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REVIEW ARTICLE

Microfluidic Techniques for Platelet Separation and Enrichment

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Abstract | One of the major areas of active research in microfluidics is in biological applications. These applications often require complex analysis of biological fluids for clinical diagnostics. One such complex multicomponent suspension is blood, a mixture of cells suspended in plasma. The cellular components constitute RBCs, WBCs, and platelets. Platelets play a fundamental role in blood clotting mechanism and their efficient functioning is of utmost importance. Platelet separation is necessary for disease diagnostics, transfusion, and research purposes. Centrifugation is commonly employed for platelet separation. However, researchers are developing techniques to enable platelet separation using microfluidics as a tool, primarily due to the various advantages offered while working at microscale. In this review, we investigate and highlight various microscale platelet separation techniques currently available, focusing on their design, working principle, and performance aspects. The issues, challenges, and further possibilities of research and development are also underscored. Our review indicates that not many microdevices for platelet separation are currently available, pointing to an important void that needs to be urgently filled. A brief discussion on the conventional method of platelet separation and platelet dynamics is also included.

Keywords: *Active, Passive, Hydrodynamic, Blood, Platelet dynamics, Margination, Platelet-rich plasma*

1 Introduction

Microfluidics can be defined as the manipulation of fluids at length scales in the range of microns to submillimeters. At these scales, the behavior exhibited by blood and some other fluids tends to greatly differ from that of the behavior exhibited at macroscale. Microfluidics provides tools for several research areas specifically in the field of biological research and clinical diagnostics[1–6]. Blood is one of the simplest gateways through which one can assess the human health condition. Blood is multiphase, non-Newtonian fluid, and is composed of cells suspended in plasma[7, 8]. The cellular portion of blood primarily comprises RBCs, WBCs, and platelets. These cell components are routinely monitored to analyze human health conditions, and several applications in the field of clinical diagnostics require their efficient fractionation[9–13]. Blood components and its composition are shown in Fig. 1, the volume fraction of RBCs is ~ 45–50%, and WBCs and platelets occupy ~ 1% of the volume fraction, remaining ~ 55% is the liquid plasma[7, 14–16]. Blood has a density of 1050–1060 kg/m^3 and a viscosity of 4–5 cP at 37 °C, with exact values dependent on shear rate[15]. RBCs form the major portion of cellular portion of blood. They are deformable, biconcave disk in shape, and have diameter ranging between 5 and 8 μm. Normal count of RBCs is between 4.5×10^6 and 5.5×10^6/μl. WBCs are spherical in shape, with diameter ranging between 7 and 22 μm and their normal count is 5000–11,000/μl. Platelets or thrombocytes are smallest cell type, typically 2–4 μm in diameter and 1 μm in thickness. Platelets are without nucleus, non-deformable, and discoid in shape.

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Platelet-rich plasma (PRP): Platelet-rich plasma (PRP) is concentration of human platelets in a small volume of plasma. PRP typically contains 3–8 times the concentration of normal platelet levels.

Hemostasis: Refers to the normal response of the vessel to injury by forming a clot that serves to limit hemorrhage.

Thrombosis: A pathological clot formation that results when hemostasis is excessively activated in the absence of bleeding or hemostasis in the wrong place.

Platelet activation: When a platelet encounters a break in the endothelium, it encounters agents that trigger its activation. These agents are collagen, thromboxane A2, ADP, and thrombin.

Point-of-care testing: Is also known as bedside testing, these are simple tests which can be performed at the bedside of the patient or at remote settings. These tests can provide quick and immediate diagnostics results. For example, the pregnancy and blood glucose tests.

Normal concentration of platelets among healthy adult ranges between 1.5 and $4.5 \times 10^5/\mu l$[16–19].

Platelets are vital for sustenance of life, and they play a very important role in blood clotting. Platelets prevent bleeding in case of injury or a cut by forming a blood clot through a series of complex processes, also known as hemostasis[10, 20–22]. The basic steps involved in hemostasis are vascular constriction, platelet plug formation, and blood coagulation[23, 24]. The clot formation is not restricted to the damaged vessel wall; clot formation can also occur within the blood vessels (thrombosis) resulting in heart attacks or strokes[22]. Platelets circulate in an inactivated state; in event of damage in the vascular wall, these platelets respond to the injury and plug the wall through a three-phase process of adhesion, activation, and aggregation[25]. Platelet separation and enrichment has numerous applications in clinical and research areas. Platelet transfusion is required for patients undergoing treatment for dengue, chemotherapy, surgery, and organ transplant. In addition, platelet diagnostic investigation is linked with various diseases and their progression. Platelet and platelet function disorders are linked to cardiovascular disease, diabetes, cancer, inflammation, hemorrhage, sepsis, von Willebrand disease, amyloidosis, scurvy, etc[21, 26–28]. Platelets also contain essential proteins that are known to be reservoirs of growth factors. Several studies have confirmed tremendous advantages gained through the use of platelet-enriched plasma (PRP). PRP is a concentrate of large number of platelets in a small volume of plasma. For therapeutic use, a platelet count of 1 million/μl in 5 ml of plasma has been defined as PRP[29, 30]. PRP has a wide range of clinical applications; it is used for wound healing, tissue regeneration, bone regeneration, dental implants, skin rejuvenation, to name a few[29–33].

Conventionally, platelets are separated and enriched using the centrifugation techniques, which includes, the buffy coat method, platelet-rich plasma and apheresis[11, 20]. Though centrifugation is widely used, it involves manual steps, is complex, and laborious[14, 16, 34]. Centrifugation may also lead to contamination and result in undesirable platelet activation[35]. Platelets can get activated on encountering mechanical stresses and may undergo irreversible morphological changes and lose their adhesive properties, which limits their further employment. The drawbacks associated with conventional methods have led researchers innovate strategies to separate platelets using microfluidic techniques. These microscale methods will result in increased portability, lower sample consumption, and reduced risk of adulteration compared to conventional methods. Also, such techniques may enable automation leading toward point-of-care analysis. The microfluidic techniques of platelet separation and enrichment can be grouped as active, passive, and antibody-based affinity methods. Active methods rely on external forces for separation, whereas passive methods are based on hydrodynamics, flow behavior, and geometry of the design[14, 16, 36]. The passive separation methods do not depend on external forces and are advantageous compared to active methods of separation[16, 36]. Currently, only a few microfluidic-based devices for platelet recovery are available. But, our survey reveals that of late such methods are gaining attention and momentum.

