Microfluidics geometries involved in effective blood plasma separation

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Abstract
The last two decades witnessed a significant advancement in the field of diluted and whole blood plasma separation. This is one of the common procedures used to diagnose, cure and treat numerous acute and chronic diseases. For this separation purpose, various types of geometries of microfluidic devices, such as T-channel, Y-channel, trifurcation, constriction–expansion, curved/bend/spiral channels, a combination of any of the two geometries, etc., are being exploited, and this is detailed in this review article. The evaluation of the performance of such devices is based on the several parameters such as separation efficiency, flow rate, hematocrits, channel dimensions, etc. Thus, the current extensive review article endeavours to understand how particular geometry influences the separation efficiency for a given hematocrit. Additionally, a comparative analysis of various geometries is presented to demonstrate the less explored geometric configuration for the diluted and whole blood plasma separation. Also, a meta-analysis has been performed to highlight which geometry serves best to give a consistent separation efficiency. This article also presents tabulated data for various geometries with necessary details required from a designer’s perspective such as channel dimensions, targeted component, studied range of hematocrit and flow rate, separation efficiency, etc. The maximum separation efficiency that can be achieved for a given hematocrits and geometry has also been plotted. The current review highlights the critical findings relevant to this field, state of the art understanding and the future challenges.

Keywords Blood plasma separation · Blood cells · Microfluidics · Separation techniques · Separation efficiency · Typical geometries

1 Introduction
In human body, the principal function of blood is to transport oxygen and nutrients through the vascular network to various body organs and tissues Fung (1981). In general, blood is a multiphase fluid consisting of two major components: plasma ~ 55 to 60% and cellular component ~45 to 50% by volume percent (Harmening 1992; Karpatin and Charmatz 1969; Merrill 1969). Figure 1 shows the cellular component of the blood which consists of red blood cells (RBCs, i.e., erythrocytes, 6-8 μm), white blood cells (WBCs, i.e., leukocytes, 12–17 μm) and platelets (i.e., thrombocytes) (Fung 1981; Merrill 1969; Evans and Fung (1972); Hou et al. 2011). For the circulation of biomarkers, plasma is seen to be one of the essential and suitable sources (Caro 2011; Mielczarek et al. 2016; Liu et al. 2021) that led to plasma separation as an important step for preparing a diagnostic test sample (Wang et al. 2012; Kersaudy-Kerhoas and Sollier 2013). Moreover, nowadays for the treatment of patients infected with COVID-19, plasma therapy is being used frequently (Duan et al. 2020; Zeng et al. 2020; Focosi et al. 2020). Thus, for a biological analysis, the separation of blood into its constituents (such as plasma and cells) is a fundamental step (Banko et al. 2019).

From Fig. 1, it can be seen that the plasma consists of ~ 91% water, ~ 7% proteins, ~ 2% inorganic substance, and various organic substances, including plasma-borne circulating markers, for instance, bacteria, fungi, microorganisms, viruses, circulating nucleic acids and metabolites, (Fung 1981; Kersaudy-Kerhoas and Sollier 2013; Gahan and Swaminathan 2008; Psychogios et al. 2011). Notably, in healthy human being, circulating nucleic acids includes DNA and RNA. On the other hand, based on the
specific health condition of a patient, DNAs can be classified as foetal (in pregnancy), viral (in infections), tumour (in cancer), and donor (in transplantation). Moreover, the specific metabolites in healthy patients can be urea, glucose, hormones, etc. For a particular concentration of plasma-borne biomarkers, please refer to the Refs. (Kersaudy-Kerhoas and Sollier 2013; Gahan and Swaminathan 2008; Psychogios et al. 2011). From the engineering and biomechanical point of view, the whole blood, i.e., pure blood, behaves as a non-Newtonian shear-thinning fluid while the plasma behaves as a Newtonian fluid (Baskurt and Meiselman 2003; Wells and Merrill 1961; Sousa et al. 2011).

Several microfluidic devices have been developed over the years for such a separation of blood into its constituents, shown in Fig. 1 (MacDonald et al. 2003; Huh et al. 2007; Laurell et al. 2007; Jung and Han 2008; Lee et al. 2009; Sollier et al. 2009; Wu and Hjort 2009; Pamme 2007; Sajeesh and Sen 2014). Over the years, it has been witnessed that a monitoring of various diseases, such as chronic hepatitis B, requires minimum 200 μL of blood plasma in a total for six-to-eight tests of a patient in the diagnostics (Gong et al. 2013). On the other hand, several millilitres of plasma are required for the testing of the circulating nucleic acids (traces), for instance, tumour DNA, viral DNA, foetal DNA, etc. (Banko et al. 2019; Chiu et al. 2011). The resulting several dozens of painful blood extraction are avoided with recently developed microfluidics that allows the analysis of the biological sample using a few microliters of the blood. Based on the above discussions, the separation of the plasma from whole blood is one of the primary objectives of the researchers in the field of biomechanics, which has its direct application in the field of disease diagnostics, therapeutic and health monitoring. Few additional applications of blood plasma separation can be found in Refs. Zorita et al. 2008; Kaloyiannia et al. 2009. In broad term, blood is a complex fluid which is also called as suspension, i.e., cells remain suspended in plasma.

The efforts in designing microfluidic devices demonstrate the development in terms of a robustness, portability, cost-effectiveness, multifunctionality, etc. In designing the hydrodynamic force-based separation system, a careful use of a syringe pump to control the inlet flow rate has been suggested by Haque et al. 2021 as it can add some unwanted source of the flow instabilities in terms of pressure fluctuations. The control of these fluctuations is challenging while separating the blood. However, it can affect separation efficiency and yield by diverting cell path, hence cell migration. Also, the exploitation of active separation techniques in microfluidic devices is being seen to address some challenges in clinical diagnostics and therapeutics due to its contactless, label-free, and biocompatible separation strategy (Li et al. 2015; Lee et al. 2015; Lenshof et al. 2009; Laurell et al. 2007; Liu et al. 2021; Wu et al. 2017; Chen et al. 2016). Overall observation suggests that the clinical/biological validation of extracted blood plasma is now the primary concern of the research communities in the last decade. Still, the biological and clinical proofs are limited; hence it limits the practical applications of the fabricated separating devices, which indicates a future scope for the researcher in niche areas. Further, many challenges still need to be overcome in designing and exploiting the separating devices for their niche applications in clinical fields, biological field, etc. The crucial challenge is the prevention of blood coagulation (Brust et al. 2013; Li et al. 2017). The inherent limitations of the separating devices that need attention are the requirement of high plasma yield, contamination, biocompatibility, long extraction time (requires particular non-clogging systems), mechanical stresses (high pressure leads to the risk of cell ruptures, starts an adverse activation as platelets).
In addition, the improvements in such devices will lead to their use on various new platforms such as lab-on-a-cell and organ-on-a-chip (Clausell-Tormos et al. 2008; Sollier et al., 2009; Kersaudy-Kerhoas and Sollier 2013; Minas and Catarino 2015; Catarino et al. 2017). In particular, retrieving a high yield of plasma for high hematocrit blood is challenging due to the increased physiological constraints in terms of the increased blood viscosity, which leads to clogging. The first endeavour to analyse the effect of elevated hematocrit on the human blood flow behaviour using microchannels with no cell lysis has been done by Haque et al. 2020. They concluded that one can observe the human blood flow in microchannels at high hematocrit with no cell lysis for a short residence time.

Figure 2 shows the various separation techniques, which have been developed over the years for the blood plasma separation, which either falls in the category of active or passive separation technique based on the utilization of external means or not (Pamme 2007; Lenshof and Laurell 2010). In general, the passive separation techniques are based on the utilization of molecular interaction between the particle (e.g., cells), i.e., hydrodynamic forces (i.e., based on inertial forces, pinch flow, deterministic lateral displacement, etc.), hemodynamic effects (i.e., based on Fahraeus effect, Fahraeus–Lindqvist effect, plasma skimming, migration of leukocytes, Zweifach–Fung bifurcation effect), flow fields, structure of the microchannel and the choice of the channel dimensions (Sollier et al. 2009; Lenshof and Laurell 2010; Tripathi et al. 2015a, b; Catarino et al. 2019; Bayareh 2020; Kim et al. 2021). On the other hand, the active separation techniques are based on the exploitation of external fields, for instance, electric field (i.e., dielectrophoretic), magnetic field (i.e., magnetophoresis), acoustic field (i.e., acoustophoresis) and optical tweezers (i.e., optical forces) for the blood plasma separation. Apart from these separation techniques, there are few more, including CD based method and paper-based method (Kersaudy-Kerhoas and Sollier 2013; Catarino et al. 2019; Haeberle et al. 2006; Songjaroen et al. 2012; Guo et al. 2020). A brief discussion on the working principle of broadly used separation techniques can be found in the next section. Moreover, both experimental and numerical analyses have been considered in the scope of the present review article, with an endeavour to present the newly introduced design aspects by researchers in the recent years.

In general, using the above discussed separation techniques (Fig. 2) along with the exploitation of various typical
geometries, the input blood sample gets separated out into two components such as plasma and RBCs through outlets, Fig. 1. The last two decades witnessed numerous researchers who have made choices among (1) separation techniques or (2) geometry of the microdevice, for the better plasma separation. Numerous review articles can be found in the literature based on the separation techniques, but to the best of authors knowledge, no explicit discussion is available on the exploitation of typical geometries (Pamme 2007; Lenshof and Laurell 2010; Tripathi et al. 2015a, b). Moreover, the performance of the typical geometries exploited in the role of plasma separation devices depends on two types of governing parameter: geometrical and flow parameters. Figure 3 shows the representative geometry of the various channel-based separation device along with the governing geometrical parameters marked in the figure such as inclination angle $\theta$ between two daughter branches, bend angle $\theta_1$, inclination angle $\theta_2$ of the inlet channel in the constriction geometry, channel dimensions (lengths, i.e., $L_{EL}$, $L_{EL1}$, $L_{EL2}$, $L_B$, $L_P$, $L_S$, $L_{CR}$, etc.; widths, i.e., $W_{EL}$, $W_B$, $W_P$, $W_S$, $d_1$, etc.; and depth, i.e., $D$), and radius of curvature in the bend and constriction region $R$ and $R_1$, as the geometrical governing parameters, and hematocrit, Hct i.e., proportion of blood volume occupied by the RBCs and flow rate ($Q_0$, $Q_1$ and $Q_2$) along with flow rate ratios, $Q^*$ for bifurcated channels are the flow governing parameters for the blood plasma separation. Along with these input governing parameters, the output parameter of interest, in this work, the separation efficiency, SE (i.e., a measure of the number of RBCs in the separated plasma against the inlet blood sample RBC count) has been noted. A rigorous review of the available literature indicates that the blood plasma separation devices are made up of various geometries of channels: T-channel, Y-channel, Trifurcation (i.e., consists of three outlets), constriction–expansion (includes tau-shaped geometries), tau channel, bend channel, curved channel, spiral channel and serpentine channel; shown in Fig. 3. Moreover, a combination of a few of the above-discussed geometries has also been utilized in the separation process by some researchers.

Although numerous review articles are available in the literature based on the several separation techniques exploited for the blood plasma separation for different microfluidic systems, a discussion based on the exploitation of the geometric configuration is largely unexplored (Kersaudy-Kerhoas and Sollier 2013; Wu and Hjort 2009; Pamme 2007; Lenshof and Laurell 2010; Catarino et al. 2019; Guo et al. 2020; Toner and Irimia 2005; Luo et al. 2018; Wloch et al. 2019; Wu et al. 2019). The main objectives of the present review article are to explore these two major concerns in this field as (1) various types of geometries being exploited by the researchers (2) the influence of the choice of the geometry on the separation efficiency for a given hematocrit (Hct, i.e., proportion of blood volume occupied by the RBCs). The present review article is novel as this will provide insights on the influence of different types of geometries and the associated geometrical governing parameter (mentioned in Fig. 3) on the separation efficiency of RBCs and plasma for various flow parameters such as $Q_0$ and Hct. The present article also demonstrates a comparative analysis of various geometries, indicating to the reader about the less explored...
geometric configurations for the blood plasma separation both on the micro-and macro-levels. Also, attempts have been made to provide important geometry-based governing parameters that should be helpful for the designers of blood plasma separators. In broad terms, suffice it to say here that the present article endeavours to answer the following questions unanswered in the available literature till now, such as (a) which geometry has been exploited most and for what range of hematocrits, Hct’s? (b) what is the optimum flow rate chosen by the researchers to use in most of the geometries to obtain a better separation efficiency, SE? (c) which geometric configuration is still to be fully explored in terms of the diluted/whole blood separation? (d) which are the most exploited geometries for the separation of whole human blood? Finally, this article also makes a concluding remark based on the comparative discussion on the utilization of various geometries for diluted blood (Hct < 37%) and whole blood (Hct ≥ 37%) (Jameson et al. 2019).

2 Plasma separation techniques

This section elucidates a brief discussion on the working principles of various separation techniques, as shown in Fig. 2 used in the recent years for the separation and sorting of biological cells for a wide variety of applications in microfluidics, point-of-care (POC) diagnostics, and micro-total analytic systems (μTAS) (Sollier et al. 2010). The choice of separation techniques for different geometries is also the focus of this section. A high separation efficiency can be achieved using a continuous microfluidic device based on either separation techniques, see Fig. 2 (Nguyen et al. 2019). Recently, based on the design principle and types of microfluidic device, Tripathi et al. (2015a, b) presented a discussion on various passive separation technique for blood plasma separation. Kaun et al. (2018) also summarized separation techniques exploited for whole blood. The advantages and limitations of such separation techniques have been discussed elsewhere (Pamme 2007; Sajeev and Sen 2014; Dalili et al. 2013).

The passive separation technique primarily includes hydrodynamic forces, hemodynamic effects, pinch flow fractionation, filtration, sedimentation, centrifugation, and lateral displacement (Tripathi et al. 2015a, b), Fig. 2. Most recent review articles based on the blood plasma separation and sorting techniques present a detailed discussion on separation techniques (Catarino et al., 2019). Hydrodynamic separation is a size-based separation technique, which includes particle size ($d_p$) as an influencing parameter to separate dispersed particles from the continuous phase. The hydrodynamic separations can be considered for a wide range of Reynolds number covering very low to high values (Sollier et al. 2009; Tripathi et al. 2015a, b; Kersaudy-Kerhoas et al. 2010a, b). If the particles are specifically blood cells such as RBCs, WBCs, etc., the separation technique is considered a hemodynamic effect. Thus, the hemodynamic effects exploit the hydrodynamic forces, which involve forces-based separation to isolate the cells from plasma from the whole blood (Sollier et al. 2009; Tripathi et al. 2015a, b; Kersaudy-Kerhoas et al. 2010a, b). In such effects, the viscous forces dominate to influence the distribution of cells through the microchannel. However, the cells in the domain choose to move in multiple branching outlets based on their size and the forces experienced (Tripathi et al. 2015a, b); see Fig. 2. Moreover, the hemodynamic effects exploit the biophysical effects, inertial effects, and geometrical effects to separate the blood and plasma from whole blood.

