al. (2012) was fabricated by printing wax patterns in chromatography paper with hydrophobic

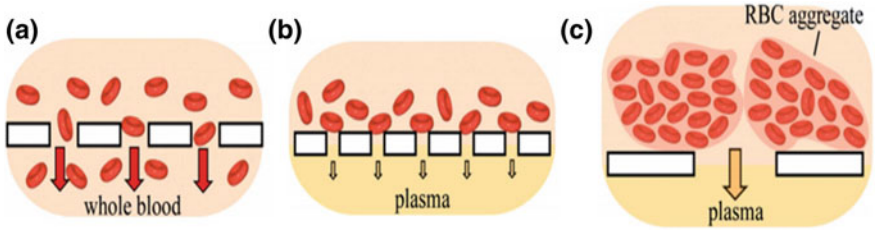


**Fig. 5.1** Schematic illustration of blood flow through varying porous media and usage of agglutination to tailor the fluidic flow of plasma excluding the cellular clusters. Blood flow dynamics through filter paper of pore diameter of **a** ~2.5 um or larger **b** smaller than 2.5 um **c** blood flow dynamics accompanied by agglutination through filters with large-diameter pores. Reprinted with permission from Yang et al. (2012), Copyright, The Royal Science of Chemistry 2012

barriers surrounding the separation zone at the center and four separate detection zones at the periphery of the respective as shown in Fig. 5.2. RBC agglutinating antibodies of about 7 ul were spotted in the center of the device (separation zone) and the colorimetric assay reagents were functionalized and dried at the peripheral detections zones. While performing the test, the sample of whole blood (~7 ul) is pipetted at the center of the separation zone. Once the whole blood sample is in contact with that of the RBC agglutinating antibodies, the RBCs starts agglutinating and the plasma is wicked out towards the peripheral region (i.e. the detection zones) where it reacted with the reagents of the colorimetric assay as shown in Fig. 5.2. Prior to Yang et al., M. S. Khan et al. studied the transport mechanisms of agglutinated blood in paper platform for instantaneous blood typing (Khan et al. 2010). The authors observed the variation in transportation of blood droplets on paper based depending on the criteria that whether the blood agglutinates or not. Simultaneous agglutination and separation of plasma from that of the RBCs ensured the blood type matching. A schematic as shown in Fig. 5.2b enunciates the working of plasma separation using agglutination used for blood typing. Blood plasma separation using agglutination was also explored by some other authors like Jarujamrus et al. for blood typing and study of blood dynamics in paper platform (Jarujamrus et al. 2012).

With increased popularity of paper based sensing platform, multifunctional microfluidic paper based assays are constantly different PoC diagnostic are devised using origami techniques. In 2012, Ge et al. (2012) devised low cost 3D Origami-based multifunction-integrated immunodevice for simultaneous detection of four tumor markers with integrated plasma separation fold as shown in Fig. 5.3. The plasma separator (chromatography paper) fold was intelligently introduced into the chemiluminescence immunoassay using multiplexed sandwiched type assembly. The following device also implemented separation of plasma from whole blood using agglutination. The consequent agglutination and vertical filtration due to