Platelet purification, however, remains a nontrivial task, and there are numerous challenges in the process of realizing a microdevice for platelet enrichment. The major challenge stems from the fact that platelets are relatively small cells (2–4 μm), studying their motion, and flow physics in microchannels involve pre-preparation

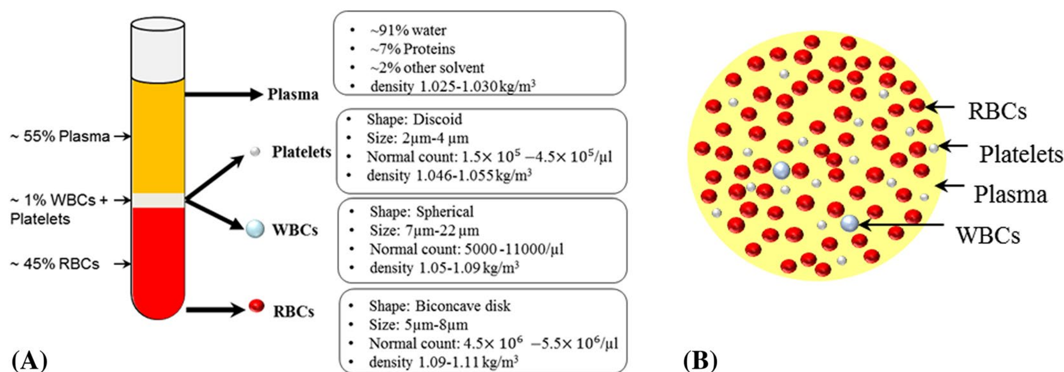

Figure 1: **A** Centrifuged blood components. **B** Schematic of blood cell visualization under a microscope.

steps and sophisticated instrumentation for visualization. The issue is further complicated by the presence of large number of RBCs, as these deformable cells impact the flow behavior of platelets. In light of the above issues, the medical community is still awaiting and exploring ways to invent an ideal device for platelet separation and enrichment. The envisioned ideal device will be one which is simple to fabricate, utilizes minimum hardware, separates platelets with high purity (minimum adulteration), and can recover most of the platelets infused and without activating them, in a continuous manner at a high throughput.

This review focuses on techniques available for platelet separation and enrichment. The emphasis is on the microscale methods of platelet separation. Although several reviews exist on the topic of cell separation and fractionation[9, 11–13, 20], the literature lacks a dedicated review on microfluidic platelet separation strategies. In this review, we discuss the microfluidic methods in detail by providing features of the existing designs, principle of operation, and their performance. This study also includes the conventional method of platelet recovery, and a separate note on platelet dynamics in microchannels has also been introduced for interested readers. Finally, the review ends with critical comments, concluding remarks, and future outlook.

2 Conventional Method of Platelet Recovery

The conventional method of centrifugation is frequently used in clinical application for separation and enrichment of platelets. The centrifugation method still remains the most popular choice to separate and prepare platelet concentrate. But, these methods suffer from certain drawbacks as discussed above. Several protocols have been reported for platelet separation using the conventional method. Platelets are prepared from whole blood using buffy coat, platelet-rich plasma (PRP), and apheresis methods[37–39]. All these methods use centrifugation for effective separation owing to the density difference among the blood components. In apheresis, blood is drawn from a donor into an apheresis machine, where blood components are fractionated by centrifugation; platelets are recovered while other components are returned back to the donor[11]. The PRP method is quite common in the United States, whereas the buffy coat method is commonly utilized in European countries[38]. The major difference between the buffy coat and PRP methods is the difference in centrifugation speeds and the order in which blood is processed. Both PRP and the buffy coat method protocols are schematically depicted in Fig. 2. No consensus has yet been reached regarding the PRP and buffy coat preparation protocols. In PRP method (Fig. 2, process-1–2–3–4–5), the initial step is to collect whole blood in an anticoagulant tube. Next, blood sample is centrifuged at constant acceleration called soft spin. Soft spin is carried out at low speed of 1000 g for 5 min[38]. Centrifugation separates the blood constituents according to their specific densities. As RBCs are of highest density, they are present in the bottom layer; the intermediate layer also called buffy layer contains mostly WBCs and platelets; and the top layer mostly contains platelets, plasma, and few WBCs. Next, the upper layer and intermediate layer are transferred to another tube and centrifuged at high speed, i.e., the hard spin. Hard spin is carried out at 2000 g for 10 min. The lower one-third portion of the solution is called platelet-rich plasma (PRP) and upper 2/3 portion is called platelet-poor plasma (PPP); the platelet-rich plasma is

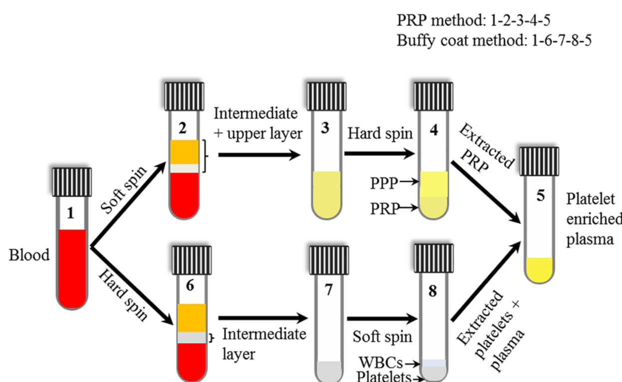

Figure 2: Schematic of the conventional methods for platelet separation and enrichment.

extracted and re-suspended in small quantity of plasma[29].

In the buffy coat method (Fig. 2, process-1–6–7–8–5), the collected whole blood is centrifuged at high speed of 3000 g for 11 min and its constituent is separated according to their specific densities. The bottom most layer contains RBCs, the intermediate layer contains WBCs and platelets, and the uppermost layer contain platelet-poor plasma. Subsequently, plasma is removed and the intermediate layer (buffy coat) is transferred to another tube. Next, the buffy coat is centrifuged at low speed of 1000 g for 5 min to separate white blood cells (WBCs) from platelets. Platelets can also be obtained by filtering WBCs at this step. Further, plasma is added to prepare the platelet concentrate. Apart from the conventional methods described above, several PRP kits are also available in the market to obtain platelet-rich plasma and are described elsewhere[40, 41]. Most of these kits work on a principal of centrifugation and have different operating protocols.

DEP (dielectrophoresis): In an inhomogeneous electric field the particle is subjected to a net force, which results in the particle movement. This movement can be toward or away from the high intensity field depending on the polarizability of the particle.

3 Microfluidic Methods of Platelet Separation

The microfluidic methods of platelet enrichment can be classified as active, passive, and antibody-based methods, as shown in Fig. 3.