In general, the cell migration happens because of two different forces; a force from the microchannel wall and a lift force (i.e., in the perpendicular direction to the flow direction). These forces, which depend on the channel dimensions, flow rate, carrier fluid rheological properties, etc., results in cell migration. Thus, to obtain an enhanced separation efficiency, few parameters such as flow rate, fluid viscosity, channel geometry, etc. can be modulated. In particular, the Fahraeus effect, which is one of the important hemodynamic effects, has been observed in a microchannel of diameter less than 300 μm, which plays a significant role in the blood plasma separation (Fahraeus 1929; Gaehgens et al. 1978; Pries et al. 1990; Goldsmith et al. 1989). This effect has been confirmed by Barbee and Cokelet (1971) for the tube diameter of the range of 21–221 μm. In this effect, the RBCs (i.e., smaller in size than the platelets and more deformable) move towards the centre of the microchannel because of the velocity gradient in the flow direction. Furthermore, if the apparent viscosity of the blood decreases with a decrease in hematocrit, the blood cells tend to move towards the microchannel centre (please refer to Fig. 3). This effect is called as Fahraeus–Lindqvist effect (Fahraeus and Lindqvist 1931). Moreover, Zweifach–Fung bifurcation effect leads to the migration of the cells towards branched channel of a bifurcated geometry (i.e., T-channel, Y-channel, etc., see Fig. 3), which has higher flow rate, see Fig. 2 (Tripathi et al. 2015a, b). Similarly, other effects discussed-above, influence the cell migration in the blood plasma separation, details can be found elsewhere (Catarino et al. 2019).

Furthermore, Pinched flow fractionation shown in Fig. 2 is generally used for the continuous separation of blood in microfluidic devices. The RBCs suspended in the plasma gets separated continuously introducing the sample into a geometry which has a pinched section along with two inlets, as shown in Fig. 2, based on the particle size exploiting the laminar flow profile. A liquid flow without any suspended particle from one inlet while from another inlet, a liquid with the suspended particle flows. Regardless of the particle size, it gets aligned towards one of the
sidewalls of the pinched section opposite to the inlet in which liquid is flowing without particles. This alignment is affected by four important factors as $Q^*$ between the two inlet branches, width of the pinched section, the angle between the pinched and followed broadened region and the flow rate of a particular branch. It is expected that with an increase in the flow rate of the inlet would increase the movement of the particle, and hence the inertial force.

In filtration, a membrane is used with pore size that allows only plasma to pass through it while the RBCs remains retain in the input section of the system. In general, the separation efficiency using filtration is seen to be smaller than the centrifugation that works based on the size of the cells and the difference in the specific gravity of the blood constituents, as shown in Fig. 2.

Sedimentation and centrifugation are one of the conventional passive separation techniques of the RBCs and Plasma (Haebeler et al. 2006; Madureira et al. 2018; Prabhakar et al. 2015; Tripathi et al. 2016). In case of sedimentation process, the gravitational force causes the separation and eventually different layers of blood constituents can be achieved in a test tube in which whole blood would be filled, for instance, RBCs at bottom of the tube while the carbohydrates, mineral salt, protein and hormone rich plasma layer would collect at the top layer. The centrifugation adds a new centrifugal force which leads to a better separation of blood, plasma and platelets, as shown in Fig. 1.

Moreover, in the lateral displacement passive separation technique, an array of pillars is used and the distance between the pillar is chosen according to the cell size to be shortened. The array is used here with the idea of forcing the cells towards a specific direction through the device for its separation as the laminar flow of the incoming fluid gets interact with the arrays of the pillars and aids to provide a specific trajectory to the cells. The cells with smaller diameter than the array gaps, moves through the gaps and remains close to the original centre streamline. On the other hand, particles with larger size than the array gap tends to move laterally and flows at an angle prefixed by the post offset distance, see Fig. 2.

Various passive separation techniques being utilized in the recent past on blood plasma separation are as follows: pinch flow fractionation (Berendsen et al. 2019; Ma et al. 2016), hemodynamic effects (Kersaudy-Kerhoas and Sollier 2013; Kaun et al. 2018; Prabhakar et al. 2015; Jaggi et al. 2007; Tripathi et al. 2013; Rodriguez Villarreal 2009; Karii et al. 2013; Tripathi et al. 2016; Tripathi et al. 2015a, b; Faivre et al. 2006; Lee et al. 2011; Zhang et al. 2015; Lopes et al. 2015), sedimentation (Haebeler et al. 2006; Forchellet al. 2018), lateral displacement (Tottori and Nissisako 2020; Kruger et al. 2014), and filtration (Gao et al. 2020). In broad term, these techniques have been utilized by various researchers as it is seen to be advantageous in terms of no requirement of external forces, no design complexities, and easy integration with biosensors.

Moreover, nowadays, to achieve maximum separation efficiency of blood and plasma separation, the active separation techniques (Fig. 2) have been favoured by many researchers (Kersaudy-Kerhoas and Sollier 2013; Laurell et al. 2007; Jung and Han 2008; Lenshof and Laurell 2010; Wloch et al. 2019; Wu et al. 2019; Shiriny and Bayareh 2020; Alnaimat et al. 2018; Kim et al. 2016; Luo et al. 2018; Yan et al. 2018; Avsiевич et al. 2020; Zhu et al. 2020; Atajanov et al. 2018; Lee et al. 2016). For the sake of conciseness, the working principle of these techniques has not been discussed here and can be found elsewhere (Sajeesh and Sen 2014; Catarino et al. 2019). Due to the higher demands for the separated blood plasma in the testing of various diseases, rampant utilization of active separation techniques has been witnessed in the literature in the recent years because of their easy application and low-cost fabrication (Luo et al. 2018; Wloch et al. 2019; Shiriny and Bayareh 2020; Alnaimat et al. 2018) Avsiевич et al. 2020; Zhu et al. 2020; Atajanov et al. 2018). Both active and passive separation techniques have their advantages and limitations in separating biological cells and plasma based on the choice of geometry, flow rate, channel dimensions, etc.

Further, from the viewpoint of geometries utilized for the blood plasma separation, it has been observed that the T-channel and Y-channel (Fig. 3a, b) both at the micro- and macro-levels has been exploited with the passive separation techniques specially the hemodynamic effects, as shown in Fig. 2 (Jaggi et al. 2007; Tripathi et al. 2013; Tripathi et al. 2015a, b; Yang et al. 2005, 2006; Huang et al. 2010). It has also been noted that the centrifugation technique also been used by only one researcher for T-channel (Madureira et al. 2018). Similarly, the passive separation technique has been the choice of the researchers while exploiting the Y-channel (see Fig. 3b) as passive separation technique (Yang and Zahn 2004; Hymel et al. 2019; Li et al. 2020). In case of trifurcation geometry, Fig. 3c, a mixed choice, i.e., both active and passive separation techniques have been utilized by the researcher to separate blood into its constituents. For the case of constriction–expansion geometries, Fig. 3d, researcher choose to mostly work with the passive separation technique such as Fahraeus effect, bifurcation law, centrifugal force, and cell-free region for the separation of plasma from human blood (Faivre et al. 2006; Sollier et al. 2009, 2010; Rodriguez-Villarreal et al. 2009; Kersaudy-Kerhoas et al. 2010a, b; Lee et al. 2011; Zhang et al. 2015; Prabhakar et al. 2015; Tripathi et al. 2016; Shatova et al. 2016). It is noted that a higher separation efficiency can be achieved using such a complicated geometry even for a very high hematocrit (i.e., Hct~62%) with the exploitation of constriction–expansion geometry, Fig. 3e (Tripathi et al. 2016). Moreover, the
bend/curved/spiral/serpentine channel geometries have also been popular among many researchers for both diluted and undiluted blood separation (Haeberle et al. 2006; Blattet et al. 2004; Chen et al. 2007; Di Carlo et al. 2008; Sollier et al. 2009; Nivedita and Papautsky 2013; Zhang et al. 2014; Robinson et al. 2017). The separation techniques mostly used with such geometries are filtration and centrifugation along with hemodynamic effects in few cases. The literature reported that such above-discussed geometries which includes a bend/curved/spiral/serpentine channel exploited the centrifugal force in the separation process.

In general, there are few possible ways to quantify the performances of the microfluidic devices in terms of separation, such as throughput, separation efficiency, and yield. Although, the most common way to predict the capability of a device in separating the whole is in terms of separation efficiency and yield. The throughput is generally defined as the product of the inlet volumetric flow rate, \( Q_0 \) and Hct, i.e., the volume fraction of cells at the inlet. Further, SE has an uncommon definition in several research articles based on \( Q_0 \) and Hct, etc. A widespread definition is found as \( [(C_s - C_p)/C_s] \times 100\) (Jaggi et al. 2007; Lenshof et al. 2009; Kersaudy-Kerhoas et al. 2010b; Tripathi et al. 2015a, b; Gonzalez et al. 2018, 2020; Li et al. 2020). Here, \( C_s \) represents the number of cells per \( \mu L \) of blood at the main channel inlet; \( C_p \) represents the number of cells per \( \mu L \) of extracted plasma from the plasma outlet. Undoubtedly, SE is a vital function of hematocrit levels, dilution factor, inlet blood flow rate, etc.; the present work has reported SE without adjusting its actual values mentioned in the companion paper. The readers should refer to the analogous literature to get the exact SE calculation method.

Further, in general, yield, i.e., the interpretation of the percentage of plasma extracted after separation, has been defined as \( [Q_2/(Q_1 + Q_2)] \), where \( Q_1 \) denotes the flow rate of the plasma cell outlet and \( Q_2 \) represents the flow rate of the concentrated cell outlet (Jaggi et al. 2007; Tripathi et al. 2015a, b, 2016; Di Carlo et al. 2008; Shatova et al. 2016). In particular, plasma yield demonstrates the volume percent of extracted plasma over the total volume of the inlet blood. Meanwhile, it is crucial to add that most research suggests an increment in the separation efficiency. SE suppresses the yield values (Tripathi et al. 2015a, b, 2016; Kersaudy-Kerhoas 2010b; Di Carlo et al. 2008). However, Prabhakar et al. (2015) noted that the yield could be significantly improved with the exploitation of the inertial-based separation techniques, which may require the dilution of blood. Also, Prabhakar et al. (2015) suggested a blood recirculation approach could help in enhancing the plasma yield up to \( \text{Hct} = 60\% \). An ideal blood plasma separation must ensure a high yield (i.e., a large amount of isolated plasma) with a minimal number of cells in the extracted plasma. Thus, there is always a limitation of the blood plasma separating devices in terms of hemolysis, extraction time, etc. These limitations can be overcome with a specific choice of channel dimensions, inlet flow rate, reduction in the flow fluctuations, etc., which can lead to a higher separation efficiency with high cell viability, but for a limited hematocrit (Lee et al. 2011; Haque et al. 2020, 2021).

3 Microchannel-based geometries exploited in plasma separation devices

The potential niche applications of separating devices in sample preparation are widespread in the field of lab-on-a-chip, point-of-care biomedical devices, etc. This paper specifically focuses on the blood plasma separation exploiting either of the active or passive separation technique. Thus, the details related to a particular biomarker such as separation of microorganisms, proteins, molecules, circulating nucleic acid, etc. has been omitted in this work, which is the future scope of present work. Though, some essential information related to the biomarkers has also been added herein. The developed microfluidic devices, in the blood plasma field is not only limited to answer in terms of a specific separation technique, hematocrit, flow rate, etc., but also in terms of a particular sample volumes as large sample volumes from transfusion (>5 x 10^5 μL), moderate volume collected from venous (~500 to 5000 μL), small volume sample collected from finger-pricks (~10 μL) (Kersaudy-Kerhoas and Sollier 2013). The details regarding the application of the plasma extraction devices for a specific sample volume can be find out in Ref. Kersaudy-Kerhoas and Sollier (2013). Eventually, this section details each specific geometry exploited in blood plasma separation.

3.1 T-channel

T-channels found to be a popular device among many researchers for separating plasma from human whole/diluted blood at both the micro- and macro- levels (Madureira et al. 2018; Jaggi et al. 2007; Tripathi et al. 2013; Tripathi et al. 2015a, b; Yang et al. 2005, 2006; Huang et al. 2010; Fenton et al. 1985). A rigorous review on the T-channel leads to the tabulation of details related to channel dimensions (\( D^* \)), separation techniques (ST), targeted/separated component (SC), flow rate (\( Q_0 \)), separation efficiency (SE) and purity; presented in Table 1 along with important remarks. This tabulation has been done with the idea of providing a quick summary of the work done with T-channel based plasma separation device.

Table 1 stands with a work done by Yang et al. (2005), who investigated the blood plasma separation from whole blood (i.e., defibrinated sheep blood) using the passive technique including hemodynamic effects, i.e., bifurcation law.
Table 1 Summary of T-channel device used for blood plasma separation

| Research groups, RG | Channel dimensions, $D^*$ (μm) | Separation techniques, ST | Separated component, SC | Hematocrit levels, Hct (%) | Flow rate, $Q_0$ (μL/min) | Separation efficiency, SE (%) | Remarks |
|---------------------|--------------------------------|--------------------------|------------------------|-----------------------------|---------------------------|-----------------------------|---------|
| Yang et al. (2005)  | $L=5000$, $W=15$ (EC), 9.6 (PC), $D=10$ | Zweifach–Fung law | Plasma | Up to 45 (particularly, 39 and 45) | 10 | 27% for Hct = 45 and 25% for Hct = 39%; (yield = 25%) | Electrical circuit analysis could be a better way to understand the flow resistances between the main channel and plasma channel. For better separation efficiency, plasma channel resistance must be higher. No cell lysis has been noted here |
| Yang et al. (2006)  | $L=5000$, $W=15$ (EC), 9.6 (PC), $D=10$ | Zweifach–Fung law | Plasma | 10 | 6:1 (flow rate ratio) | ~ 100% at Hct = 10 %; yield = 25% | The choice of higher flow rate ratio between the plasma and main channel outlet leads to a better separation efficiency |
| Jaggi et al. (2007) | $L=NA$, $W=14,000$, $D=20$ (d$_1$) and 50 (d$_2$) | Hemodynamic effects | RBC | 4.5, 15 and 45 | 2000 and 5000 | 92% at Hct = 4.5%; 30% at Hct = 45% with d$_2$ at 5000 μL; (yield = 2.5% at Hct = 4.5%) | Higher flow rate can enhance or suppress the recovery of the RBC depending upon the hematocrit level. High flow rate and low Hct leads to ~ 100% RBC separation |
| Huang et al. (2010) | $L=2857$, $W=50$ (EC), 40 (DC), 204 (OC), $D=40$ | Bifurcation law | Plasma | 45% | 0.3 m/s (inlet velocity) | NA | Channels including the converging and bending effects is the best design in between the proposed four layouts |
| Tripathi et al. (2013) | $L=2000$, $W=200$ (EC); 100 (PC), $D=50$ | Bifurcation law | Plasma | 2, 3, 5, 10, 16, 22, 45 | 150 | ~ 100% for low Hct as upto 24 and ~ 67% for 45% Hct using 2-stage T-channel (yield 1.68%) | Feed hematocrit, main channel width and flow ratio plays an important role in plasma separation. Higher aspect ratio channel can enhance the plasma recovery at high Hct and flow ratio as 67% with 45% Hct |
| Research groups, RG | Channel dimensions, $D^*$ (μm) | Separation techniques, ST | Separated component, SC | Hematocrit levels, Hct (%) | Flow rate, $Q_0$ (μL/min) | Separation efficiency, SE (%) | Remarks |
|---------------------|-------------------------------|--------------------------|------------------------|----------------------------|--------------------------|-----------------------------|---------|
| Tripathi et al. (2015a, b); (T-200/100) | $L=NA$, $W=200$ (EC) and 100 (PC), $D=60$ | Bifurcation law | Plasma | 7, 24, 45% | 400 | 90% (Hct = 7%), 63% (Hct = 24%) and 54% (Hct = 45%); (yield = 3.8%) | Used elevated channel dimensions as 200 μm of the inlet blood channel and 100 μm of the outlet plasma channel |
| Tripathi et al. (2015a, b); (T-400/100) | $W=400$ (EC) and 100 (PC), $D=60$ | Bifurcation law | Plasma | 7, 24, 45% | 400 | 80% (Hct = 7%), 59% (Hct = 24%) and 42% (Hct = 45%); (yield = 1.81%) | Increased channel and compared the effect of inlet channel width and reported suppression in the separation efficiency for higher channel width |
| Tripathi et al. (2015a, b); (T-cascading) | $W=200$ (EC) and 100 (PC) for first bifurcation; Similarly, 100 and 60 for second bifurcation; $D=60$ | Bifurcation law, Fahraeus effect | Plasma | 7, 24, 45% | 400 | 94.44% (Hct = 7%), 64% (Hct = 24%) and 62% (Hct = 45%); (yield = 5%) | Used two bifurcations in a T-channel which enhances the separation efficiency |
| Madureira et al. (2018) (ovine blood, heparin treated) | $L=10,000$, $W=400$ (EC); 120 and 200 (OC), $D=50$ | Centrifugation (2000 rpm for 15 min at 4 °C) | RBC | 5 | 150 | NA | Curved T-channel is a better choice for the blood plasma separation than that of the straight channel |