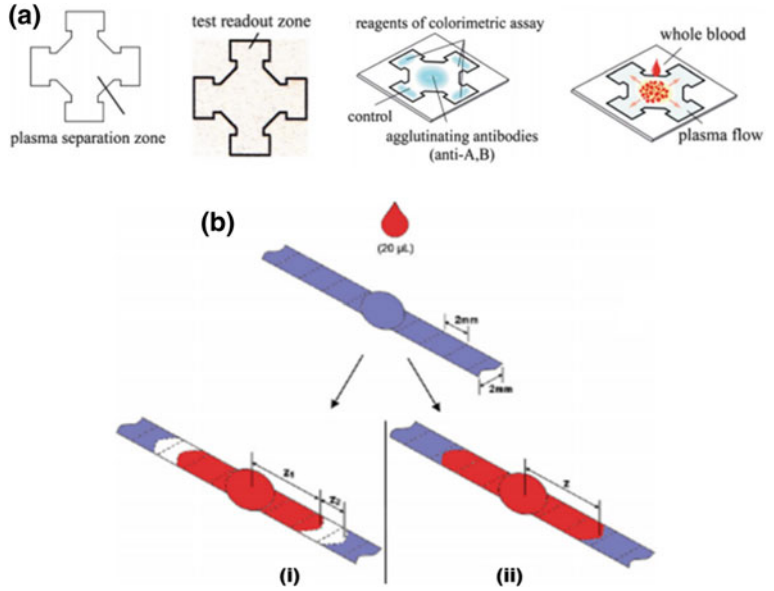


**Fig. 5.2** **a** Design and enunciation of agglutinating and readout zones in a microfluidic paper-based analytical device Reprinted with permission from Yang et al. (2012). **b**. Schematic representation of the wicking of blood (i. AB+, ii. B+) on paper treated with a specific and a nonspecific antibody (anti-A). Reprinted with permission from Khan et al. (2010), Analytical Chemistry 2010

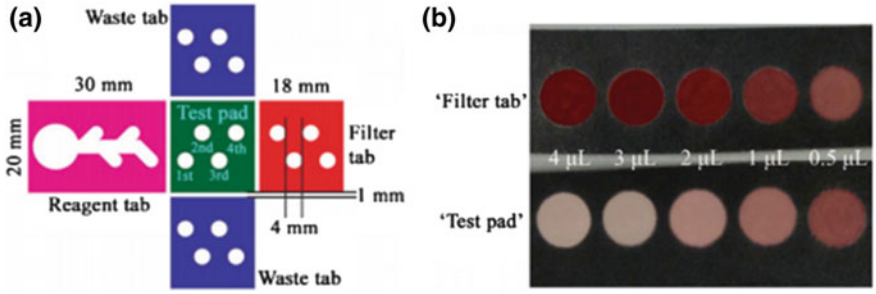


**Fig. 5.3** **a** The schematic representation 3D origami-based device. **b** Images of the separation of red blood cells from the pretreated whole blood samples in the paper micro-zones. Reprinted with permission from Ge et al. (2012)

intelligently spatially attacked paper folds the efficient separation of plasma was achieved. The following assay used blood samples treated with Anti D were used to obtain separated plasma at the reaction fold of the device.

The paper based plasma separation assays discussed so far works on the basic principle of agglutination based separation which relies upon the existence of a

strong specific antibody–antigen interaction for its efficient working. In case of certain blood groups such as O-blood group, the assay fails to provide a strong specific antigen-antibody reaction using A, B and Rh antibodies. Additionally, this agglutination based method also requires preparatory blood typing to know whether antibody–antigen interactions are able to induce blood agglutination. Furthermore, antibodies used requires extra maintenance to retain its bioactivity resulting an increase in material cost. Thus, to overcome the limitations faced by the method of agglutination for blood plasma separation researchers preferred an alternative of implementing the method of aggregation of RBCs for plasma separation. Most recently, Li et al. used calcium chloride solution to aggregate RBCs in rabbit blood samples (Li et al. 2014). The following invention describes how calcium ions are able to destabilize the suspension of RBCs in blood by suppressing the electric double layer on the RBCs surfaces, reducing the charge repulsion between RBCs (Ataullakhanov et al. 1994). Following the concept of RBC aggregation using salt solutions introduced by Li et al., Nilghaz et al. also developed a plasma separation assay based on aggregation via salt solutions. Strong salt concentrations causes a hypertonic medium for the erythrocytes by increasing the osmotic pressure around the cell membranes and further resulting in a compression of cell's electric double layer (EDL). Loss of EDL of the respective cells results in close packing of RBCs. The close packing of RBCs with a fibrin network leads to formation of immobile RBC aggregates. Further, the immobility of RBC packs results in differential velocity of the cellular components with that of plasma. Thus, maintaining a mobile phase for separated plasma which further wicks and reaches the detection zone as shown in Fig. 5.4. The clinical validation of the respective method was verified by performing successful colorimetric detection of glucose from the separated plasma. The schematic blow describes the working of the saline functionalized uPAD developed by Nilghaz and Shen (2015).