The active separation methods of platelet removal are limited to dielectrophoresis (DEP)[42, 43] and acoustic methods[44–46], where external forces such as electric field and acoustic waves are generated to enable platelet separation. In contrast, the passive methods of separation do not require external forces rather utilize inherent hydrodynamic and geometrical effects for its operation[14, 16, 34, 36, 49]. Passive methods for platelet separation include inertial methods[50], hydrophoretic techniques[21, 51], deterministic lateral displacement (DLD) method[52–55], hydrodynamic methods[56], and filtration techniques[57]. The antibody-based strategy of platelet removal is the third category[58]. In this method, platelets adhere to the protein matrices patterned on the device surface. These microfluidic methods for platelet separation and related microdevices are discussed in detail in the following paragraphs.

3.1 Active Separation Methods

Active separation method is essentially the separation of cells based on external forces acting on them. Pommer et al.[42] reported platelet separation from diluted whole blood using a dielectrophoresis-based microfluidics device, as shown in Fig. 4A. The two-stage device fabrication involves patterning of quadrupole electrodes with 20 nm of titanium and 200 nm of gold on top of a 4-in glass wafer. The bottom substrate was coated with polyimide layer, and microchannels of 20 µm height and 1.75 mm width were patterned using optical lithography process. Diluted whole blood and buffer were introduced into the channel by syringe pump at 150 µl/h. A sine wave actuation voltage was applied at 100 V_{p-p} at 1 MHz. The actuation of electrodes produces dielectrophoresis forces on the cells based on their size and this force was controlled by varying the voltage. In addition to DEP forces, the cells were also subjected to hydrodynamic forces which were controlled via flow rate variation. The basic

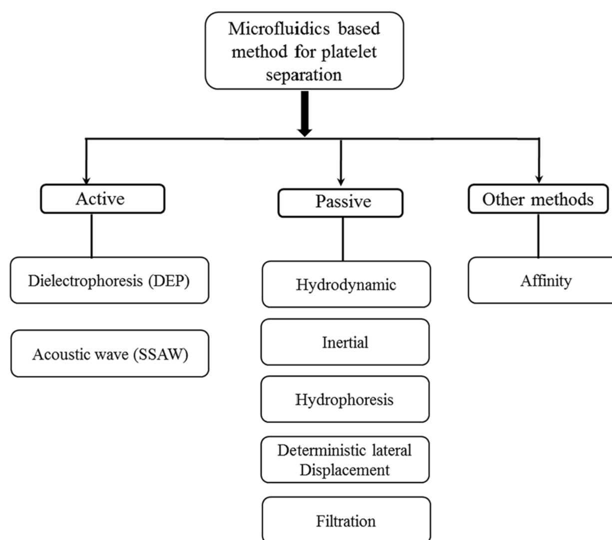

Figure 3: Classification of microfluidic methods for platelet separation.