$L=$ channel length; $W=$ width; $EC=$ entrance channel; $OC=$ outlet channel; $DC=$ daughter channel width; $D=$ depth; $RG=$ Research Group (Blood quality); $D^*$=major dimensions (μm); $ST=$ separation techniques; $SC=$ separated components; $Hct=$ hematocrit, Hct (%); $Q=$ flow rate (μL/min), $R=$ recovery (%)
(Zweifach–Fung effect) for up to 45% Hct of blood. The methods used by their study are analytical (based on electrical circuit analysis), experimental and numerical (using CFDACE). In general, the T-microchannel consists of three branches: a whole blood inlet, a concentrated blood outlet and a plasma outlet (Fig. 3a). This study, in addition to a single T-channels, also looks at the performance of five plasma-skimming channels included in the incoming blood sample channel (Ref. Yang et al. 2005 for actual figure). In the analytical analysis, since both outlets are exposed to the atmosphere, the blood plasma separation device has been treated as an equivalent electrical circuit, with the inlet flow rate of the whole blood acting as the current source, and each channel of the plasma skimming channels being modelled as an electrical resistor (Yang et al. 2005). The analysis uses the fundamental law of flow, i.e., flow equals the ratio of the driving force (pressure drop) and the resistance (electrical resistance), and thus, the flow resistances were controlled at each bifurcation of plasma skimming, in order to control the flow rate ratio at each bifurcation of the channel (i.e., plasma skimming channel). The study reports that, in order to form a critical separation streamline (the line that demarcates the blood component and plasma segregation region; for a 15 μm wide channel, it occurs at 1 μm) between the main and plasma channels, it is necessary to maintain $Q^*$ between them as 14:1. Similarly, to achieve a 14:1 current ratio, it is required to maintain the main channel resistance as 210 times smaller than that of 9.6 μm wide plasma channel. For the numerical solution, blood with 39% Hct was assumed to behave as a non-Newtonian homogeneous fluid. The numerically simulated results showed three major key results: (1) as Hct decreases, the mass flow rate of the main channel increases, (2) as the blood moves in the separation device, the plasma skimming (by volume %) decreases, (3) the volume percentage of the plasma skimming drops with the blood hematocrit. This is due to the fact that, in plasma channel, the plasma skimming and the mass flow rate have a direct dependence on each other. This agrees with the observations of the study for the two hematocrits of 45% and 39% where a plasma recovery of 27 and 25% were noted, respectively.

Further, Yang et al. (2006) extended their work to introduce a microfluidic device for continuous and real-time blood plasma separation, consisting of a blood inlet, and two outlets, one each for plasma and concentrated cell as shown in Fig. 3a. A similar geometry with same channel dimensions and separation technique, as discussed above in case of Yang et al. (2005), was adopted. They demonstrated that their device could, regardless of the blood dilution, achieve ~100% of plasma selectivity using image analysis. They found that 15–25% plasma separation (by volume %) was achievable as the inlet Hct increases. Their study used a device wherein the main channel dimensions were 35×35 μm² wide and deep, and the two daughter branches had appropriate width to maintain $Q^*$ as 1:1 and 8:1. The effect of particle size (Type-I: 16 μm diameter fluorescent particles and aspect ratio of 0.46; Type-II: 8–10 μm human C8161 melanoma cells) on the separation efficiency, SE was also studied. They observed that Type-I particles achieved SE of 87.2% with a $Q^*$ of 2.5:1, however, if one needs to obtain 100% efficiency for the same $Q^*$, a particle diameter of 20 μm is required. Further, by modulating $Q^*$ as 4:1, 6:1 and 8:1, they achieved a SE of 95.68, 100, and 100% using Type I particles. Type-II particles gained a SE of 88.7, 98.9, 100, and 100% with $Q^*$ as 2:5:1, 4:1, 6:1, and 8:1, respectively. The two important observations from the above analysis are as follows: (1) if one uses a $Q^*$ = 6:1, 100% SE is achieved with either particle size; (2) smaller particle size achieves a higher SE. In broad terms, $Q^*$ greater than or equal to 6:1 provides 100% SE. Based on these observations, and $Q^*$ = 8:1 for T-channel geometry (Fig. 3a), the critical separation streamline placement was found to be at 1.67 μm from the main channel wall (15 μm wide). As discussed earlier, the electrical circuit analysis was performed to obtain the optimal flow resistance ratios. An analytical study was done to choose the optimum channel dimensions, i.e., flow resistance ratio requirement (Yang et al. 2005). In overall terms, the appropriate choice of the channel dimension with flow rate ratio variation in the range of 8:1 to 9:2:1, leads to 100% separation with up to 25% plasma yield.

Moreover, Jaggi et al. (2007) experimentally observed the separation of RBCs from whole blood using high volumetric flow rates (i.e., 2–5 mL/min) in high aspect ratio microchannels using hemodynamic effects, detailed in Sect. 2, and Table 1. Such a microchannel system has a direct application as it imitates the blood flow behaviour in a living organism. Their experimental analysis used an “inverted” T-channel consisting of one inlet and two outlets, as shown in Fig. 3a. In this inverted T-channel, the collection channel extended horizontally at a particular length. The channel width chosen by Jaggi et al. (2007) as 20 and 50 μm is much higher than the previous works (Yang et al. 2005, 2006). Such a choice of high aspect ratio was made herein to enhance the plasma yield. A wide range of fixed volumetric flow rates (i.e., ratio of collection channel, i.e., RBC outlet versus feed/blood sample channel, Fig. 3a) of 0.01 < $Q^*$ < 0.18 along with various Hct ~ 4.5 to 45% were studied, Table 1. The model reported by Fenton et al. (1985) was used here to understand the cell depletion process in the microchannel. It was observed that by fixing $Q_0$ and depth as 2 mL/min and 20 μm, respectively one could get a SE of 52 and 37% for 15 and 45% Hct, respectively. On the other hand, for the T-channel with 50 μm depth, SE decreases up to 42 and 17%, respectively. Thus, suffice is to say here that SE decreases with channel depth. Another key message from their study is that at a fixed channel depth, SE increases significantly with
an increase in $Q_0$ for low Hct. In contrast, for high Hct, SE decreases with $Q_0$, although with a weak effect. They also reported that a single bifurcation is generally more effective in plasma quality and yield for low flow ratios ($Q^* < 0.2$). Also, the cascading series of the single bifurcation channel could work better for some specific conditions.

Furthermore, a three-dimensional T-microchannel designs (almost similar to Fig. 3a T-channel), has been numerically used by Huang et al. (2010) for the plasma extraction from whole blood. They designed four different types of three-dimensional T-microchannel designs, with converging and bending channels, namely, Type I, II, III, and IV (Huang et al. 2010). Important details of this work are presented in Table 1. They also presented the similar channel depth as exploited by Jaggi et al. (2007). These geometries were different to each other on the basis of difference in the inlet channel structure along with backward facing for the plasma extraction from whole blood sample (Huang et al. 2010). They also used a converging inlet channel to enhance the flow velocity at the bifurcation point. A T-microchannels design incorporated with a bend/curved ($R_1 = 455 \mu m$, Table 1) inlets were also used herein in order to introduce the centrifugal forces to enhance SE as discussed above. They also presented details on SE and plasma extraction flow rate. In the simulation, the inlet Reynolds numbers were chosen from 1:2 which corresponds to the inlet flow rate ratio as 1:20. Their study decided the separated plasma quality on the basis of minimum radius of cell passing through the plasma channel outlet, see Fig. 3a. Here, the critical particle size separating from the devices has been fixed as 1 μm as the platelets has the smallest radius in between all blood components. The details of channel dimensions can be found in Table 1. Their results show that the converging channel design is more efficient in separating the particles of smaller size. Also, the introduced centrifugal force through the bending channel enhances SE. Thus, it was found that the combination of the bending and converging channels, i.e., Type IV improves SE as it allows to achieve the lowest values of particle radius and highest values of the fraction of the volumetric flow rate at the plasma outlet.

Later, an elevated T-microchannel geometry has been experimentally studied by Tripathi et al. (2013), Fig. 3a using hemodynamic separation, basically Zweifach–Fung bifurcation law as discussed in Sect. 2. They choose the elevated dimensions, Table 1, to ensure that there is no clogging in the microfluidic device but with the same channel depth as used by Jaggi et al. (2007). They elucidated (for the first time) the applicability of the bifurcation law to the high aspect ratio channels (in mm, Table 1 and channel dimensions much higher than that of the particle size), Ref. (Jaggi et al. 2007). Their device has been fabricated using PDMS, which is a biocompatible material. They also reported various features of plasma separation in a T-channel. The study also introduced the 2-stage (includes two plasma channels as outlets) and multiple plasma channel designs to increase SE with undiluted blood (~45% Hct) (Tripathi et al. 2013). The effect of flow ratio, main channel width and feed Hct on blood plasma separation was analysed. A plasma SE of ~100% was reported at high $Q_0$, Table 1. Notably, SE achieved with the 2-stage T-channel is 67 with 45% hematocrit (i.e., whole blood) using main channel width as 400 μm and plasma channel as 100 μm with $Q^*$ of 54:1. Also, for the fabricated devices, $Q^*$ ranges from 8:1 to 108:1. This work showed a better SE than Yang et al. (2006) and Jaggi et al. (2007). The study reported that if the main channel width is small, the lower flow rate ratio leads to a better plasma recovery, and also showed that a 2-stage T-channel leads to ~100% SE for low Hcts of up to ~24%. However, they reported that in a single bifurcated T-shaped microchannel, SE decreases with an increase in Hct, see, Table 1. Also, they reported a yield of 1.81%. They further extended their work for a cascading T-channel and reported a better SE as 67% for Hct = 45%.

Furthermore, Tripathi et al. (2015a, b) extended their work to improve SE and reported three different T-channels, similar to Fig. 3a including T-200/100 (width of main and plasma branches are 200 and 100 μm, respectively), T-400/100, and T-channel cascading, Table 1. The design named T-200/100 was more effective than T-400/100 in separating plasma for a fixed Hct and $Q^*$, i.e., the flow rate ratio in the main blood channel inlet to plasma outlet. They reported a 2% yield using T-200/100 microchannel at $Q_0$ of 0.4 mL/min. Thus, it can be said that for a fixed plasma channel width, Hct, and flow rate ratio, a better SE can be achieved with a smaller inlet channel width than a larger one. Such analysis influenced them to analyse a cascading T-channel, consisting of two bifurcations with different plasma channel width as 100 and 60 μm. Thus, two T-microchannels in series, i.e., T-200/100 and T-100/60, were used to fabricate the T-channel cascading. A better performance in terms of SE of T-cascading design compared to the single T-microchannel was observed in this work, detailed in Table 1.

Furthermore, Madureira et al. (2018) extended the work of Tripathi et al. (2013) for higher channel dimension for the separation of RBCs (Ovine RBCs and dextran 40) for a 5% Hct. As previously reported (Jaggi et al. 2007; Yang et al. 2005, 2006), the microfluidic devices, i.e., straight T-channel (Fig. 3a with a constriction region close to the junction) and curved T-channel studied consisting of an inlet upstream channel contraction. Also, all microchannels length as 1 cm and inner diameter as 1 mm for curved channel. Image analysis has been used as the counting method. The key focus of this study is that a straight and curved T-shaped geometry is compared in terms of SE using the similar $Q_0$ as 150 μL/min. They experimentally observed the curvature effect on
cell free layer (CFL) and SE. It was reported that no CFL was observed in the upstream channel, while it can be clearly seen at the right wall of outlet 1. Also, the curved geometry is seen to enhance CFL thickness and hence the RBC separation, as the introduced centrifugal force moves the cells farther away from the wall. They also mentioned that even curved channel is not sufficient for the complete separation of RBCs from plasma with higher channel dimensions. They discussed about the possibility of decreasing channel dimension for better SE.

Thus, based on the above discussions, suffice it to say here that the T-channel geometry leads to a better plasma extraction ~ 100% SE if one chooses a significantly modified geometry in terms of channel dimensions, the inclusion of curved/bend channels and converging channel for a specified parameter such as $Q^*$, Hct, etc. For a better understanding of the performance of T-shaped microchannel, some critical remarks of the above-reviewed articles have been tabulated in Table 1. Further, Fig. 4 demonstrates the obtained SE with respect to Hct from various research articles using the multiple T-shaped microchannel designs as a blood plasma separation device.

Over the years, it can be noted that approximately 100% SE has been achieved only by Yang et al. (2006) and Tripathi et al. (2013) using a T-channel microfluidic device for low Hct ≤ 35%. Interestingly, both have utilized more than one bifurcation in their plasma channel device but with a different plasma channel width. Also, it can be seen from Fig. 4 that the T-channel has been widely exploited to separate whole blood (i.e., Hct ~ 45%) by many researchers (Jaggi et al. 2007; Tripathi et al. 2013; Tripathi et al. 2015a, b; Yang et al. 2005). The maximum SE as 67% for Hct = 45% using a two stage T-channel device has been reported by Tripathi et al. (2013). It was concluded from these studies that elevated channel dimension, which confirms no cell lysis; can be a better choice for such separation process (SE=92% for low Hct) at a fixed $D=50 \, \mu m$ (Jaggi et al. 2007). By Having $D=20 \, \mu m$, SE is improved by 37% with no cell lysis but at low $Q_0$. The maximum SE as 67% for Hct = 45% using a two stage T-channel device has been reported by Tripathi et al. (2013). An improved SE achieved in Tripathi et al. 2013, even for Hct=45%, may be attributed to the large difference, i.e., ~4 times between the main and plasma channel width. This considerable difference in channel dimensions may induce a substantial resistance in the plasma channel for the movement of RBCs in that channel; hence, gained better separation. Thus, suffice it to say here that the channel dimension plays a vital role in SE.