Unlike, salt based differential osmosis based RBC aggregating methodologies, Kar et al. tried manipulating the hemodynamics using PBS solution and bifurcating geometry to separate plasma in paper porous media (Kar et al. 2015). They fabricated an H-shaped channel device where whole blood and PBS solution were simultaneously dispensed. During the simultaneous flow of the PBS and whole blood through the linear region of the device, the lighter molecules suspended in the bloodstream diffused into the PBS stream. This resulted in RBC-rich and plasma-rich components being separately collected in each of the two outputs of the device. A clear perspective of the working can be obtained from Fig. 5.5 enunciated.

In addition to cellulose paper (filter paper or chromatography paper) some researchers also tried amalgamating filter or chromatography paper with plasma separation membrane (PSM) to achieve efficient separation of plasma without the use of any RBC agglutinating or aggregating reagents in single step. A single step plasma separating device was engineered by Songjaroen et al. (2012) using an overlapped combination of PSM and chromatography as shown in Fig. 5.6a. PSMs made of polyvinyl alcohol-bound glass fiber filters like MF1 and LF1 were assembled with Whatman Grade 1 filter paper using wax dipping method.

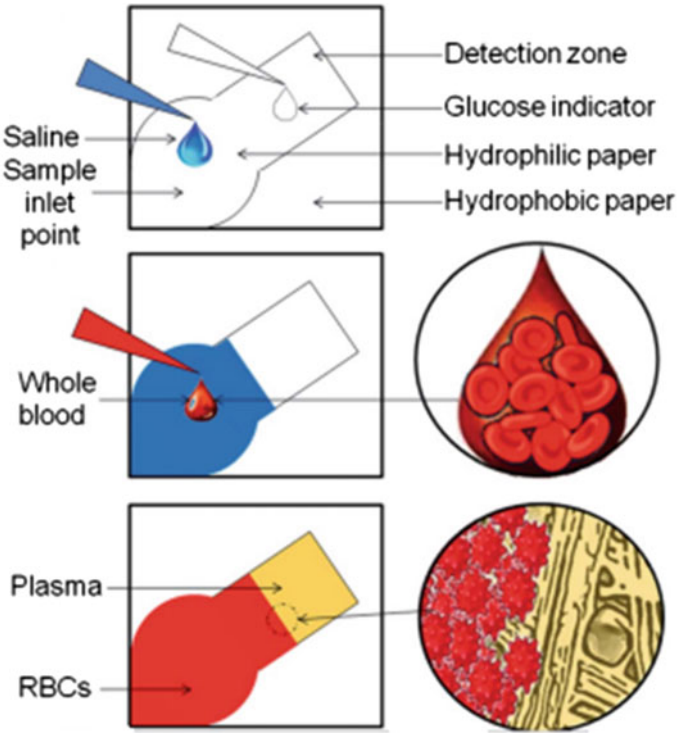


**Fig. 5.4** Schematic illustration of the plasma separation method on saline functionalized mPAD. Reprinted with permission from Nilghaz and Shen (2015)

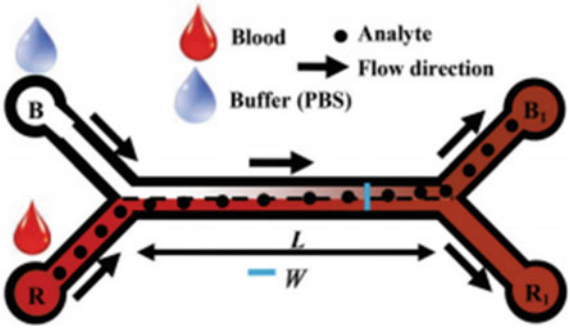


**Fig. 5.5** Schematic representation of the H–type plasma separator with two reservoirs R and B at rear end for whole blood and PBS respectively resulting in separation of plasma and cellular components. Separated plasma is dispensed at reservoir B1 whereas the cellular components gets dispensed at reservoir R1. Reprinted with permission from Kar et al. (2015)