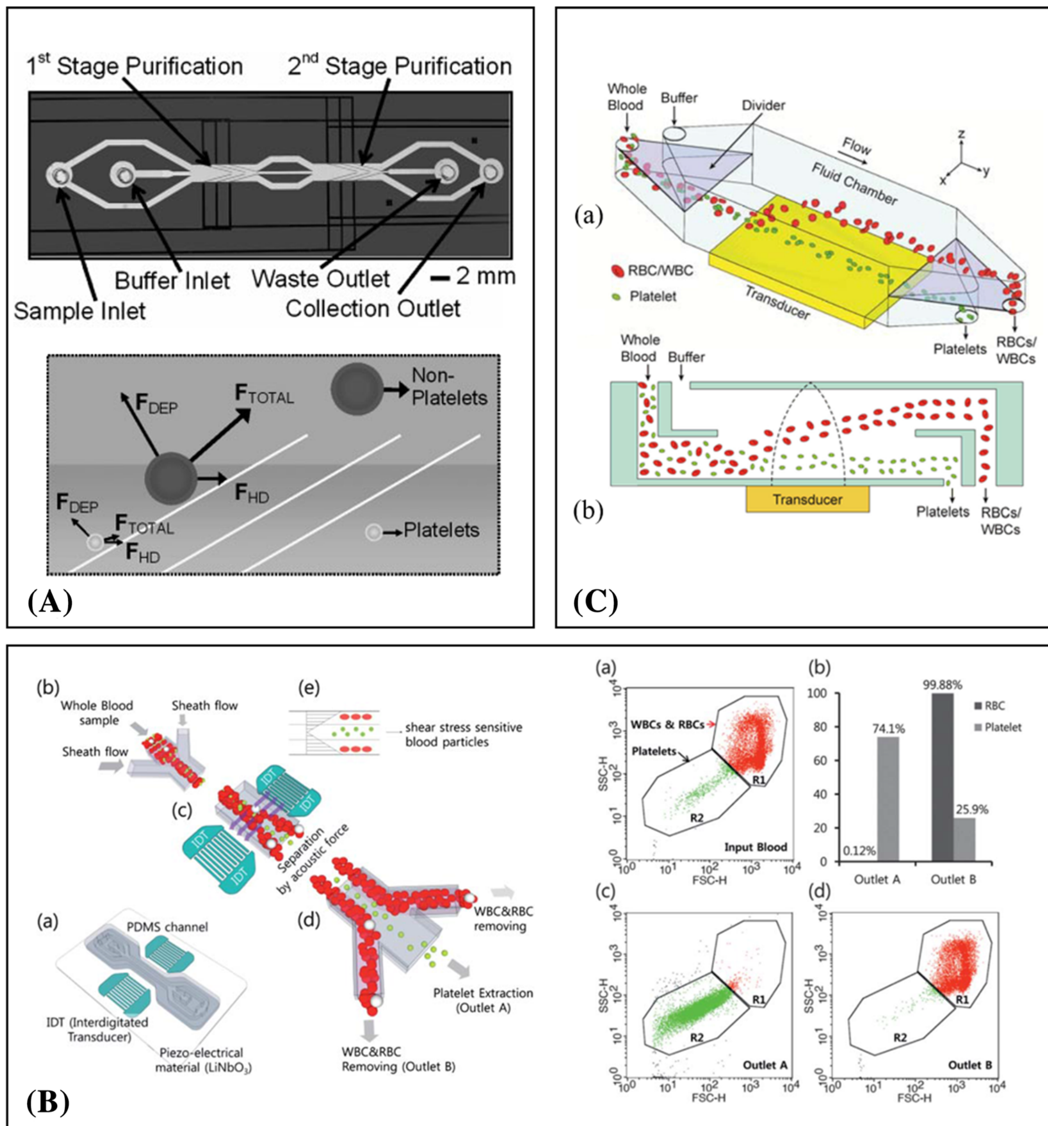

Figure 4: Platelet separation microdevices based on active separation techniques. **A** (Top) Dielectrophoresis activated cell sorter microdevice architecture showing integrated two stages to enhance platelet purification. (Bottom) Schematic representing separation principle; large cells (non-platelets) are subjected to higher DEP forces resulting in sufficient deflection and enter the waste outlet, whereas platelets are subjected to insufficient deflection and collected in collection outlet. Adapted from Ref.[42] by permission from John Wiley and Sons. **B** (Left) Schematic of the platelet separation standing surface acoustic wave (SSAW) microdevice. (a) Image of the acoustic device. (b) Hydrodynamic focusing of whole blood using sheath fluids. (c) Working region of SSAW and cell separation; forces on RBCs and WBCs are large compared to platelets. (d) Platelets collected in center. (e) Shear effect on platelets is less as they flow in center of the microchannel. (Right) Performance analysis represented using scatter diagrams obtained by flow cytometry. (a) Whole blood sample at inlet. (b) Recovery ratio at outlets A and B. (c) Cell distribution in outlet A post-separation showing small number of unwanted RBCs and efficient collection of platelets. (d) Cell distribution in outlet B post-separation showing the presence of large number of unwanted RBCs. Adapted from Ref.[44] by permission from The Royal Society of Chemistry. **C** (Top) Schematic of the acoustic separation device, the fluid chamber, inlets, and outlets. (Bottom) Schematic of the side view of the device; RBCs and WBCs are deflected upward, whereas platelets remain at bottom due to pressure gradients and acoustic forces. Adapted from Ref.[45] by permission from The Royal Society of Chemistry.

principle of separation relies on the dielectrophoretic forces and hydrodynamic drag forces acting on the cells. Both these forces are dependent on cell size. The ratio of dielectrophoretic force and hydrodynamic drag force is higher for larger cells (RBCs and WBCs) compared to smaller (platelets), this provides large deflection for RBCs and WBCs which were collected in waste outlet, whereas platelets were collected in collection outlet (left) due to insignificant deflection. Sample collected in collection outlet after single pass through two-stage device was processed in a flow cytometer. They reported platelet purity of 95% at a throughput of 2.2 × 10^4 cells/s per channel. Furthermore, the research group also verified that the buffer used had no adverse effect regarding platelet activation.

Flow cytometer: Used for analysis of cells in motion. It can be laser based or impedance based and is frequently employed in disease diagnostics.

Platelet purity: Number of platelets captured to total number of cells (including RBCs and WBCs) in that collection reservoir. Platelet purity of 100% signifies that all RBCs and WBCs are removed.

In yet another study on dielectrophoresis, Piacentini et al.[43] demonstrated platelet separation in their microdevice. They used hydrodynamic focusing in combination with DEP. They reported a single-stage process for device fabrication. The H-filter-shaped device consists of a 4-in glass substrate patterned with 200-nm-thick electrodes patterned on 20-nm-thick titanium adhesion layer by lift of process. The microchannels (40 µm width and 40 µm high) were fabricated by SU8 and covered with PDMS slab; patterned electrodes were also connected to the microchannel. Concentrated (2 × 10^8 cells/ml) platelets and RBCs were introduced from left inlet and focusing buffer from right inlet at 134 and 853 µm/s flow speed, respectively. Electrodes were actuated with a voltage of 10 V_{pp} at 100 kHz. Again, the disproportionate deviation of cells on account of DEP forces acting on cells was used for cell separation. The buffer forces the cells toward one side of the channel and the DEP force acts on the suspended cells. Larger cells (RBCs) show higher deflection compared to the smaller cells (platelets) in the microchannel; the RBCs and platelets are collected in different reservoirs based on their position in the flow stream. Video processing of platelet collection at the outlet shows 98.8% purity of platelets with a recovery rate of 98% while analyzing 5000 cells. Platelets activation was not reported by this group.

Hydrodynamic focusing: Hydrodynamic focusing can be described as the squeezing of a sample fluid using sheath fluid. It is a simple, yet effective technique for flow (sample) focusing and control. It provides means of transporting, controlling, and guiding the sample of interest.

Recovery rate: It is defined as number of platelets collected to total number of platelets injected. Platelet recovery of 100% signifies that all the infused platelets contained in the inlet sample are recovered.

Nam et al.[44] presented a microdevice for the separation of platelets from undiluted whole blood using standing surface acoustic waves (SSAW). The microdevice consists of PDMS-based channel (150 µm width, 50 µm depth, and 10 mm length) bonded to a SSAW substrate and integrated with interdigitated transducers (IDTs) on lithium niobate wafer (Fig. 4B). The microchannels are designed to hydrodynamically focus the sample to the midstream of the channel to minimize shear-induced activation of platelets. Whole blood and focusing fluid are supplied to microchannel at 0.25 and 5 µl/min, respectively, by syringe pump, AC voltage (439 mW) was applied to the IDT electrodes to generate surface acoustic waves, and superimposition of two opposite acoustic waves leads to constructive and destructive interferences resulting in standing surface acoustic wave with pressure nodes (maxima and minima). These acoustic waves produce acoustic forces on the blood cells according to their size. Cells are also subjected to viscous drag forces. Considering the dependence of the acoustic force and drag forces on the size of particle, it is found that the resulting net force on the larger cells (WBCs and RBCs) is higher compared to the smaller cells. Therefore, RBCs and WBCs were moving faster in the channel and collected in side outlet leaving the platelets, which were collected in middle outlet as shown in the figure. Flow cytometry results were reported by analyzing 10,000 cells. The results are also shown in Fig. 4B (right), 74.1% platelets are obtained in outlet A, with 0.12% of RBCs, and purity of platelets is reported as 98% (defined as ratio of number of platelets to total number of particles at outlet A). Outlet B shows 99.88% of RBCs and 25.9% platelets. The throughput of the device is 2.7 × 10^4 cells/s. Experiments also confirmed that platelets did not get activated.

Recently, Chen et al.[45] have also reported the separation of platelets from whole blood using the acoustic separation method. The microdevice is made of stainless steel sheet (60 mm × 20 mm) of 75 µm thickness. Channels of dimension 375 µm height and 17 mm width were fabricated by laminating various patterns of seven stainless steel sheets sealed with epoxy, and a composite transducer is attached at the bottom of the channel to form a resonator. Two inlets are provided for introducing the blood and buffer samples using a peristaltic pump, as shown in Fig. 4C. The separation principle of this device is similar to the device by Nam et al.[44]. As the transducer activates at its resonance frequency, pressure nodes and pressure antinodes are generated along the vertical direction above the buffer and below the blood sample, respectively. As the blood cells enter in the channel, they are subjected to acoustic radiation forces in the vertical direction. Due to large size of RBCs and WBCs, they are subjected to higher acoustic force than platelets, and they move toward the upper layer in the buffer and are collected from top outlet. Whereas, platelets remain in the bottom of the channel and are

collected from bottom outlet as shown in Fig. 4C (bottom). The most highlighting feature is the separation at a very high throughput of 10 ml/min. By optimizing the voltage and buffer, they achieved a platelet purity of 88.4% and recovery of 86.2% at throughput of 5 ml/min and an applied voltage of 46 V_{pp}. They used flow cytometer for data collection. This group has also compared this technique with the centrifugal method, and they found that the activation level of platelets in their acoustic separation device is lesser in comparison to the centrifugal technique. Further, they reported morphology score and hypotonic shock response showing that the separated platelets are of greater integrity and viability compared to the centrifugal method.