### 3.2 Y-channel

Figure 3b shows a simple representative schematic of the Y-channel, found useful by many researchers for the blood plasma separation (Yang and Zahn 2004; Hymel et al. 2019; Li et al. 2020), detailed in Table 2. The microfluidic device has one inlet along with two outlets, similar to T-channel, Fig. 3a which has an inclination angle of $\theta=90^\circ$ but Y-channel has a different $\theta$. Firstly, Table 2 presents experimental and numerical observations by Yang and Zahn (2004), for the separation of particle and melanoma cells (human C8161 melanoma cells of size 8–10 μm diameter) through Y-channel, see Fig. 3b. They modified the simple Y-channel, Fig. 3b in terms of including two different channel widths in one of the daughter branches to modulate $Q_0$. They explored a wide range of flow rate ratios and reported SE for them mentioned in Table 1. The separation was based on the Zweifach–Fung effect because of the presence of the bifurcation in the cell separator geometry, see, Fig. 3b. Due to such effect and modification in the geometry, RBC choose to travel to the daughter branch, which has a higher flow rate, as discussed in Sect. 2 (Yang et al. 2006). In this work, the inlet channel has a dimension of $35 \times 35 \, \mu m^2$, while the two outlet channels have different dimensions to introduce the range of flow rate ratios, as discussed above. To ensure that the cells are alive throughout the experiments, these were conducted within 15 min of trypsinization. Numerical simulations were also performed using COMSOL and CFD Research Corporation software. They observed that for $Q^*=4:1, 6:1$ and $8:1$, and for a wide range of the cell diameter to channel width ratio (0.23–0.29), SE was ~98.9%, i.e., 297/300 cells choose to travel to the high flow rate branch, see Fig. 12b.

Later, Hymel et al. (2019) studied the cell sorting and isolation in a Y-shaped junction, (slightly different from Fig. 3b) numerically as also reported by Yang and Zahn (2004). They used the three-dimensional Y-channel for the

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**Fig. 4** Comparison of various T-channel geometries in terms of hematocrit and separation efficiency (%). [1]: Jaggi et al. (2007); [2]: Tripathi et al. (2013); [3]: Tripathi et al. (2015a, b); [4]: Yang et al. (2005); [5]: Yang et al. (2006)
| Research groups, RG | Channel dimensions, $D^*$ (μm) | Separation techniques, ST | Separated components, SC | Hematocrit levels, Hct (%) | Flow rate, $Q_0$; $Q^*$ (μL/min) | Separation efficiency, SE (%) | Remarks |
|---------------------|---------------------------------|--------------------------|--------------------------|----------------------------|-------------------------------|-------------------------------|---------|
| Yang and Zahn (2004) (human melanoma cells) | $L_E = 35$, $L_P = 200$, $L_B = 200$; $W_E = 35$ \(\theta = NA\) | Zweifach–Fung effect | Cells | 45% | 2.5:1, 4:1, 6:1 and 8:1 (flow rate ratios) | 88.7% (with 2.5:1), 98.9% (with 4:1), 100% (with 6:1), and 100% (with 8:1) at Hct=45% (yield = 15–25% at Hct=45%) | In the analysis of a wide range of the flow rate, a critical flow rate exits which leads to better separation |
| Hymel et al. (2019) | $L_E = 140$, $L_P = 210$, $L_B = 210$; $W_E = 70$, $W_P = 35$, $W_B = 35$; $D = 70$ \(\theta = 30°–180°\) | Inertial microfluidics | RBCs | NA | 14.29 cm/s, 1.43 cm/s (flow velocities) | NA | Studied the effect of flow velocity, cell diameter, elasticity, etc. on the deformable cells trapping efficiency using Y-shaped microchannel |
| Li et al. (2020) | $L_E = 40$, $L_P = 50$; $W_E = 15$, $W_P = 15$, $W_B = 9.6$; $D = 70$ \(\theta = 30°–180°\) | Hybrid method, SDPD-IBM | Plasma | 5.2%, 7.8%, 13%, 15.6%; | 13.3 μL/min | 100% (Hct = 5.2%); 100% (Hct = 7.8%); 64% (Hct = 10.4 with multiple bifurcation) | They studied the effect of single and multiple bifurcations and found 100% plasma purity for a specified flow rate |
computational domain. In their study, they used cell diameter, the initial separation distance between the two particles in the flow direction, i.e., $X$; and the initial offset distance of the cell’s centre from the centreline, i.e., $Z$ as a parameter to study its influence on the blood plasma separation. The effect of bifurcation angle, $\theta$ (from $30^\circ$ to $180^\circ$, Fig. 3b) on SE was analysed based on the inertial focusing in the microfluid device. The channel dimensions with other important details are presented in Table 2. The analysis was carried out using a single cell or two spherical shapes under the flow condition. The work aims to find out the critical lateral and longitudinal cell-to-cell separation distance for the effective entanglement of RBC cells. Their results show that a longer time is needed for a larger particle to accelerate to one of the daughter branches. Also, as the distance between the cells increases, a longer time is required for the above-discussed reason to approach a fixed flow velocity (14.29 cm/s) and cell elasticity (1000 Pa).

Further, they observed that the critical separation distance, $Z_{crit}$ decreases with the bifurcation angle for $\theta \leq 120^\circ$ and, it starts to increase for $120^\circ \leq \theta \leq 150^\circ$. They also observed that $Z_{crit}$ is higher for the sharp tip than the curved tip; also, for a fixed shape, it decreases with bifurcation angle till $\theta = 180^\circ$. Their results suggest that a junction with a sharp and rough tip would be inefficient in cell sorting. This is because of the high probability of the cell collection at the junction which prevents such cell sorting. Hence, SE gets suppressed. Further, a higher trapping efficiency was observed for RBCs at $\theta = 120^\circ$. A similar analysis has been done here for two identical and non-identical cells. A conclusion has been made here for identical cells with a curved tip junction, the critical offset distance is slightly reduced at $\theta < 120^\circ$ and shows no influence for $\theta \geq 120^\circ$. In general terms, a more considerable separation distance is required to move the different cells into various Y-channels branches, which leads to a lower trapping efficiency. The numerical model also predicts that the trapping efficiency significantly enhances at $\theta = 120^\circ$. It has also been predicted here that Y-shaped microfluidics gives better performances in terms of the cell sorting for the cells that differed in cytoplasmic viscosity, cytoskeletal shear elasticity, cortical tension, and/or size.

Recently, Li et al. (2020) numerically investigated the highly efficient blood plasma separator device using SDPD-IBM. In the microfluidic device, they used a single bifurcation such as Y-shaped channel at different inclination angles, $\theta$ as well as multiple bifurcations, Fig. 3b. As reported in the work of Hymel et al. (2019), to optimize the flow parameter and bifurcation angle, $\theta$, the study first numerically investigated a single bifurcation as an important component of the studied device. SE of the device was compared against the experimental one and reported a higher than that. Different flow rate ratios, $Q^* = 1, 2.5, 4$ and 6 were studied herein. The key results of their work are as follows: The study showed that for $Q^* = 1$; the plasma and blood distribute themselves equally to the daughter branches, as shown in Fig. 3b. Further, for $Q^* = 2.5$ and 4, they found lower separation efficiency than the experimental results with a deviation of 4%. This may be attributed to the different quality of RBCs used in the experiments and the simulation. Further, at $Q^* = 6$, they mentioned 100% SE, as also reported by the experiment. The study also reported 100% plasma purity for Hct ≤ 10.4. Moreover, the effect of the inclination angle, $\theta$ (as shown in Fig. 3b) is reported as follows: in general, for a given $Q^*$, the plasma extraction either increases with an increase in the branch angle or remains almost similar. For instance, at $Q^* = 2.5, \theta = 120^\circ$, a 100% purity is obtained but this has not been observed at $\theta < 120^\circ$. Moreover, at $Q^* \geq 4$, 100% purity is achieved for all inclination angles. Furthermore, for $Q^* = 1$, the extraction of plasma in terms of purity is not 50% for either of $\theta$. This may be attributed to the narrower plasma outlet, which restricts the movement of RBCs due to the lower flow rate of the plasma outlet (Hyakutake and Nagai 2015; Pries et al. 1990; Ye et al. 2019).

The above-discussed results belong to the single bifurcation channel. This was also extended for the multiple branches and again analysed the effect of $Q_0$ and Hct (5.2%, 7.8%, 13%, 15.6%; at the initial stage, which corresponds to 2, 3, 5, and 6 RBCs in the inflow domain). Further, they adjusted the flow rate in this device using the externally applied gravitational accelerations for a wide range such as from 162 to 263 in the inflow domain, see Fig. 3b. The acceleration generates a wide range of flow rates in the inflow. Results show that as Hct increases, the SE first rises and then starts decreasing. The maximum purity of 100% is achieved at Hct = 10.4%. Also, the plasma purity remains 100% for Hct < 10.4%, but decreases with higher Hct. Furthermore, 100% purity can be found at a flow rate of less than 13.3 μl/h with 64% SE. Thus, based on the above discussion, it was concluded that a combination of high aspect ratio geometry and high bifurcation angle (i.e., $\theta = 120^\circ$) with an average flow rate ratio of 6:1 or 8:1 results in a better plasma separation ~ 100% with 15–25% yield for Hct = 45% for sheep blood (Yang et al. 2006; Yang and Zahn 2004; Hymel et al. 2019; Li et al. 2020). Eventually, in conclusion, from the Table 2, it can be said here that a 100% separation can be achieved by exploiting the Y-channel geometrical configurations for both diluted/whole blood.

3.3 Trifurcation geometry

Over the years, the trifurcation geometry has also been exploited by many researchers in separating blood and plasma. Figure 3$c$ represents a basic trifurcation geometry which consists of one inlet and three outlets. In general, the trifurcation geometry consists of two different daughter
branches alongside the main channel, and uses some external force to drag RBCs at the central of the domain. Hence, the separated RBCs gets collected from the main channel outlet while the plasma is extracted alongside the channel edges, while the RBCs are extracted through the centre of the domain, i.e., main channel. Through a rigorous literature review, important information related to the trifurcation geometry has been summarized in Table 3.

The discussion of trifurcation geometry starts with the work of Han and Frazier (2006). They studied experimentally, single-stage and three-stage cascade micro separators in terms of a trifurcation geometry, based on paramagnetic capture (PMC). They used magnetophoresis as a separation technique and its basic principle can be found somewhere else (Alnaimat et al. 2018). The separation process was based on the fact that the magnetophoretic velocity depends on the particle size and magnetic susceptibility. Thus, the separation process depends on the magnetic properties of the blood cell components (paramagnetic RBCs and diamagnetic WBCs). They have specified SE of RBCs for diluted whole blood (1:10) for both single-stage and three-stage PMC micro separators as 91.1 and 93.5%, respectively, at a flow rate of 5 μL/h. They have also mentioned SE of WBCs from whole blood using a three-stage PMC micro-separator as 97.4% at a similar flow rate. To confirm the choice of the PMC mode of the device, they did a theoretical analysis with a rectangular ferromagnetic wire.

Furthermore, an active separation technique as acoustophoresis has been used by Lenshof et al. (2009) for the plasma extraction in a trifurcation geometry as shown in Fig. 3c. The details of the separation technique can be found somewhere else (Wu et al. 2019). Here, the device consists of one inlet and five outlets, a diamond shape outlet, named as A, B, C, D, and E with four channel designs, namely, 1, 2, 3, and 4 M; and different channel lengths as 56, 108, 166, and 224 mm, respectively. The details of the dimensions are presented in Table 3. This geometry has been included in this section as at the channel end, there is basically three outlets, one for RBC extraction while the other two outlets for the plasma get merged to form a single outlet. All the devices such as 1, 2, 3 and 4 M were actuated with a transducer at a frequency of 2.05 MHz and net input power ~300 mW as the active separation technique has been chosen in this work. Each outlet flow has been fixed as 20, 20, 15, 15, and 10 μL/min with a net $Q_0$ of 80 μL/min. The first two outlets have been maintained at high $Q_0$ for quick removal of the high blood concentration, leading to decreased Hct levels. This device demonstrates yield of high plasma (contains less than $6 \times 10^9$ RBCs/L) with low blood cell content. They used complex geometry, which included a trifurcation outlet, wherein the blood was extracted from the channel centre, and plasma from side branches. Herein, it can be concluded that for low Hct such as 10%, 1, and/or 2 M device results in a 100% SE.

However, it must be noted that separation efficiencies are only relative to the total amount of particles processed per minute, which means that there can still be a relatively high number of cells present in the plasma fraction even though SE is close to 100%. But as the blood concentration increases, these two devices with short channel length start losing blood cells into the plasma outlet, hence SE becomes low. The low SE tendency increases with a further increase in blood concentration; however, for these conditions 3 and 4 M devices with higher channel lengths were used, and they were able to achieve ~100% SE. Thus, one can choose any of the described devices according to the required purification of the low and/or high Hct blood. Further, they checked the device efficiency with high Hct, i.e., 40% (whole blood), and concluded that the better SE at high Hct as 40%, can be achieved by using 3 or 4 M devices with longer channel lengths in comparison to 1 and 2 M device with short channel length which gives SE as ~96.2% and ~97.7, respectively.