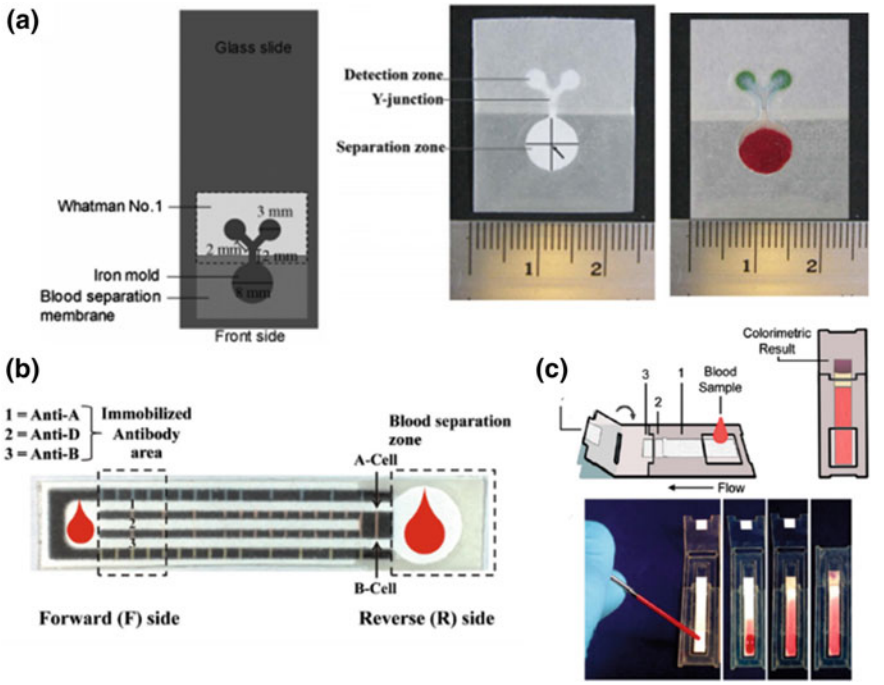


**Fig. 5.6** **a** The assembly of the mPAD for plasma separation from whole blood using wax dipping method (top view) and the working mPAD for whole blood separation composed of separation zone and detection zone. Reprinted with permission from Songjaroen et al. (2012). **b** The PAD approach for simultaneous determination of Rh typing and the forward and reverse ABO blood groupings. Reprinted with permission from Noiphung et al. (2015). **c** Prototype of disposable paper-based device (Robinson et al. 2016 Copyright MDPI)

The assembly consisted the placing of filter paper over the PSM with a small overlapping area. The blood separation in the following device is achieved by combining two separate types of paper where the lateral fluidic flow is governed by Lucas-Washburn model. The following device was proven to be functional at higher hematocrit levels as well ($\sim$55% HCT). It was capable of separating plasma within 2 min of application of 15–22 ul of whole blood. In addition to efficient separation of plasma the device was capable of colorimetric detection of plasma protein concentration with high reproducibility [RSD = 2.62% (n =10)]. Similarly, Noiphung et al. (2015) used the combination of chromatography paper and MF1 blood separation paper for blood plasma separation in paper based assay for simultaneous determination of Rh typing and forward and reverse ABO blood group as shown in Fig. 5.6b. Wax printing and wax dipping were performed to fabricate the following device. A combination of PSM and traditional cellulose/ filter was also utilized by Robinson et al. (2016) for separating plasma for semi quantitative Phe (phenylalanine) detection in whole blood for PKU therapy

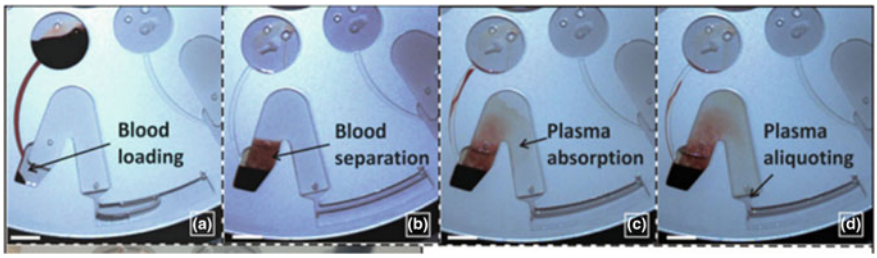


**Fig. 5.7** Schematic illustrating the several steps in leading to blood separation process using paper siphon mechanism. Reprinted with permission from Godino et al. (2014)

monitoring as shown in Fig. 5.6c. The separation was executable due to the layering of PSM over the porous substrate with an addition of Mylar folding housing with a small overlap of 1.5 mm.