Another potential method involving active separation is the microfluidic-centrifugal platform also known as CD (compact disk) format[14], [47]. It is a miniaturized version of conventional centrifugation process. This method involves channels cut on CD/disk facilitated with valves. This arrangement is rotated using small motor, and density gradients are formed based on the density of cells and collected from different valves. Recently, Moen et al.[48] have reported successful separation of WBCs and RBCs from whole blood. In addition, they have also mentioned that by utilizing weak centrifugal forces platelets can also be separated, but any data regarding platelet separation have not been reported.

3.2 Passive Methods

Passive methods are known to be advantageous compared to active methods as they do not involve external forces and are based on hydrodynamic forces. But, these methods require a thorough understanding of the physical properties of cells and their motion within the microchannel. Understanding the theoretical mechanisms underlying blood flow in microchannels is the key to design effective platelet-enriched plasma microdevices based on hydrodynamic flow separation. A brief summary of passive microdevices is presented in this section.

Dicarlo et al.[50] presented an asymmetric microfluidic filtration device which allows for continuous separation of particles of varying sizes at high throughput using inertial focusing. The device design is shown in Fig. 5A; the PDMS-based microdevice consists of a coarse filter, curving channels (31 in number) having width of 350 and 650 μm on smaller and larger radius, respectively, with an average radius of curvature 325 and 890 μm respectively. The depth of the microdevice is 50 μm. These curving channels are arranged in three segments having five different outlets. The basic principle behind their device is to exploit differential inertial forces. The inertial forces constitute the lift and drag forces acting on particles/cells. The lift forces are responsible for lateral migration of particles away from the channel center, and the presence of a curvature in the geometry generates secondary flows in a microchannel and gives rise to an additional drag force, named as Dean drag. Equilibrium separations were achieved using the balance between the lift and drag forces developed within the microdevice. Design rules were determined for focusing particles dependent on their size. Experiments were carried out to study separation of different particles and cells. In one such experiment, platelet separation was observed using diluted blood (Hct 2%) at 0.9 ml/min. The flow cytometry results indicate that the initial fraction of platelets, 0.04, in the input blood was increased to 4.2, thereby enriching the platelets 100 folds in one of the outlets, as shown in Fig. 5A (right).

Dean drag: Presence of secondary flow in curved channels due to centrifugal and inertial forces; magnitude of secondary flows can be described by Dean number which depends on the Reynolds number, hydraulic diameter of channel, and radius of curvature of the channel.

Choi et al.[21] invented a novel hydrodynamic technique to achieve platelet separation, this technique used slanted microgrooves and anisotropic obstacles. This particle ordering technique was called hydrophoresis. The PDMS microdevice fabrication involves two layers of photolithography: a linear channel structure and patterns of slanted grooves with depth of 8 and 14 μm, respectively. Slanted grooves 530 in number were placed at an angle of 80°. Obstacle thickness is 27 μm with pitch length as 33 μm, gap height of obstacle is 8 μm, and channel height is 22 μm, as depicted in Fig. 5B. The width of the microchannel is kept at 1000 μm; the use of wide width of the device assists in maintaining shear stress to permissible limits to avoid platelet activation. The underlying mechanism of operation is based on inducing rotational flow, vortices, and cell obstacle interaction to separate cells based on size. The hydrophoretic ordering is controlled by the anisotropic obstacles (steric barrier), obstacle hinder rotational flow, and lead to cell ordering. The anisotropic fluid resistance (obstacles) results in rotational flows and vortices. Cells being subjected to vortices reach the sidewall, they collide with the obstacles, and the larger cells move toward sidewall, as shown in the figure, whereas the platelets remain unfocused and move upward while being subjected to recirculation. The obstacle gap height is a critical parameter and its maximum height should be less than twice the diameter of the particle for effective