Further, Kersaudy-Kerhoas et al. (2010a) experimentally investigated the RBC separation from plasma using biophysical effects, i.e., Zweifach–Fung effect discussed in Sect. 2 and geometric details has been summarized in Table 1, through trifurcation device, as shown in Fig. 3c. They worked on two blood samples, mussel and human blood, and analysed the effect of variation of laminar flow rate and geometries. The device has been designed for both the low and medium Reynolds numbers varying $Q_0$ as 0.01 and 1 mL/h, respectively. The study varied the flow rate from 1 to 30 μL/h in this work while their device can sustain up to 100 μL/h. Most of the experiments done in this work used an average $Q_0$ of 10 mL/h, which is notably higher than earlier works (Yan et al. 2006; Faivre et al. 2006). Also, the main channel includes a constriction section, as shown in Fig. 3c, which introduces a high shear stress causing the cells to migrate to the centre of the channel, hence, achieving a better SE. Also, the choice of the microchannel ensures the presence of laminar flow in the device and a particle-free layer. To enhance plasma SE, the device includes twenty bifurcation branches on each side of the main branch. Thus, the flow rate ratio has been varied in such a way that no plasma will pass through the centrally placed branch of the device. Interestingly, a discussion of drift induced by constriction has also been done using Ref. (Faivre et al. 2006). It is also interesting to note here that the capillary length ratio variation of plasma outlet capillary and cell outlet capillary, named, outlet length ratio (OLR) will also affect SE. Thus, it has also be chosen here as a parameter. The constriction introduced in the channel design is expected to enhance SE. But it did not work well as expected even
| Research groups, RG     | Channel dimensions, \( D^* \) (μm) | Separation techniques, ST                  | Separated components, SC | Hematocrit levels, Hct (%) | Flow rate, \( Q_0 \) (μL/min) | Separation efficiency, SE (%) | Remarks                                                                 |
|------------------------|-------------------------------------|--------------------------------------------|--------------------------|-----------------------------|---------------------|-----------------------------|--------------------------------------------------------------------------|
| Han and Frazier (2006) | \( L_{BG} = 30,000 \), \( W_{BG} = 360 \), \( D = 50 \) | Magnetophoresis                           | RBCs and WBCs            | 10%                         | 0.083               |                             | 91.1% RBCs with single separator; 93.5% for RBCs and 97.4% for WBCs with three-stage separator | Worked on single PMC and three stage PMC micro separator |
| Lenshof et al. (2009) | \( L_{BG} = 56,000 \) (1 M), \( L_{BG} = 108,000 \) (2 M), \( L_{BG} = 166,000 \) (3 M), \( L_{BG} = 224,000 \) (4 M); \( W_{BG}, W_p, W_B = 350 \), \( O_h \approx 233 \), \( D = NA \) | Acoustophoresis                        | RBCs                      | 10, 20, 30, 40% | 80                  |                             | ~100% for all Hct values                                                   | Tested four different designs using acoustophoresis and finalized one which worked well with high Hematocrit as 40% (i.e., whole blood) |
| Kersaudy-Kerhoas et al. (2010a) (mussel and human blood) | \( L_{CR} = 300 \); \( W_{EL} = 100 \), \( W_B = 20 \), \( W_{CB} = 25 \); \( D = 20 \) | Geophysical effects, experimental; Zweifach-Fung bifurcation law | Plasma                    | \( \leq 3\% \)     | 167                  |                             | 76.3% (for mussel blood); 53% (for human blood) at Hct \( \leq 3\% \) (yield = 40%) | A constriction channel has been used here to induce high shear stress resulting in the movement of cells towards the centre of the channel |
| Xue et al. (2012)      | \( L_{BG} = 7000 \); \( L_{CR} = 300 \); \( W_{BG} = 100 \), \( W_p = 20 \), \( W_B = 10 \), \( W_{CB} = 25 \); \( D = 20 \) | Bifurcation law                         | RBC and plasma            | 45%                         | 1.2                 |                             | 91.23% at Hct = 45%                                                      | The effect of inlet and outlet boundary conditions has been highlighted |
| Kaun et al. (2018)     | Detailed in text (not mentioned here for the sake of conciseness) | Bifurcation law, cross-flow method, hydrodynamic flow | Plasma, RBC, WBC          | 1 (PBS buffer) | 0.3                 | NA                          | Discussed about the simultaneous extraction of RBCs, WBCs and plasma     | |
| Gonzalez et al. (2020) | \( L_{BG} = 50,000 \); \( W_{BG} = 700 \), \( W_B = 230 \); \( D = 700 \) (for entrance), 230 (for blood and plasma outlets) | Acoustophoretic                        | Plasma                    | 2.25, 4.5, 9.0, 22.5, 45% | 0–80                |                             | 90% at Hct \( \leq 9\% \)                                                | Studied the combined effect of hydrodynamics and hematocrits |
though the Reynolds number in this work is less than one (~0.69). This is attributed to the fact that the channel bifurcations can cause a force to act on the RBCs and drag them back towards the main channel walls. In general terms, the present work used $Q_0$, as 0.167 mL/min for low Hct as 3%. SE has been noted as 76.3% for mussel blood and 53% for human blood for such flow parameters. The plasma purity was observed to be 40%. Later, Kersaudy-Kerhoas et al. (2010b) extended their work for the extraction of DNA on-chip using extracted plasma exploiting PCR and gel electrophoresis using the same trifurcation geometry (Fig. 3c). In this work, they included several constriction regions, which enhances the blood plasma separation before the bifurcation region. The plasma purity was increased to ~100% at $Q_0$ of 10 μL/min, but the yield gets suppressed up to ~8%. They reported SE = 99% for plasma for Hct ~45% using a hybrid design consisting of a series of networks of constriction expansion. They exploited bifurcation law, Fahraeus effect and high flow rates herein.

Subsequently, a similar type of trifurcation geometry as reported by Kersaudy-Kerhoas et al. (2010a, b) but with different dimensions (Table 3), has been used by Xue et al. (2012). They numerically investigated the effect of microchannel design, a trifurcation geometry, on the bulk flow behaviour of blood, and optimized the channel design for better SE. They assumed blood as a single-phase Newtonian, incompressible, and homogeneous fluid. The variation in cell concentration with the number of bifurcations used in the geometry, was discussed, as shown in Fig. 3c. The effect of inlet and outlet boundary conditions on the flow rate ratios of the main and daughter channels, was also studied, in three particular designs of the trifurcation geometry (1) push design ($Q_0 = 72$ μL/h; equal exit pressure at all branch outlets) (2) pull design (pressure-driven, inlet pressure = 0 Pa, main branch outlet pressure = −68 Pa and side branch outlet = −68 Pa) (3) push design with varying outlet pressures ($Q_0 = 72$ μL/h, main branch outlet = 0−35 Pa and side branch outlet = 0 Pa). Thus, the flow rate ratio as a function of the outlet pressure drop was studied, as it was expected that varying the pressure drop could maintain the better flow rate ratio across all the channel branches. They optimized the channel design through theoretical analysis. The study demonstrated a relationship between the particle concentration at the bifurcation in the main channel and the inlet and flow rate ratio. In general, the geometry consists of an inlet, a constriction to accelerate cells towards the centre of the channel using the viscous force, i.e., inertial focusing, one cell outlet, and two plasma outlets, which has been fabricated on a planar substrate with 7 × 10 mm² (Xue et al. 2012).

In this study, a high aspect ratio of the channel length and cross-section is chosen by Xue et al. (2012). The introduced constriction results in cell-depletion layers, which reduces the cell concentration in the constriction region when compared to the main branch. Thus, the flow viscosity also reduces in the constriction section. The choice of constriction would help in the separation process as the cell movement would be faster than the surrounding fluid because of the two reasons: (1) the same number of particles would be placed on either side of the channel constriction (2) as fluid enters the constriction region, the velocity gradients become sharp. Two characteristics of the microchannel geometry were also identified by the study: (1) large surface to volume ratio of the cross-section (2) high aspect ratio of the length to the cross-section. Thus, a high-pressure drop is required to drive the flow through the device if the wall surface to flow volume, and channel length to cross-section increases. This is because their two-channel design enhances the flow resistances, hence the low flow velocity through the device. In this work, it was concluded that for all three designs, a higher flow rate ratio than the required one, i.e., an 8:1 ratio, has been achieved at each bifurcation (Yang et al. 2006). But the flow rate ratios obtained from design (2) are 8–10% less than what was predicted by design (1). Further, by using design (3), an increase in the flow velocity magnitude could be obtained than design (1), but no significant change in the difference in flow velocity between each side branch channel was observed.

A slightly different trifurcation geometry than traditionally in use as shown in Fig. 3c and used in the Refs. (Lenzhofer et al. 2009; Kersaudy-Kerhoas et al. 2010a, 2010b), has been used by Kaun et al. (2018) to analyse experimentally the simultaneous isolation of RBC, WBC, and plasma from whole blood. The device consists of a blood inlet, a buffer inlet followed by a bifurcation region, which leads to three outlets, namely, RBC, WBC, and plasma outlet. They fabricated a microfluidic device which exploits the cross-flow method, bifurcation law and hemodynamic effect for the simultaneous separation of RBCs, plasma and WBCs. They have also tabulated the state of the art regarding the active and passive separation methods. Under the buffer inlet, the study chooses the plasma channels dimension smaller than RBCs ($d_p = 6.2 ~ 8.2$ μm) and WBCs channel dimension ($d_p = 2 ~ 2.5$ μm). All the side branches have been tilted with a 60° angle relative to the main branch to maintain a uniform flow pattern. A series of hemodynamic based WBC trapping unit has two inner geometries as rectangular and triangular (Kaun et al. 2018). The microfluidic device has the following dimensions: $W_{EL} = 20$ (main channel), 3 (neck channel) 23.5 (side channel) 30 (gap); slit distance between triangle-rectangle (rectangle-rectangle) = 2.5 (10) μm; rectangle edge length = 30 μm; row-to-row distance = 60 μm; offset distance = 10 μm. However, the study confirmed successful separation of the three blood components through simulation, also, the extracted plasma has a low dilution.
factor (i.e., 0.755x) and a low hemolysis effect. The device performances for RBC and WBC trapping (~1800 WBCs in 20 min) were tested and it was identified that the device could process a low volume of whole blood such as 6 μL without requiring any external force such as electrical force, magnetic force, etc.

Recently, the trifurcation geometry, as shown in Fig. 3c, has also been used by Gonzalez et al. (2018) for the isolation of rare tumour cells from WBCs blood using ultrasonic separation slightly different than others (Kaun et al. 2018; Lenshof et al. 2009; Kersaudy-Kerhoas et al. 2010a; b; Xue et al. 2012), Fig. 3c, also detailed in Table 3. It shows SE greater than 80% at \(Q_0=80\) μL/min. Most recently, the geometry has been chosen by Gonzalez et al. (2020), and they introduced a new separation technique for plasma extraction, such as an ultrasonic standing wave. In general, the geometry includes one inlet and three outlets, Fig. 3c. They used the heparin-treated blood samples taken from a healthy individual and acoustophoretic as a separation technique (Lenshof et al. 2009). Gonzalez et al. (2020) performed experiments in a glass capillary with a frequency of 1.153 MHz, which results in a 2-D orthogonal standing wave of half-wavelength to the channel length (Gonzalez et al. 2020). The outlets dimensions attached at the channel end are 230×230 μm². The single pressure node is developed at channel centre axis, promoting the cell exit by the radiation force while the clear-plasma exits from the channel outlets close to the walls. The two significant forces influencing the separation process are the primary and secondary acoustic forces. A 90% recovery of plasma for low Hct (≤9.0%) in a short time of 10–12 s for the wide range of the optimum flow rate of 20–80 μL/min was reported. They also observed that for a flow rate ratio of 1:20, plasma recovery increases with time up to some particular value. Beyond this, the ultrasonic time of actuation does not affect SE regardless of \(Q_0\). Also, for the effective time range, a higher plasma separation can be achieved in less time with a high \(Q_0\). Further, if the flow rate ratio reduces to 1:2, only ~55% SE can be achieved, regardless of \(Q_0\). It can also be noted that the microfluidic device is ineffective for high \(Q_0\) and high Hct’s for the time range of 22 s. This is because the time of cell circulations within the channel provided by the hydrodynamics must always be longer than the drift time for a good SE. Here, the separation time can be reduced by cascading of the capillaries.

A graph representing SE studied in every case of trifurcation geometry with respect to Hct has been plotted, see Fig. 5. It can be observed that SE of 100% has been obtained by only one researcher using such geometry (Lenshof et al. 2009). It can be noted from Fig. 5 that to separate the whole blood, the trifurcation geometry has been used only by Xue et al. (2012). On the other hand, the researchers mostly utilized it to separate diluted blood, i.e., Hct ≤ 10% (diluted blood). Thus, a wide range of Hct is still open for exploration in the future. Also, all the reported studies on plasma separation are experimental except a numerical study by Xue et al. (2012). Thus, there is a scope for numerical studies using trifurcation geometry. Moreover, a high aspect ratio microchannel (similar to Tripathi et al. 2013) and a high flow rate ratio, i.e., \(Q^*\) (similar to Yang et al. 2006) has been used by Xue et al. (2012) leading to a similar SE either for RBCs or plasma. Thus, such a combination of high AR and high \(Q_0\) could also be the choice of researchers in blood plasma separation even for future studies.

### 3.4 Constriction–expansion

This section includes the influence of modification in geometries in terms of constriction, constriction–expansion, etc., on the various flow parameters and SE of the blood plasma separation. Such modifications in the geometry results in various forces to enhance the blood separation process, such as Fahraeus effect. The inclusion of the constriction in a particular geometry enhances the cell-free layer because of the increased Fahraeus effect. A tau-shaped constriction–expansion geometry has been shown in Fig. 3d. Although, the constriction–expansion can also be included in the separation devices in various other forms, few are shown in Fig. 6. An important remark of the reviewed articles related to such a geometry has been tabulated in Table 4.

The discussion initiates with the study of Faivre et al. (2006) that involves constriction–expansion in terms of including a pinched section of a particular width (Fig. 2) in a micro-channel Faivre et al. (2006). In particular, the study investigated the geometrical focusing of blood cells (i.e., ellipsoidal cells) through the constriction section in
the microfluidic device to separate blood and plasma in a shear flow. In overall terms, the study explored the influence of the geometry, flow rate, surrounding fluid viscosity, hematocrit and mechanical properties of cells on the increment in the cell-free layer downstream of the constriction, Fig. 6a. This geometry is generally known as a pinched-flow fractionation as shown in Fig. 2c. In this work, the modification in the spatial distribution of cells in the channel was also observed because of the modulation in the geometry coupled to the cell deformability. The blood cells have been centrifuged using PBS solution twice to avoid the aggregation and wall adhesion. The test was run with 0.1 and 2.6% v/v Hct, while only one run with Hct = 16%. In this work, a significant enhancement in the cell-free layer was observed with the cell concentration, constriction length, cell deformability, cell volume, and suspending media viscosity. Moreover, the cell-free layer was seen to increase with the decrease in the constriction width. An insignificant effect of the flow rate was observed on the cell-free layer in the downstream. In conclusion, a SE of 100% has been reported herein for 16% of Hct along with 24% plasma yield. Furthermore, such experimental results have been exploited to design a blood plasma separator based on the modulation in the length and width of the channel. Kersaudy-Kerhoas et al. (2010b) carried out plasma extraction using multiple constriction regions in the plasma channel to exploit hydrodynamic forces. They proposed for the first time such a geometry that exploited the series of constriction and expansion to get better separation. They have also done the biological testing of the extracted plasma by analysing the human free cell DNA in it. The channel dimensions have been reported in Table 4. They worked for a wide range of entrance Hct upto 30% for a $Q_0$ of 2, 5 and 10 mL/h. They noted SE of first plasma branch in between 95 to 100% for low Hct, i.e., 1.8–10%. For Hct greater than 10%, SE decreases from 95 to 32% at Hct = 33.7%. A 30% plasma yield has been reported herein with 20 μm wide plasma channel dimensions. By decreasing plasma channel dimensions up to 10 μm and with 2 mL/h flow rate, ~99% SE has been noted for whole blood with 5% plasma yield.