In contrast to previously discussed hybrid devices for plasma separation working on differential flow in lateral flow assays, Godino et al. engineered a novel hybrid paper-polymer device using paper siphon. The respective device relies on two main interplaying forces to create unique valving and liquid sampling under centrifugal microfluidics (Godino et al. 2014). The author has utilized the differential behavior of centrifugal force to obtain paper siphoning. At elevated speed the fluid is found to accumulate at the edge whereas at sufficiently low speeds the fluid tends to wick inwards along the curved geometry. Thus, distinct modes of flow modes can be accounted for different fluid with varying viscosity. The device mimics the conventional method of centrifugation for plasma separation in paper-polymer composite platform. The two main physical forces responsible for separation action are capillarity action and the centrifugal field. The assurance of efficient interplay of the forces is obtained by deciding the optimized frequency for centrifugal force required for efficient siphoning. The stepwise separation through siphoning is enunciated using Fig. 5.7 shown.

## 5.4 Summary and Future Perspectives

The chapter describes several paper based plasma separation assays developed in last few years (2010 onwards). It also enunciates several assisting methodologies such as agglutination and aggregation used to achieve efficient separation. Apart from reagent assistance the porous media structural architecture of paper which affects the flow dynamics of blood fluid through the same is also enunciated. The detailed explanation of the devices developed along with its working mechanisms is elaborated. These paper based devices are often found to follow Darcy's Law in porous media resulting in dynamics defined by Lucas Washburn to undergo passive capillary driven flow through the porous paper substrates. Further the separation

efficiency of the cellular components from plasma is either assisted using reagents causing agglutination/ aggregation of RBCs or some geometrical modification achieved by tailoring the shape of the paper substrate. Often the non-reagent based separation physical separation is achieved by creating a hybrid or creative composite of paper with that of the PSMs or by tailoring the geometrical shape of the platform itself. The intelligent tailoring of the substrate shape leads to manipulation of the flow dynamics (e.g. flow rate, centrifugal forces or other driving forces) of the blood itself as performed for the device with H-channels or the device developed by Godino et al. to act as paper siphon. Thus, the following chapter presents the researchers with detailed advances in the domain of paper based assays for plasma separation to present a clear understanding and knowledge about the advances. The researchers may further use the following knowledge to tailor new and efficient devices with added modified characteristics. The future researchers should understand that to achieve the maximum working and separation efficiency of the engineered device, the following must be exclusive or independent of the variations in hematocrit content of the blood as hematocrit content largely affects the rheological dynamics or properties of the blood. Efforts should also be made to explore various geometrical shapes which would be capable of manipulating the flow of blood and effective in separating out plasma from the same without any usage of reagents which are costly and require challenging preservation platforms. Scientists may also explore the domain of developing paper based composites capable of separating plasma but comparatively cheaper to the existing PSMs (LF1, MF1, VF1 and VF2). Methods of improvements in the existing fabrication methodologies (ink-jet printing, wax-printing etc.) to develop more intricate and narrow micro-capillaries in paper platform is also suggested. Narrower and intricate micro-capillaries will lead to usage of small sample volume for characterization/ analysis resulting in maintenance of the homogeneity of the sample and reduction in errors caused due to hematocrit variations. Simultaneously, attention should be paid in dimension of the micro-capillaries to avoid edge effects. Conclusively, there exists a huge scope of future developments in the following domain to obtain a fully functional/ efficient and low cost paper based plasma separation device which can further assist several other PoC diagnostics.

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