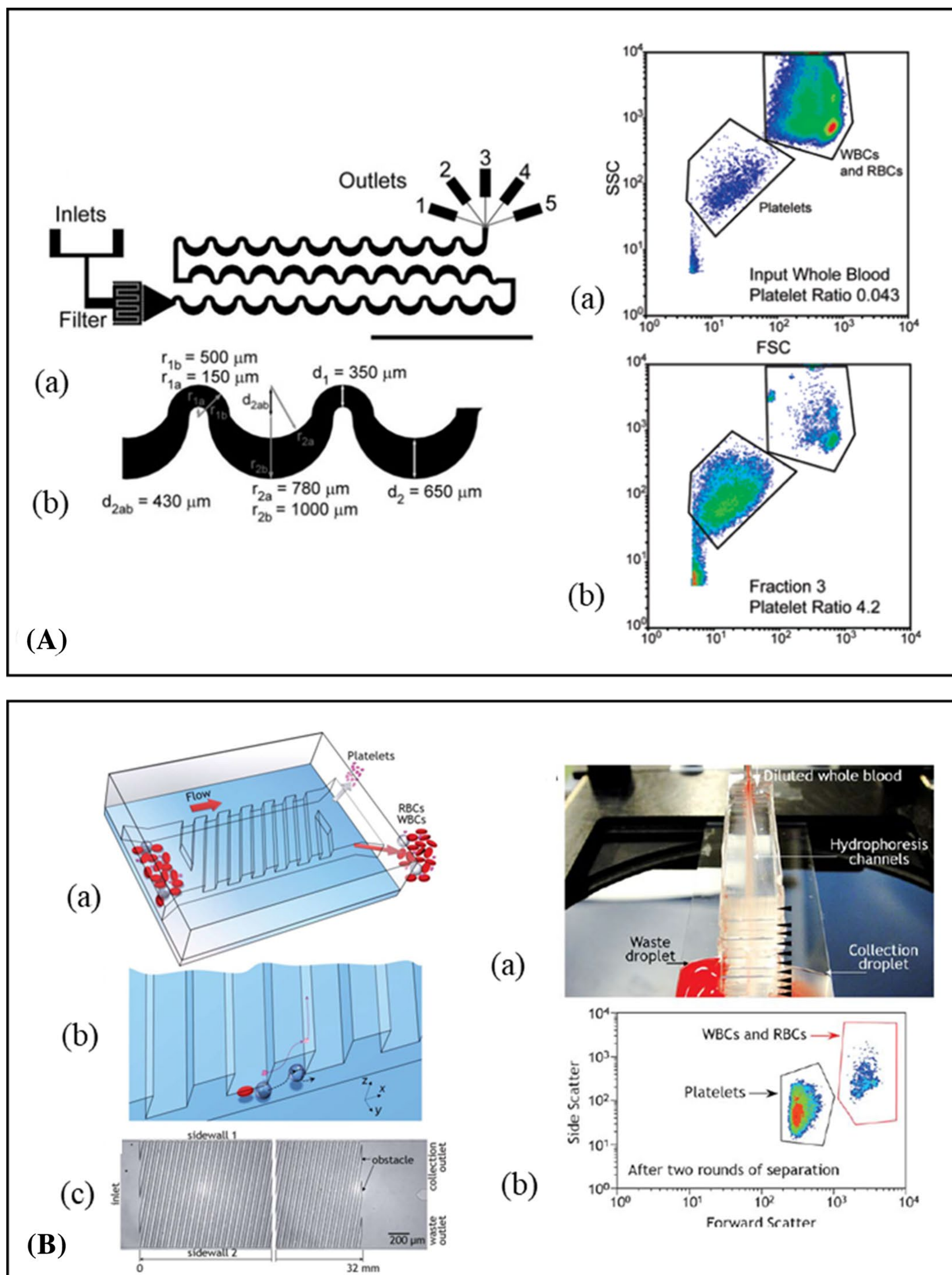

Figure 5: Platelet separation microdevices based on passive separation techniques. **A** (Left) Design of the differential inertial focusing microdevice. (a) Design showing inlets, filter, and outlets along with the asymmetric turns arranged in three rows. (b) Close-up view of the asymmetric turns showing details of the geometry with related dimensions. (Right) Performance analysis using flow cytometry. (a) Side scatter and forward scatter plots for input blood, platelet ratio of 0.04. (b) Scatter plots for blood obtained from outlet 3 enriching platelets by 100 folds, platelet ratio of 4.2. Adapted from Ref.[50] by permission from American Chemical Society. **B** (Left) Schematic of the hydrophoretic microdevice. (a) Illustration of device with anisotropic obstacles and separation of platelets. (b) Larger cells (WBCs and RBCs) stay near sidewall 2 owing to steric hinderance effects. (c) Arrangement of obstacles at an angle 80° to the bulk flow. (Right) Parallelized hydrophoretic device and its performance. (a) Experimental photograph showing ten layers of hydrophoretic device stacked on top of each other. (b) Flow cytometry scatter plot indicating platelet enrichment. Adapted from Ref.[21] by permission from The Royal Society of Chemistry.

hydrophoretic ordering. Experiments were carried out to target platelet separation using diluted rat blood (1:9) at a flow rate of 20 μl/min. Quantified results using flow cytometry indicated that platelet purity reached 36.7% from an initial purity level of 3.1%, after one round of sorting and the second round of hydrophoresis resulted in purity level of 82.8%, with each round 41% of platelets were collected. Here, purity was defined as the percentage ratio of number of platelets to total number of cells, and recovery rate was defined as number of target cells collected to number of target cells injected. Later, the research group parallelized the microdevice (ten stacks of the hydrophoretic microdevice). With this high throughput, parallelized microdevice platelets were enriched to 76.8% from an initial purity of 2% at a throughput of 2.9 million cells/s, which is also shown in Fig. 5B (right).

Li et al.[54] used the DLD (deterministic lateral displacement) technique for platelet separation. Using the DLD method, cells can be separated based on their sizes, deformability, and shape[52, 53]. Herein, obstacles or micropost arrays are arranged in a specific manner (rows are shifted horizontally relative to the upstream rows) to control the trajectory of the particles or cells. The reported PDMS-based microdevice consists of two stages: the first stage has 36 rows with an inter-row shift of 2 μm and the second stage had 49 rows with an inter-row shift of 5 μm. The gap between the two obstacles was kept as 20 μm with center-to-center distance between two adjacent obstacles as 80 μm. The critical separation diameters obtained were 3.8 μm and 6.1 μm for the first and second stages, respectively. The basic idea is that when blood cells flow through the arrays, the cells (platelets) which are smaller than a critical size will follow the initial streamline, whereas the larger cells (RBCs and WBCs) undergo greater deflections. Sample blood (50 times diluted) and buffer were injected at rate of 0.1 and 1 μl/min. Initially, platelets were separated at a distance of 100 μm from the WBCs and RBCs in the first stage and later on, RBCs were separated from WBCs in the second stage. The ratio of separated RBCs, platelets, and WBCs reported was 470:36:1. Utilizing DLD principles, Inglis et al.[55] have also reported a microfluidic device to separate and capture morphological changes in platelets.

Geislinger et al.[56] proposed a simple microdevice utilizing purely viscous hydrodynamic effects to separate RBCs, platelets, and microspheres in a continuous and label-free manner. The proposed microdevice consists of a single layer of PDMS and is easy to fabricate. Microchannels are rectangular in cross section, the sample inlet width is 30 μm, and sheath inlet width is 110 μm. Depth of the microchannels is kept at 110 μm. The basic principle behind the cell separation in the microdevice is to exploit the difference in size and deformability properties of the cells. Blood of Hct 0.1% is used as sample fluid and PBS-Dextran is used as sheath fluids for the experiments; two separate syringe pumps are used. The cells entering the microchannel are squeezed by the sheath fluid. Non-inertial cell–wall interaction causes the RBCs to lift at a greater height compared to the platelets and microspheres. Heights of the cells are measured at two locations: immediately after focusing and at the end of the microchannel, as shown in figure. The results indicate that RBCs adopt a height 20.6 μm (total flow rate $\geq$ 100 μl/h), and platelets and microspheres adopt a height of 12.5 and 7.2 μm, respectively (total flow rates $\geq$ 150 μl/h) when viscosity of the solution was 7.2 mPa s, where the total flow rate is defined as the sum of sheath and sample flow rate. Also, it is observed that the partition between the cells improves with increase in sheath flow rate. The cells occupy a different height downstream the microchannel and can, therefore, be used for their sorting. The size difference is identified as the most important parameter for the separation of RBCs from platelets; deformability and shape for separation of platelets from microspheres. The results also indicate improvement of separation with increase in sheath flow rates and increase in viscosity of the solution. The group extended the height variation findings to sort RBCs and platelets successfully. Quantified results were, however, not provided by this group.