**Fig. 6** a Representative schematic of the geometry including constriction for the flow of blood cells with side and top view used in Ref. (Faivre et al. 2006). b Constriction–expansion array of the microchannel with low aspect ratio leads to a balance between inertial lift force ($F_L$) and dean drag force ($F_D$) which results in the particle migration direction across the channel according to the particle size Reprinted with permission from (Lee et al. 2011). c Particle migration principle at two aspect ratio values (AR) as AR = 1 and < 1 at three cross-sectional points shown in b. Reprinted with permission from (Lee et al. 2011). d Schematic diagram of the experimental setup and line diagram of the experimental device along with the dimensions along with a microscopic image to show the cell free layer in the expansion region of the device (Shatova et al. 2016). Reprinted with permission from (Shatova et al. 2016). Copyright (2016) American Chemical Society
| Research groups, RG | Channel dimensions, $D^*$ (μm) | Separation techniques, ST | Separated components, SC | Hematocrit levels, Hct (%) | Flow rates, $Q_0$ (μL/min) | Separation efficiency, SE (%) | Remarks |
|---------------------|--------------------------------|--------------------------|--------------------------|---------------------------|-----------------------------|-----------------------------|---------|
| Faivre et al. (2006) | $L_{CR} = 50–300$, WEL = 100, WCR = 15–50; $D = 75$ | NA | NA | 0.1%, 2.6%, 16% | 0.167–166.67 | 100% at all Hct values; (yield = 18% with Hct = 16%) | Studied the flow behaviour of flow through a constriction expansion geometry coupled with cell deformability |
| Kersaudy-Kerhoas et al. (2010b) | $L_{CR} = 5000$, WEL = 100, WCR = 10–20; $D = 20$ | Hydrodynamic forces | Plasma | Upto 30% | ~ 33, 83, 166 | ~100% for Hcts upto 30% at flow rate of 33 μL/min (yield ~ 30%) | Multiple constriction in the microfluidic system has been exploited for the blood plasma separation. The biological validation of the extracted plasma has been done by analysing human cell free DNA |
| Lee et al. (2011) | $L_{CR} = 300$; WCR = 50; $D = 20$ and 50; | Inertial focusing (Dean force and lift force) | RBC | ~45% | 220 | 60% at Hct = 45%; (yield = 62.2%) | Used constriction–expansion array. Utilization of inertial lift and Dean drag forces to decide the separation cut off of the RBC particles |
| Prabhakar et al. (2015) | It is an intricate geometry; details can be found in text | Combined effect of Fahraeus effect, bifurcation law, centrifugal force, cell-free region | Plasma | 7–45% | ~100% (for diluted blood, Hct = 20%); ~80% for Hct = 45%; (yield = 3% at Hct = 45%) | Used a device which can exploit the combined effect of various separation effects and reported a better separation efficiency. Such device called as “τ-channel” |
| Zhang et al. (2015) | $L_{CR} = 2500$; WEL = 360, WCR = 100, $D = NA$ | Surface acoustic wave | Plasma | 50× diluted human blood | 0.006 | NA | Investigated blood plasma separation with a surface acoustic wave (SAW) and without SAW |
| Tripathi et al. (2015a, b); τ-90) | $\theta_1 = 90^\circ$; $R = 3000$; LB, LP = 2000, WEL = 200, WB = 300, WP = 100, WCR = 100; $D = 60$ | Biophysical effects, hemodynamic separation, bifurcation law | Plasma | 7–45% | 400 | 100% at Hct = 7%; 93.65% at Hct = 24%; 78.34% at Hct = 45%; (yield = 2.53% at Hct = 45%) | Studied the performance of a plasma separator, (an intricate design of the microchannel, namely, τ-channel) to analyse the effect of the flow rate, Hct and geometries |
| Research groups, RG | Channel dimensions, $D^*$ (μm) | Separation techniques, ST | Separated components, SC | Hematocrit levels, Hct (%) | Flow rates, $Q_0$ (μL/min) | Separation efficiency, SE (%) | Remarks |
|---------------------|---------------------------------|--------------------------|------------------------|---------------------------|--------------------------|-----------------------------|---------|
| Tripathi et al. (2016) | $\theta_1 = 180^\circ; R = 300$, WEL = 200, WB = 300, WP = 60, WCR = 100; $D = 60$ | Biophysical effects, hemodynamic separation, bifurcation law | Plasma | 7–42%; 42–62% (in correlation) | 300–500 | ~100% at Hct<42%; 99.66% at Hct=42%; 84% at Hct=62%; (yield = 1–6% at Hct=42%) | Exploits the elevated dimensions of 100 μm. Tested a wider range of hematocrit as 62% |
| Tripathi et al. (2015a, b); (τ-135) | $\theta_1 = 135^\circ; R = 3000$, $W_E = 200; W_B = 300, W_P = 100; D = 60$ | Biophysical effects, hydrodynamic forces, and bifurcation law | Plasma | 7, 24, and 45% | 400 | 93.05% at Hct=7%; 74.14% at Hct=24%; 61.02% at Hct=45%; (yield = 7.14% for all Hct) | Discussed the wide range of hematocrits including the diluted and whole blood |
| Tripathi et al. (2015a, b); (τ-160) | $\theta_1 = 160^\circ; R = 3000$, $W_E = 200; W_B = 300, W_P = 100; D = 60$ | Biophysical effects, hydrodynamic forces, and bifurcation law | Plasma | 7, 24, and 45% | 400 | 70.83% at Hct=7%, 58.04% at Hct=24%, 54.92% at Hct=45%; (yield = 3.22% for all Hct) | Discussed the wide range of hematocrits including the diluted and whole blood |
| Tripathi et al. (2016); (τ-low expansion microchannel) | $\theta_1 = 90^\circ; R = 3000$, $W_E = 200; W_B = 300, W_P = 100; D = 60$ | Biophysical effects, hydrodynamic forces, and bifurcation law | Plasma | 7, 24, and 45% | 400 | 97.22% at Hct=7%; 84.87% at Hct=24%; 67.91 at Hct=45% | Discussed the wide range of hematocrits including the diluted and whole blood |
| Tripathi et al. (2015a, b); (τ-low expansion microchannel) | $\theta_1 = 90^\circ; R = 3000$, $W_E = 200; W_B = 300, W_P = 100; D = 230$ | Biophysical effects, hydrodynamic forces, and bifurcation law | Plasma | 7, 24, and 45% | 400 | 84.72% at Hct=7; 64.87% at Hct=24; 35.03 at Hct=45% | Discussed the wide range of hematocrits including the diluted and whole blood |
| Rodriguez-Villarreal et al. (2009) | $L_{E_1} = 30,000$, $L_B = 25,000$, $L_P = 20,000, 20,500$, $L_E = 20,000$, WEL = 400; WB = 400, WP = 200, D = 40 | Hydrodynamic forces | RBCs | 30 and 45% | 200 | 97% at Hct=30% at 37 °C and 95% at Hct=45% at 26.9 °C | Discussed the combined effect of temperature and flow rate over a wide range of hematocrits |
| Shatova et al. (2016) | $L_{E_1} = 3000$, $L_B = 2000$, WEL = 30, WB = 200, WP = 20 and 50 | Passive separation | Plasma | 40 and 50% | 50 | Separation ratio of 2800 (9% yield for all Hcts); 5–30 μL/min throughput, | A gradual expansion region was observed to enhance the plasma skimming separation |
A fully integrated immunoassay has been used by Lee et al. (2009) for the plasma extraction. They reported 33% of plasma yield from whole blood. Although, a pink colour of extracted plasma demonstrates the presence of RBCs in the extracted plasma. Furthermore, Lee et al. (2011) designed an array of constriction-expansion in a microchannel for exploiting the inertial forces for the separation of blood and plasma in a low aspect ratio (AR) geometry (defined as the channel height to constriction channel width), Fig. 6b. The device consists of two inlets, one for blood and another for PBS (which is used as a focusing fluid), the constriction region consists of six rectangular structures of 700 μm width. The device is based on the balance between the inertial force and Dean drag force that can be modulated by the presence of constriction-expansion, which controls the direction of the migration of the cells and hence, blood plasma separation. The choice of low AR channel results in the dominance of lift force over the Dean drag force. The RBCs accelerate towards the lower and upper walls, entangled in Dean’s vortex, and tends towards one of the side walls due to the dominance of the lift forces. Herein, AR of 0.4 and 1 were explored for $D = 20$ and 50 μm, respectively. The basic reason for the modulation of the channel aspect ratio at the constriction region (which leads to a change in the magnitude of the inertial lift forces of the RBCs), is the force balance between the Dean drag and inertial lift. This balance leads to a control on the particle size cut off during separation. In overall terms, inertial focusing is found to be promising in enhancing cell-free layer as well as address the plasma extraction challenges because of the simple fabrication, economical cost, high purity performance, high throughput and applicable in separating high volume samples.

Figure 6b shows the principle of particle migration across the channel cross-section. If AR is low, a higher shear-rate is induced between the top and bottom of the channel than that of the left and right of the channel. Such a condition leads to a strong inertial lift forces that accelerate the particles towards the top and bottom of the channel wall. Thus, variation of AR leads to a modulation in the shear-rate and hence the direction of the particle migration. From Fig. 6b, it is also clear that if AR = 1, the input sample will be affected by the dominant Dean drag force, i.e., $F_L < F_D$, and hence will migrate towards the side wall 2 (s2). On the other hand, for AR < 1, the particles move towards the sidewall 1 (s1), i.e., the top and bottom of the microchannel because of the high shear-force induced inertial force than that of dean force ($F_L >> F_D$). Further, a slightly different geometry has been used here for the blood plasma separation after testing various particle sizes from very small to 10 μm as shown in Fig. 6c, where the channel outlet is bifurcated into two branches for the collection of blood and plasma separately. Due to the dominance of the inertial force, the cells move towards the upper outlet while passing through the constriction-expansion array. In contrast, the plasma dominated by the Dean drag force will be accelerated towards the bottom outlet. In conclusion, a SE of 60% was observed with the blood and PBS flow rate of 1.2 and 12 mL/h, respectively. Also, the plasma yield of 62.2% and throughput of 1.2 mL/h ($\sim 1.0 \times 10^8$) has been noted. However, SE of 100% is reported in their work.

Further, a hybrid geometry, i.e., τ-shape (τ-C1, τ-C2, and τ-C3 with three different diameters) almost similar to the figure shown in Fig. 3d that includes a constriction-expansion to exploit the Fahraeus effect, centrifugal force, cell-free region has been proposed by Prabhakar et al. (2015) for the separation of plasma from human blood. The bio-compatible device utilized in this work has been fabricated using PDMS, detailed in Table 4. As discussed in the section of Trifurcation, Kersaudy-Kerhoas et al. (2010a) used the combined effect of the bifurcation law, high flow rates along with the constriction close to the channel bifurcation, and reported SE of 100% for the diluted blood (up to Hct = 30%). Prabhakar et al. (2015) exploited relatively larger channel dimensions and combined the above discussed five separation effects in a novel manner, and reported a high SE even for the undiluted blood of 80% (Hct = 45%) and 100% for the diluted blood along with the yield as 3%. A hematocytometer was used here for the counting of the blood cells. It was noted that the performance of their device deteriorates as Hct increases. In particular, the maximum SE was observed with the device, namely, τ-C3 and $Q_0 = 400 \mu$L/min detailed in Table 4.

In this work, the microchannel is designed in such a way that it can exploit the effect of the bifurcation law through T-shape, centrifugation through the bent shape, constriction through constricting the microchannel in the bend section, and expansion through expanding the dimension immediately just after the bend region, as shown in Fig. 3d. The device consists of one inlet for the blood sample flow and three outlets, including two for plasma (although, Fig. 3d shows only two outlets but this work uses three outlets, Prabhakar et al. (2015)) and one for blood. The purpose of fabricating the second plasma outlet was to increase SE, but due to high flow resistance, no significant increment in SE has been observed. As shown in Fig. 3d, the name τ-C is the representative of the perpendicular connection of the first plasma outlet at the blood outlet and constriction section. Further, it has been observed in this work that SE shows a strong dependency on the plasma channel resistance, which in turn is directly related to the length of the plasma channel. Thus, a long plasma channel and the multiple bends in the device has been chosen to enhance the plasma channel resistance, see Fig. 3d.

In broad terms, for a constant dimension, the bend channels have almost twice the resistance than that of the
straight one. A slight angle has also been used at the channel inlet for encompassing the reservoirs in the design constraints, which was also helpful in recording the movement of the cell through the microchannel. Due to such intricate design of the device, the direction of movement of the blood cells and plasma is in the swift axial flow and in the marginal stream, respectively. Also, the average velocity of the plasma is seen to be smaller than that of the blood cells. The velocity difference is found to be increasing with a decrease in the channel dimension. Also, the blood viscosity is reduced. Thus, the increased velocity difference and reduced viscosity with a smaller channel dimension in the end of the constriction zone may lead to an increment in the momentum of the blood cell aggregates. Further, the expansion just after the constriction provides a larger trajectory to the blood cells because of the higher momentum of the blood. Hence, the blood cells move away from the plasma molecules before the blood outlet region. Thus, the blood and plasma get separated from each other.

The key conclusions from this complex arrangement of the geometry are as follows: a critical flow rate has been observed, above which SE is seen to be decreasing, while below that it is seen to be an increasing function of \( Q_0 \) i.e., \( \sim 500 \mu \text{L/min} \). At low Hct, i.e., 7–15%, SE is found to be 100% even at a low flow rate of \( Q_0 = 300 \mu \text{L/min} \), also reported in Table 4. For higher Hct (> 20%), SE is seen to be increasing with \( Q_0 \) but only for the \( \tau \)-C2 arrangements, while for the \( \tau \)-C1 and \( \tau \)-C3; the flow rate shows a dramatic effect on SE. Also, SE is seen to be decreasing with Hct, regardless of the three constrictions used in this work and the flow rate. Thus, their device has an essential feature of compactness, which includes utilization of an elevated dimension of the order of microns as well as the bends in the plasma channel. Moreover, the device can operate continuously and reliably for a longer period of time without any contamination or clogging.

Furthermore, Zhang et al. (2015) used constriction–expansion in the device, different than Fig. 3d followed by a bifurcation microchannel for the human blood plasma separation using a surface acoustic wave (SAW) through sinusoidal signal. The separation was also done here without using SAW. The device used consists an expansion section followed by two outlets as Y-splitter (see, Fig. 3b) with an angle of \( \theta = 30^\circ \) with the overall channel dimensions of 28.7 \( \times \) 24.8 \( \mu \text{m}^2 \). A sudden expansion in the device has been introduced, which leads to hemodynamic effects influencing the separation of the particle. Results show that the blood cells are accelerated towards one side of the channel wall experiencing the acoustic field. They found an acoustic power of 20.43 dBm to be sufficient for the plasma isolation for 50x dilution of blood with \( Q_0 = 0.006 \mu \text{L/min} \). They observed that as the flow rate increases, a higher acoustic power is required.

Notably, at a fixed SAW, the enhancement in the cell rich layer has been observed as the blood flow increases from 0.006 to 0.02 \( \mu \text{L/min} \). This indicates the perturbation of the SAW field with the flow rate.

Later, Tripathi et al. (2015a, b) extended the work of Prabhakar et al. (2015) and studied the various blood plasma separator devices using the hemodynamic separation techniques such as used in T-channel configuration, as shown in Fig. 3a (Tripathi et al. 2013). The performance of such devices has been analysed in terms of separation efficiencies with respect to the modification in the microchannel design, feed hematocrit, and microchannel geometry. A tau-shaped geometry, as shown in Fig. 3d was used as also exploited by Prabhakar et al. (2015) with an elevated dimension of 100 \( \mu \text{m} \), which reduced the problem of blood clogging. Also, an idea of better plasma separator device was found from the previous studies of T-channel (Tripathi et al. 2013), constriction–expansion, \( \tau \)-centrifugal, etc. Various channel designs have been explored as T-200/100, T-400/100, T-cascading, constriction–expansion, \( \tau \)-centrifugal, \( \tau \)-135 microchannel (it keeps \( \theta_1 = 135^\circ \), \( \tau \)-160 microchannel (\( \theta_1 = 160^\circ \)), \( \tau \)-90 microchannel (\( \theta_1 = 90^\circ \)) \( \tau \)-low expansion microchannel. Few of them have been discussed in T-channel Sect. 3.1 (Tripathi et al. 2015a, b). Each channel has been tested with various flow rates and for the studied wide range of hematocrits. However, \( \tau \)-90 microchannel, similar to Fig. 3d, but with different dimensions mentioned in Table 4, for instance, \( \theta_1 = 90^\circ \), was observed as the best device in separating the blood and plasma in terms of SE. In general, the design consists of one inlet, one outlet for RBCs and two outlets for the plasma, as also reported in Ref. Prabhakar et al. (2015). The SE of \( \tau \)-90 microchannel was observed as 100, 93.65 and 78.34% for Hct of 7, 24 and 45%, respectively at the flow rate of \( Q_0 = 400 \mu \text{L/min} \) along with 2.53% yield. A comparison of SE achieved in the study was made with the previous works and showed a better SE than the others for the pure blood, i.e., \( \sim 80\% \) with yield 3% for Hct as high as \( \sim 45\% \).

Moreover, few more designs of Tripathi et al. (2015a, b) have been discussed here as these can play a vital role in separating the low Hct blood samples, for instance, \( \tau \)-centrifugal microchannel, \( \tau \)-135 microchannel, \( \tau \)-160 microchannel and \( \tau \)-low expansion microchannel, detailed in Table 4. These geometries differ from each other on the basis of bend angle, i.e., \( \theta_1 \), while the bending radius, i.e., \( R \) is same as 3 mm. Also, the devices such as \( \tau \)-135 and \( \tau \)-160 microchannels uses the expansion ratio as 1:3. On the other hand, \( \tau \)-low expansion microchannel has an expansion ratio of 2:3 with bend angle and radius as \( \theta_1 = 90^\circ \) and \( R = 3 \text{ mm} \), respectively. Also, it has been tested with two channel depths as 60 and 230 \( \mu \text{m} \). In general, all the above discussed designs exploit the centrifugal forces to influence the cell free regions through the constriction region. These devices have been fabricated in such a way that they can...
utilize the density difference between the cell and plasma. The separation efficiencies obtained using each above-discussed devices can be found in Table 4, which represents that the smaller bend angle results in better plasma separation. The τ-centrifugal channel, similar to Fig. 3d is the best design among all the tested one.

Furthermore, Tripathi et al. (2016) extended their work for the high Hct of human blood as 62% in a tau-shaped geometry with one inlet and two outlets, i.e., one for RBCs and another for plasma with $\theta = 180^\circ$ and $R = 300$ μm, as shown in Fig. 3d (Tripathi et al. 2013). The Zweifach–Fung bifurcation law was exploited here at the elevated dimensions as the channel dimensions are in the range of 21–221 μm. In Tripathi et al. 2016, the effect of the lift and drag forces was observed along with the other parameters as curvature ratio, confinement ratio, Dean number, channel Reynolds number. The hydraulic diameter of the curved channel was 75 μm and a radius of 250 μm. In this work, the particle Reynolds number is found to be less than one along with the confinement ratio greater than 0.07, suggesting the presence of inertial focusing at the particular degree. Details on flow rate and Hct levels explored herein can be found in Table 4. The results show that at a low Hct of 7%, SE was 100%, regardless of the flow rate, see Table 4. Further, for high Hct of 24–42%, a drop in SE was observed at the low (~100 μL/min) and high flow rates (~800 μL/min). On the other hand, at 500 μL/min, for Hct = 31%, ~99.66% separation efficiency was achieved, while for pure blood, it is found to be 99.55 ± 0.35%. Further, an empirical relationship was also proposed here between the separation efficiency and the hematocrit at the flow rate of 500 μL/min. This correlation suggests a separation efficiency of 84% for inlet hematocrit of 62%.

Furthermore, Rodriguez-Villarreal et al. (2009) fabricated a hybrid microfluidic device, which exploited the hydrodynamic forces to extract the plasma and blood cells from human whole blood through a constriction–expansion channel design (please refer to Rodriguez-Villarreal et al. (2009) for figure). The effect of temperature and flow rates on SE was investigated here. The device used herein consists of one inlet (for blood) and two outlets (one for blood and another for plasma), as like T-channels, shown in Fig. 3a. In this device, the plasma channel width was reduced ~20 times than the inlet channels and constructed a small constriction zone. A wide range $Q_0 = 50–200$ μL/min and temperature as 23 to 50 °C was studied. A comparison of the constriction plasma channel with a straight plasma channel was also made herein. In the constriction region, it was observed that the velocity is ~13.36 times higher than that in the inlet/outlet channel. The straight lateral plasma collection channel has two parts such that the first part has the channel length and width as 800 and 65 μm, respectively. The other part has channel length and width as 2 cm and 200 μm, respectively, while the serpentine plasma channel has different channel dimensions, mentioned in Table 4. Such choice of the channel dimensions caused the flow resistance of RBCs (~5.68 times) higher in the main channel for the effective blood plasma separation. A wide range of Hct’s was explored herein, but SE was reported only for Hct = 30% as 97% at 37 °C, while for Hct = 45%, it was reported as 95% at 26.9 °C at 200 μL/min. Notably, SE at the lower flow rate, i.e., 100 μL/min was smaller than that of the higher one. Thus, it can be said here that a better SE has been achieved at higher $Q_0$ and higher temperature. The distinct features of this device are that it exploits the Zweifach–Fung effect, Fahraeus effect and pinched flow fractionation effect through different sections of the device to successfully isolate the plasma and blood from whole blood.

Shatova et al. (2016) studied experimentally the constriction–expansion-based blood plasma separation device, Fig. 6d. A slightly different constriction–expansion channel has been used by Shatova et al. (2016), which includes two inlets, one for buffer and another for blood and a Y-splitter ($\theta = 5–45^\circ$) along with three outlets (two for plasma and one for blood). A separation ratio (defined as the ratio of the number of RBCs per mL in the inlet to the plasma outlet) of 2800 was observed with a 9% yield using a high $Q_0$ of 5–30 μL/min. Depending upon the plasma channel resistance at the skimming, the plasma outlet channel width has been further expanded to $W_p = 50$ from 20 μm for individual designs. Here, the microchannel design consists of a Y-splitter (Fig. 6d) in which the entered blood with bubbles is washed away with the buffer connected with the splitter. The result shows that the expansion angle, $\theta \geq 20^\circ$ did not give the expected SE. This may be attributed to the presence of the vortices at the higher angles, while the lower angles as $\theta = 5–15^\circ$ results in higher SE such as 100% with 15–5% yields.

Figure 7 indicates the separation efficiency (%) with the different hematocrit levels (%). It is interesting to note here that using a constriction–expansion geometry, one can achieve a high SE ~ 100% for the diluted blood (Prabhakar et al. 2015; Tripathi et al. 2016; Tripathi et al. 2015a, b; Faivre et al. 2006). Even for the whole blood (Hct ~ 45%), the best SE achieved using such a geometry is 95% (Rodriguez-Villarreal et al. 2009). It is clear from Fig. 7 that a researcher studied such a high Hct of 62% and reported SE of 84% but with the expense of such an intricate constriction–expansion geometry (Tripathi et al. 2016). Moreover, it can also be said here that many researchers restricted themselves in exploring the diluted blood, i.e., Hct ≤ 20, using such an intricate geometry. This section concludes that mostly experimental observations have been performed using constriction–expansion devices to extract plasma. Numerical investigation is fully unexplored herein, hence could be the choice of researchers with an appropriate...
geometric modification. Unlike the trifurcation geometries, a low AR microchannel with $D = 50 \mu m$, has been exploited by Lee et al. (2011) to enhance shear-rate; hence, 60% SE for $Hct > 37%$. One of intricate design, namely “Tau-channel” (Fig. 3) with an elevated channel dimension was exploited widely by the researchers and, results in SE up to 80% for pure blood and ~100% for diluted blood. The device performance gets deteriorate as blood concentration increases. Also, using the similar kind of geometry Tripathi et al. 2016 reported 84% SE at 62% Hct. Suffice it to say that with an expense of a complicated geometry, one can get the best performance in terms of plasma extraction between all the above-discussed geometries.

### 3.5 Bend/curved/spiral channels

Figure 3e, f, g present a representative schematic of the bend/curved/spiral channels which are also seen to be choice of many researchers to separate cells from whole blood as well as diluted blood. This section combines the above-discussed three geometries and in addition to the same, the centrifugal force which helps in separating the RBCs and plasma, has been exploited herein. Moreover, Table 5 presents a short summary of the bend/curved/spiral channels which have been exploited in blood plasma separation to the reader.

In 2004, a new RBC separation technique was introduced by Blattert et al. (2004) for both whole human blood (fresh venous blood) and diluted blood (used sodium chloride) samples using experimental analysis. The effect of microchannel geometry was observed in this work for a wide range of Hct. As shown in Fig. 3e, the device used in this work has a bend shape, which includes a bifurcation close to the channel outlet. The device consists of one inlet and two outlets (cell outlet and plasma outlet), detailed in Table 5. Various chip designs (which differ in the entrance and plasma channel length, and flow rate ratio) was investigated to enhance SE of the cells. The cell concentration of inlet and outgoing blood samples was noted herein. A better SE was observed for the diluted blood (Hct= 5, 10 and 17%) than the whole blood (Hct = 48%), Table 5. An enhancement in SE for low Hct and $Q^*$ (i.e., ensures a larger flow rate difference between the plasma and cell channel) was observed. This is because the different flow rate causes the variation in the shear force for various cells, leading to a higher flow rate direction, i.e., the plasma skimming effect (Svanes and Zweifach 1968; Yen and Fung 1978; Ditchfield and Olbricht 1996). Also, a larger density difference between the cells and surrounding particles is expected for lower Hct’s, which results in the higher centrifugal force, hence SE. The highest SE was achieved as 90% for Hct= 10%. Also, using such a curved bifurcated geometry, an improvement in SE of whole blood was observed from 28 to 57% in comparison to previous work (Blattert et al. 2003) due to the exploitation of the small channel dimension and reduced flow rate ratio. Such a bifurcated curved geometry is more preferable as it assists the continuous sample extraction along with rapid separation times.

Moreover, a rotating disk, which generates the centrifugal forces, and influences the plasma isolation from whole blood has been exploited by Haeberle et al. (2006). The model structure is different here than previous studies, as shown in Fig. 8a. The separation of two phases occurred in the duration of the sedimentation followed by a decanting process, in which purified plasma gets collected in a reservoir, Fig. 8a. The device consists of a metering chamber, connected to a drain channel (located at 32 mm radius on the rotating disk of 120 mm) followed by two subsequent chambers for the sedimentation and plasma collection, respectively, Fig. 8a. The Hct level was varied from 42 to 52% (for males) and 36–48% (for female) in this work. Here, the centrifugal separation worked with two different frequencies. At the moderate spinning frequencies of 40 Hz, the technique supplies 2 μL plasma from 5 μL of whole blood within 20 s. The purified plasma contains less than 0.11% residual cell concentration and 40% plasma yield, which does not depend on the rotation frequency. Furthermore, a comparison of pillar type and weir type geometry fabricated using curved channels has been made by Chen et al. (2007) to choose better separator. They used filtration and centrifugation technique for separation process as also reported in Table 5, which also includes channel dimensions and other important key points related to this work.
Table 5 Summary of bend/curved/spiral devices used for blood plasma separation

| Research groups, RG | Channel dimensions, $D^*$ ($\mu$m) | Separation techniques, ST | Separated components, $SC$ | Hematocrit levels, Hct (%) | Flow rates, $Q_0$ ($\mu$L/min) | Separation efficiency, SE (%) | Remarks |
|---------------------|-------------------------------------|--------------------------|---------------------------|---------------------------|-----------------------------|-----------------------------|---------|
| Blattert et al. (2004) (venous blood); bend channel | $R_1 = 500$; $\theta_1 = 90^\circ$, $L_{el} = 3000$, $L_p = 3000$; $W_{el} = 42$; $WB = 97$, $WP = 28$; $D = 116$ | Centrifugal force and plasma skimming | RBC | 5 and 45% | 60–120 | 90% at Hct = 5% and 58% at Hct = 45%; (yield = 3% at Hct = 5%) | Proposed curved bifurcated geometry which is preferable as it includes the rapid separation times and separation of small sample volume |
| Haeberle et al. (2006) | Mentioned in the text itself | Centrifugal force | Plasma | 36% to 52% | NA | NA | Used sedimentation process to separate plasma from whole blood |
| Chen et al. (2007) (rat blood); curved channel | $L_{el} = 160,000$ (tortuous channel); $W_{el} = 200$ (tortuous channel); $D = 30$ | Filtration and Centrifugation | RBC | Diluted whole blood with $10^6$ cells $\mu$L | 5 | 91.2% for weir type geometry; 82.37% for pillar type geometry | From the viewpoint of separation efficiency, weir-type chip is more efficient than the pillar-type |
| Di Carlo et al. (2008); curved geometry | $d_1 = 350$ (small radius turn of 325 $\mu$m), $d_2 = 650$ (large radius turn of 890 $\mu$m); $D = 50$ | Filtration and Centrifugation | Platelets | 2% whole blood in PBS | 0.9 | $\sim$ 100% at Hct = 2% | Cascading geometries could be useful for separating the particles |
| Sollier et al. (2009); curved and spiral geometry | $L = 120,000$; $W =$ 200, 200–400; $D =$ 200 (main and spiral channel), 0.5, 1.4 and 9.5 (filtering depth); | Filtration and Centrifugation | Plasma | 10% | 1:20 (flow rate ratio) | 60% with Snake chip and 25% with U-Filter Chip at Hct = 10% | Addition of geometric singularities can help in lateral migration of the particles which helps in improving the separation |
| Nivedita and Papautsky (2013); Spiral geometry | $L = NA$; $W = 250$ (device 1); 500 (device 2); $D = 75$ (device 1) and 110 (device 2) | Inertial focusing (rotating dean drag and hydrodynamic forces) | RBCs | $\sim$0.45% | $\sim$1000 | $\sim$100% at Hct = 0.45% (with device 1); (yield = 17%) | Used a spiral channel and exploited the Dean drag force along with the hydrodynamic forces |
| Zhang et al. (2014); serpentine channel | Details can be found in the respective text | Inertial focusing | Plasma | 2.25, 4.5, 9% | 350 | $\sim$100% at Hct = 2.25%; throughput = $7 \times 10^8$ cells/min (yield = 46%) | Utilizes two-sided secondary flow that aided inertial focusing for cell migration towards the channel side walls |
| Robinson et al. (2017) (bovine blood) | $L = 1,085,000$ $W = 500$ (for single spiral) (250 $\mu$m for double spiral) $H =$ 60 | Inertial focusing (dean vortices); Zweifach–Fung effect, filtration | RBCs | 2% | 1250 | 99% (double spiral filter) at Hct = 2% | Used a cascaded inertial microfluidic device in the form of two 5-loop Archimedean spirals in series for the separation |
The device has been fabricated using the MEMS technique, and it is capable of handling 1 μL whole blood (i.e., rat blood). The device consists of microfilter, micropillar array, microchannel, microwire, microchamber and porous matrix, Fig. 8b. The crossflow filtration was suggested to separate blood cells as it will avoid clogging or jamming (Crowley and Pizziconi 2005). The device consists of two major parts as microfilter for cell separation, Fig. 8b(A), and microchannel for cell lysis and purification of DNA, Fig. 8b(B). Figure 8b(II) shows the integrated microfluidic chip, composed of three different layers discussed in detail in Ref. Haeberle et al. (2006). The silicon wafer used in the device mainly consists of cell separation, cell lysis and DNA purification regions. The cell separation first region consists of a tortuous channel, which is further divided into three subchannels, i.e., two parallel filtration barriers including pillar type (diameter = 20 μm; 6.5 μm separation gap) and weir type (height = 26.5 μm; width = 20 μm; 3.5 μm separation gap). Figure 8c represents the two-dimensional CFD simulations which was run using Fluent to compare the straight channel and a "tortuous" channel in terms of flow pattern. For the tortuous channel, a lateral flow in the entrance region as well as in the bend region were observed, which generates a higher tangential flow rate than that of the straight channel in which lateral flow occurs only close to the entrance region. Such a higher tangential flow rate results in better SE. The pillar-type and weir-type geometry has been compared using the inlet diluted blood flow rate, i.e., $Q = 5$ μL/min. The images and videos of cell separation were observed in the duration of experiments, which emphasized that the separation of cells occurs both in entrance and bend region, which was also observed numerically. The SE of RBCs was noted as 91.2% with weir-type geometry, while using a pillar-type geometry reduced SE to 82.35%. The reduction in SE can be attributed to the reason that there may be a diffusion of filtered RBC back to feed stream.