Dickson et al.[57] worked toward enrichment of platelets utilizing a filtration-based macroscalable microdevice. The main idea behind this approach is that cells will not pass through openings or pores, which are smaller than the cell size. However, it is worth noting that RBCs are deformable and may pass through the pores, and may lead to clogging issues[14, 16]. The reported filtration microdevice is based on cross-flow filtration, device design is shown in Fig. 6A, and the microsieves are 3 mm long and 3 mm wide. Two-step filtration is employed, and the first filter removes platelets from RBCs, whereas the second filter separates plasma from pool of platelets; furthermore, the plasma is recycled to the blood and the process is repeated till desired concentration of 1.1×10^6/μl of platelets is reached. Experiments were performed with whole blood using filter pore size of 0.45 μm for separating platelets and plasma and pore sizes of 1.2–3.5 μm for retaining

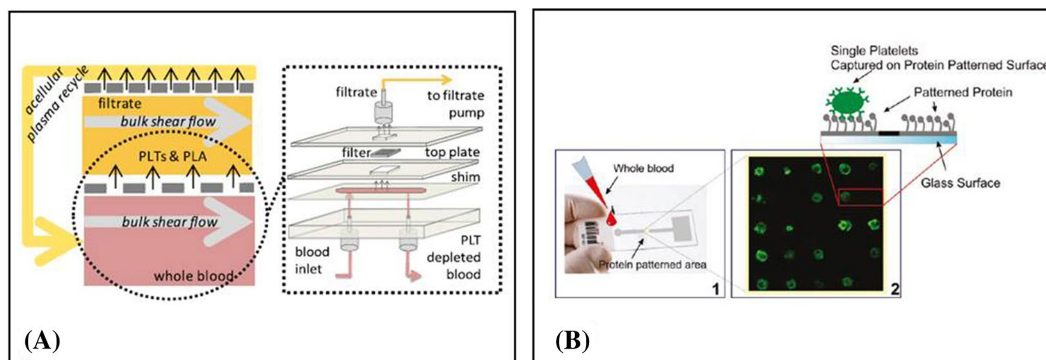

Figure 6: Platelet separation microdevices based on passive separation techniques. **A** (Left) Microfiltration device design showing two filters: larger pore size filter separates platelets and plasma from RBCs, and the finer filter separates plasma. (Right) The platelet extraction test setup. Adapted from Ref.[57] by permission from Springer Nature. **B** (Left) Schematic showing whole blood incubation on the interfacial platelet cytometry substrate which is patterned with platelet-specific protein for capturing platelets in a single step. (Right) Closer view of the protein pattern area proteins enabling single platelet capture for direct optical observation and analysis. Adapted from Ref.[58] by permission from American Chemical Society.

RBCs. Highest extraction fraction (115%) of platelets was achieved using 3.5 µm pore size of filter; the extraction fraction was defined as the ratio of filtrate concentration of platelets to concentration in the inlet blood. The concentrations were determined using a hemocytometer. Testing was carried out for 30 min at a shear rate of 16,000 s^{-1} and flow rate of 100 µl/min. The RBC leakage was found to be below 2000/µl. In addition, the research group envisioned that the study can be transformed into a macroscale device to obtain 50 ml of platelet-rich plasma in 30 min.

Another interesting way to separate platelets from whole blood was proposed by Desmonts et al.[58] This method is different from the active and passive methods. Herein, platelets are selectively captured using micro-patterned platelet-specific proteins on a glass substrate in a single step and without the need for sample preparation, as shown in Fig. 6B. Direct optical observation and quantification are possible using this technique. The three proteins studied include fibrinogen, von Willebrand factor (VWF), and anti-CD42b antibody. The number, size, and interaction between platelets can be controlled via protein patterning. The experiments reported that single or multiple platelets can be captured depending on the protein spot size (2–24 µm). This microdevice is useful for studying parameters such as platelet activation, platelet adhesion, morphological changes, receptor levels, biological and physical properties, and for detecting enlarged platelets.

Table 1 summarizes the prominent features of the platelet separation microdevices invented by various researchers. It includes the design principle, use of diluted or whole blood, their performance in terms of purity and recovery, throughput achieved, counting method, and information on activation of platelets separated. The analysis of the table indicates that active methods of platelet removal provide high purity and recovery of platelets, but they involve use of buffers, use of external forces for their operation, and intricate fabrication steps. It is noteworthy that the passive methods involve simple geometry, hydrodynamic principles, and reduced hardware (pumps etc.) for effective platelet separation. Except for the hydrophoretic design, all other passive microdevices are still in their development stage and demand further research. Table 1 also shows a disparity among the researchers in using the definitions of purity and recovery rates of platelets in their microdevice. Use of a common definition may enable comparison among the microdevices with much more clarity.

4 Brief Background of Blood Flow and Platelet Dynamics

Blood flow in microchannels is a complicated phenomenon, primarily due to the multiphase nature of blood. The major portion of the cellular fraction of blood is occupied by RBCs. Therefore, the motion of blood is predominantly affected by the presence of the deformable red blood cells. When flowing through microvessels, RBCs have a tendency to migrate toward the center of the

Table 1: Platelet separation microdevices and their performance.

Research group	Design principle	Input sample (blood)	Recovery	Purity obtained	Initial purity	Through-put	Cell counting method	Platelet activa-tion test
Pommer et al.[42]	DEP	Diluted + buffer	–	95%	18%	2.2×10^4/s/channel	Flow cytom-eter	Yes
Piacentini et al.[43]	DEP + HF	Diluted + buffer	98%	98.8%	–	–	Video imaging	No
Nam et al.[44]	Acous-tic + HF	Whole + buffer	74.1%	98%	5%	2.7×10^4 cells/s	Flow cytom-eter	Yes
Chen et al.[45]	Acoustic	Whole + buffer	88.4%	86.2%	2.62%	5 ml/min	Flow cytom-eter	Yes
Di Carlo et al.[50]	Inertial effects	Diluted	100 folds of enrichment		0.04 (frac-tion)	1 ml/min	Flow cytom-eter	No
Choi et al.[21]	Hydro-phoretic (single device)	Diluted	41%	82.8%	3.1%	2×10^5 cells/s	Flow cytom-eter	No
	Hydropho-retic (par-allelized device)			76.8%	2%	2.9×10^6 cells/s		
Desmonts et al.[58]	Antibody	Whole	–	–	–	–	Optical	Yes
Geislinger et al.[56]	Hydrody-namic lift	Diluted	–	–	–	–	–	No
Dickson et al.[57]	Filtration	Whole	Extraction fraction 115% with RBC leak-ages below 2000/μl		–	1.67 ml/min	Hemo cytom-eter	No

Purity obtained is defined as number of platelets captured to total number of cells in the collection reservoir; initial purity refers to purity in infused sample at inlet. Extraction fraction is defined as per concentration ratios and recovery is defined as number of platelets collected to total number of platelets injected. The targeted microdevice will recover all the platelets (recovery 100%), with no adulteration of other cells (100% purity) at high throughputs

DEP dielectrophoresis, *HF* hydrodynamic focusing

– Not reported

vessel, leaving a cell-free layer (CFL) devoid of RBCs along the vessel wall[59–63]. The phenomenon of cell-free layer formation leads to Fahraeus and Fahraeus–Lindquist effect[64–66]. The flow dynamics of blood is known to be influenced by various factors such as viscosity, deformability of cells, hematocrit, geometry of vessel, and shear rate[15, 16].

Platelet transport in microchannels plays a key role in understanding and developing methods for platelet separation and enrichment using passive separation techniques. Platelets occupy approximately 0.3% of the blood volume, in comparison RBCs occupy almost 45% of the blood volume[67]. Also, platelets are one-third the diameter of RBCs and are fewer in number compared to RBCs. Therefore, the presence of platelets does not affect the overall behavior of blood, but platelet motion is significantly affected by the presence of RBCs[22, 68, 91]. Extensive studies have been carried out to study platelet dynamics to understand hemostasis and thrombosis. It is well known that in case of an injury, platelets get concentrated near the microvessel wall to form a clot and prevent bleeding[69, 90]. Several experimental[70–76], theoretical, and computational studies[22, 77–81] confirm near-wall concentration of platelets in microvessels attesting to non-uniform distribution of platelets in the radial direction. This near-wall excess or lateral drift of platelets is termed as platelet margination[75]. Platelets concentrate near the wall and reside within the cell-free layer, as shown in Fig. 7. Platelet margination or enhanced diffusion is vital for platelet plug formation and for regulating the bleeding. The exact mechanism underlying this behavior

Fahraeus–Lindquist effect: Viscosity of blood decreases with decrease in tube diameter (10–300 μm).

Fahraeus effect: Concentration of RBCs in glass tubes (diameter < 300 μm) is lower in comparison with the concentration in a larger feed or supply tube, provided that the flow rates are high enough to avoid aggregation of cells.

Hematocrit: The hematocrit (Hct) or packed cell volume (PCV) or erythrocyte volume fraction is the ratio of red blood cell volume to the total blood volume.

Margination: The lateral movement of platelets resulting in higher platelet concentration near the wall compared to their presence in the center, also known as near-wall excess or enhanced platelet diffusivity.

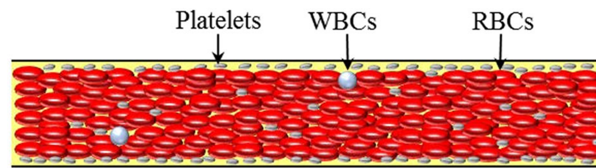

Figure 7: Schematic representing near-wall excess of platelets or platelet margination in the presence of RBCs.

has not yet been completely understood. However, researchers report that the excess transport of platelets toward the vessel wall is due to exclusion and shear-induced mixing[74, 75, 82, 83]. Exclusion is attributed to the drift of platelets toward the microvessel walls due to formation of the cell-free layer; herein the platelets are pushed toward the vessel wall as RBCs move toward the center of the vessel. An alternate reasoning for platelet margination is referred as shear-induced mixing, which is by virtue of hydrodynamic interactions between RBCs and platelets[72, 84, 85, 92].

Platelet margination is influenced through various factors such as hematocrit, shear rate, shear stress, initial cell concentration, RBC deformability, particle or cell size, and microvessel size. However, the major factors dominating margination are hematocrit, shear rate, and RBC deformability[72, 84, 86]. Experimental and computational studies (using platelet sized latex beads) report that platelet concentration near the wall increases with increase in hematocrit[72, 74, 75, 78, 81, 86]. Experiments carried out with 50-µm-wide channel report near-wall excess of almost five times the concentration in the central region of the vessel for hematocrit ranging between 15 and 45%[73, 74]. No margination was observed in the absence of RBCs and platelets marginated to an equilibrium position of 0.6R (*R* is the radius of the tube), also known as the tubular pinch effect[72, 87]. Non-linear dependence of average tube platelet concentration on near-wall excess is also reported. It was observed that the average tube concentration of the platelets 50×10^3/µl and 50×10^4/µl was augmented to concentrations of 450×10^3 platelets/µl and 150×10^4/µl, respectively, near the walls at a hematocrit of 40% at shear rate of 1260 s^{-1}[72]. Platelet excess near the wall is also strongly dependent on the shear rate; the near-wall excess was large only for shear rate greater than 430 s^{-1}[72, 74]. Also, large near-wall excess is reported at elevated shear stresses[75]. Platelet margination or near-wall excess is also effected by physical properties of the platelets (or beads) and suspended RBCs. Experiments conducted using bead size of varying diameters report the absence of margination for bead diameter less than 1 µm, whereas large near-wall excess was observed for bead diameter in range of 2.2–5.2 µm[74, 88]. Furthermore, computational studies using lattice-Boltzmann methods show that the spherical particles tend to migrate more quickly compared to the disk-shaped particles, and the presence of rigid RBCs decreases the near-wall excess of platelets[86]. Platelet concentration is also influenced by the channel geometry; platelet migration can be enhanced by employing a constriction–expansion geometry[22]. In a recent study, enhanced platelet margination has been reported in the flow separation region of a sudden expansion microchannel[89].

5 Future of Platelet Separation Microdevices: Concluding Remarks and Outlook

This review has presented a brief overview of the current technologies available for platelet separation at microscale. The study reveals that there exist only few microdevices for efficient platelet separation, and developing such microfluidic methods still remains a challenge. The active separation techniques are capable of isolating platelets with high purity and recovery, but their major drawback is low throughput, complex fabrication, and use of buffers. In contrast, the passive methods of platelet separation seem to have an edge over the active methods, but still require further improvements in design and performance. Among the passive methods, the hydrodynamic and inertial methods of platelet separation are potential techniques. However, the employability of such methods is based on comprehending the underlying physical mechanisms behind platelet dynamics in a microflow, which itself remains largely unexplored. In our opinion, the application of the separated platelets will dictate the microdevice and its design strategy. For instance, to study isolated platelets and their functions, small amount of blood is enough

to extract relevant information; therefore, the active techniques or affinity-based methods can be employed in such cases. However, for applications requiring platelet-rich plasma (PRP) where large volume is requisite, filtration, hydrophoretic, or inertial methods can be further improved and employed. Although, centrifugation technique is associated with certain limitations and drawbacks, it still continues to be widely used for platelet separation. On the other hand, microfluidic techniques though quite promising require further investigation, improvement, and testing for its transformation from the research to the usability state.

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