Later, an improvement in the curved channels for the better whole blood separation, has been made by Di Carlo et al. (2008) in terms of inclusion of an asymmetrically curved channels for the blood cell separation, as shown in Fig. 3f (Blattert et al. 2003; Chen et al. 2007). The hydrodynamic forces based on the inertial focusing of the cells of different sizes was exploited for such a separation process (Di Carlo et al. 2007). Here, a theoretical background has been developed for emphasizing the underlying forces (Di Carlo et al. 2008). A size cut-off was determined here for the separation process. A semi-empirical correlation demonstrating the relationship of the channel geometry with the cut-off has been proposed. In this work, the microfluidic device includes a filter region, an asymmetrically curved section, and five outlets (Di Carlo et al. 2008), which is slightly different geometry than that shown in Fig. 3f. Thus, as shown in Fig. 3f, the geometry consists of two radii of curvature, i.e., $d_2=890$ μm (wide) and $d_1=325$ μm (narrow), along with the Dean drag force, which is approximately 8 times higher in the small diameter, $D_1$ turns than the larger diameter turns, $D_2$. Numerous experiments have been done to optimize the flow rate, i.e., $Q_0$ along with all the channel dimensions, see Table 5. The cascading separation was also part of this work, which demonstrated 99.9% purification with 2% diluted in PBS, using such a device. Also, the separation of platelets of the size of 2–4 μm from the other blood components can be done using this device. A comparison of curved structure with a straight channel was also made here to show the effect of channel curvature, and found a curved design better than the straight channel. This is because the curved channels introduce an additional drag force on the cells, and it causes secondary flows perpendicular to the flow direction because of the nonuniform fluid inertia. However, the extra force aids in improving SE. The microfluidic filtration device works on the cell size and improves SE from 90 to 100%, along with a high throughput of 1 mL/min.

Furthermore, Fig. 8d represents an innovative lab-on-chip device, i.e., named as U-filter chip (see, Fig. 8d(A)) and the snake chip (see, Fig. 8d(C), based on the lateral migration of RBC and cell-free plasma layer of an experimental work of Sollier et al. (2009), detailed in Table 5. Such migration of cells is known to create the geometric singularity (i.e., a corner edge) and locally enhance the clear plasma region. In this study, a systematic analysis and comparison of various separation techniques such as microfiltration, centrifugation, filtration in terms of advantages and disadvantages for both beads and blood were made. The presence of Dean vortices was expected to hinder the centrifugal force present in the curved channels, which could not be effective for the blood cell separation. Both U-filter chip, and snake-chip devices (see, Fig. 8d) have been investigated for three different filtering depths of 0.5, 1.4, and 9.5 μm. Table 5; in contrast, the main channel depth is similar for each device as 200 μm as shown in Fig. 8d(B) and (D). The various length and widths have been tested to understand the existing resistances in the geometry. It was observed that U-Filter chip can separate 10 μm beads from liquid with an extraction rate of 0.5–1% for $D=0.5$ μm, while for $D=1.4$ μm, it is seen to be 10–25%. The best SE noted in this work was 25% using U-Filter and 60% with Snake-chip device at $Q_0=10$ μL/min and $D=9.5$ μm. Furthermore, two geometries, such as spiral design, as shown in Fig. 3g and a 180° bend (similar to Fig. 8c with 180° bend angle) was used with the centrifugation separation technique for plasma extraction. It was observed that the spiral geometry for blood separation was a bad choice due to the presence of the Dean vortices that quickly appears in the separation process making it difficult to achieve the desired separation, Fig. 3g. On the other hand, the bend geometry is seen to be quite effective in separating the beads as the short bend of 180° exploits the Dean
Sollier et al. (2010) suggested that by using geometric singularities such as sudden geometric enlargement in the channel, the cell-free plasma layer can be locally expanded because of the lateral migration of the cells in the device. Such modification in geometry can lead to 18% plasma yield at high $Q_0$, i.e., 100 μL/min for diluted blood (1:20) with ~90% SE. The device has also been varied biologically. Moreover, for the separation of 1/20 diluted blood cells, the presence of the Dean vortices influences the migration of the red blood cells towards the inner wall at the short bend end. In broad terms, a device has been used based on the lateral migration of cells leading to a cell-free layer as an efficient one with 100 μL/min flow rate. The geometry was modified here in terms of the addition of geometric singularities, which leads to an expansion of clear-layer dimensions and, hence made plasma extraction easier. Thus, suffice it to say here that each blood separation device has its pros and cons but has its application in some particular areas. Moreover, the continuous separation of cells has been experimentally observed by Nivedita and Papautsky (2013) using a rotating Dean drag and hydrodynamic forces acting on cells in the laminar flow in a curvilinear microchannel geometry. They reported 17% plasma yield with 90% SE for a Hct of 0.45%. As discussed by Haeberle et al. (2006), the Archimedean spiral devices, which was small in size (<1 in$^2$) was used in this work to isolate the cells in a downstream focusing length of less than 8 cm. A cell being suspended in a spiral microchannel experience an inertial lift force caused by rectangular cross-section and a traverse Dean drag force because of the two major Dean vortices. Two devices have been fabricated here using PDMS with different dimensions. Each device has one inlet while three outlets in device 1 and four outlets in device 2, as shown in Fig. 3g. The details of the dimensions can be found in Table 5. For the separation of blood cells from plasma, device 1 with a spacing of 250 μm between the loops and a focusing length of ~6 cm was used. The focusing length plays a vital role in determining the total size of the microfluidic device if the inlets and outlets size are fixed. The length also helps to determine the required number of spiral loops to accommodate the needed channel length for cell focusing.

Further, for the given spiral length, wide channel dimensions are necessary to separate the WBCs and RBCs and a more extended focusing range to separate the cells in different streams. Thus, the following sizes was used here as
using a serpentine channel of low aspect ratio as AR = 0.21. They developed an inertial passive microfluidic device exploited by Zhang et al. (2014) for the blood plasma separation. For the blood separation, the blood was filtered and the integrated device results in maximum plasma volume as 488\(V_{\text{chamber}}\).

Furthermore, Robinson et al. (2017) discussed the importance of various biological fluids in point-of-care (POC) diagnostics. The importance of separation, identification, and quantification of small molecules circulating in the blood plasma suspension for various diagnostic purposes has also been discussed in this work. Particularly, the rapid separation of RBCs from blood plasma suspension was observed here. A cascaded inertial microfluidic device in the form of two 5-loop Archimedean spirals in series was used for the separation process, see, Fig. 3g for single spirals with one 5-loop. The microfluidic chip was designed in such a way that it can exploit the Dean force-induced migration to separate the blood. Furthermore, two different spiral channel networks such as a single filter and a double spiral channel filter was used herein (Robinson et al. 2017). It is essential to satisfy the dimension criteria for fluid particles to flow through the spiral channel, i.e., the ratio of the particle diameter and hydrodynamic diameter of the channel must be greater than or equal to 0.07 (Kuntaegowdanahalli et al. 2009). Also, the channel length plays a vital role in such filtration process; it is important to choose such a filtering length so that the hydrodynamic forces could accelerate the particles to their final focusing positions, i.e., 108.5 mm, Table 5. The device has three outlets to collect the blood and waste. Such channel dimensions were selected here to allow filtration of the particles of size 7.5 μm only. Thus, the second loop dimension in the five-fold spiral has used as 2.8 mL/min, throughput as 7 × 10⁸ cells per min, and yield was found to be 46%.

Furthermore, Zhang et al. (2017) extended their previous work (Zhang et al. 2014) of blood plasma separation using inertial focusing in a serpentine channel, as shown in Fig. 8e to analyse the combined effect of two functions, i.e., parallel inertial microfluidic channels and membrane filters with filter-on-top configuration (Zhang et al. 2014). In this work, the impact of the integrated microfluidic device was observed experimentally, i.e., a combination of membrane filtration and particle inertial focusing on the efficient separation of the blood plasma. It was observed that using such an integrated device, the quality of extracted plasma meets the standard plasma quality through centrifugation. Also, the integrated device provided better plasma extraction than that of the inertial focusing microfluidic device alone. The device was tested using the diluted whole blood (i.e., 1/10, 1/20). In general, using filter-on-top configuration, the theoretical maximum volume of the extracted plasma is 1.22\(V_{\text{chamber}}\) for Hct ~ 45%. Also, as discussed earlier in Zhang et al. (2014), using an inertial focusing device, 99.75% of blood cells can be separated from plasma. On the other hand, a membrane filter was implemented before the inertial focusing device, and the integrated device results in maximum plasma volume as 488\(V_{\text{chamber}}\).

Furthermore, a slightly different geometry than the discussed-above, a serpentine channel, as shown in Fig. 8e was exploited by Zhang et al. (2014) for the blood plasma separation. They developed an inertial passive microfluidic device using a serpentine channel of low aspect ratio as AR = 0.21 to separate RBCs from plasma at \(Q_0 = 350 \mu L/min\). The device relies on the two-sided secondary flow techniques that aided inertial focusing of the cells in the used serpentine channel, see Fig. 8c. In the separation device, the RBCs start moving towards the two side channels while the cell-free plasma moves towards the centre outlet, see Fig. 8e. For such a separation, two different channel geometries such as straight and curved channels were used herein. First, the straight channel with AR as 0.84, 0.42 and 0.2, and length as 70,000 μm is chosen. Secondly, a serpentine channel has the dimensions of 200 × 42 μm². Due to the curvature addition, the secondary flow will generate in the serpentine channel, which leads to accelerate the cell towards the two side walls of the channel, and facilitates particles movement towards the inertial equilibrium positions, as shown in Fig. 8e. In the straight channel, due to the absence of secondary flows, the particle cannot reach the inertial equilibrium position because of the weak inertial lift forces. Thus, introducing the curvature in the channel helps in particle migration and inertial focusing. For the blood separation, the blood was collected from a healthy donor adult male and treated it with heparin. With a single process, the extracted plasma purity was noted as 99.75%. Subsequently, through the second process, the purity was improved and reported as 99.95%, i.e., ~100%, Table 5. In order to process a large volume of the samples, the parallelization of eight serpentine channels was done, which results in the enhancement in the flow rate of various dilution factors. Device 1 gives 100% plasma separation with dilution of 0.45% with \(Q_0 = 1000 \mu L/min\). However, this microfluidic device could not be employed in practical applications as it has been tested for very diluted blood only. They concluded that SE first increases with \(Q_0\) for a fixed value of dilution factor; after that, it decreases with increased flow rate. This critical flow rate is seen to be different for each dilution factors. It was also noted that for high dilution factors, i.e., \(\geq 500x\), the SE is ~100% at \(Q_0 \sim 800 \mu L/min\). As the separation is based on the sorting method, the size of the cell/particle is an important factor in their experimental results. A particle of similar diameter as of the blood cells was used in the experiments, i.e., polystyrene particles of \(d_p \sim 7.32, 10, 15, \) and 20 μm for a wide range of \(Q_0 = 1000–3000 \mu L/min\).
as $W_S = 250 \mu m$, which restricts the filtration of particles with $d_P < 6.78 \mu m$.

Importantly, the inertial focusing depends on a shift in the velocity profiles to predict the maximum velocity of the channel. It has been assumed herein that there were no secondary Dean flows in the channel. The Hct of 2% of whole bovine blood was used here for a wide range of $Q_0 = 0.75 – 1.5 \text{mL/min}$. Their results show that SE achieved by the single spiral filter is 55%, while the double spiral filter shows ~ 99%. This may be attributed to the dominance of the Zweifach–Fung effect at the secondary bifurcation (~10% contribution of Zweifach–Fung effect in the separation) than the first bifurcation (~4%). It has also been noted here that if the dilution factor is increased to 100×, the single spiral filter approaches SE as maintained by the double spiral filter.

Finally, a clearer picture of SE versus Hct has been plotted in Fig. 9 to demonstrate the best SE achieved using such geometries. From Fig. 9, it can be concluded that almost every previous work related to such geometries limits itself to the highly diluted blood (Hct \leq 5%). On the other hand, only one researcher exploited such geometry to separate whole blood, i.e., Hct = 45% (Blattert et al. 2004). Thus, a wide range of hematocrits ($5 < \text{Hct} \leq 45$) is still open to be explored using such bend/curved/spiral geometries. This section concludes the following key points as: a curved channel should be the choice over straight channel as it enhances SE up to 57% from 28% for pure blood (Blattert et al. 2004, 2003). A tortuous channel separates whole blood up to 91.2% SE of RBC’s over the straight channels (Chen et al. 2007). The curved geometries with short bend ~ 180° was found to be quite useful than the spiral one leading to better SE. The Dean vortices that quickly appear in spiral geometry makes the separation process difficult (Sollier et al. 2009), still it has been used by Nivedita and Papautsky (2013) and reported a SE ~ 100% for diluted blood. This is because their device exploits not only Dean drag forces but also the hydrodynamic forces. Furthermore, choice of double spiral geometry could be better than single one (Robinson et al. 2017). In conclusion, spiral geometry with an appropriate channel dimension can lead to enhance the plasma extraction.

### 4 Comparative analysis of various channel-based microfluidic

As per the discussion in Sect. 3.1, a wide range of flow rates, $Q_0$ and hematocrit levels, Hct have been chosen for separation of the blood and plasma using various types of channel-based microfluidics geometries and the different separation techniques. It is difficult to compare the various geometries as they have different channel dimensions, flow rate, hematocrit levels, etc. A comparison has been made here based on the separation efficiency, SE of different devices in terms of diluted and whole blood. This analysis is presented in two separate sub-sections for whole and diluted blood. It is well known that the hematocrit level of an adult woman and man ranges from 37 to 47% and 43 to 54%, respectively (Jameson et al. 2019). Therefore, the range of hematocrit level is chosen as follows: Hct \geq 37% for whole blood and Hct < 37% for diluted blood.

#### 4.1 Comparative analysis for whole blood (Hct \geq 37%)

From Fig. 10, it can be said that each geometry with different channel dimensions has been exploited widely by many researchers to separate the blood and plasma. From preceding sections, one can note that the channel dimensions, especially channel depth plays a very significant role in blood plasma separation. Also, each of them provides different separation efficiencies as per the choice of various flow and geometric parameters. In overall terms, it is difficult to say that which geometry is the best one for such a separation process as each of them has their limitations. Although, a meta-statistical analysis has been carried out in the subsequent section to answer best geometry that can serve better in separating blood plasma for different values of Hct. Also, we found that the least used geometry in blood plasma separation is $Y$-channel, which is studied by only few researchers (Yang and Zahn 2004). Yang and Zahn (2004) got 100% SE for bovine blood using a channel cross-section...
of 35 × 35 μm² and a different plasma channel. Furthermore, a slight reduction in the channel cross-section has been used by Yang and Zahn (2004) leading to ~100% SE. A flow rate ratio of 6:1 and 8:1 in a Y-shaped microchannel, leads to SE ~ 100% with 15 to 25% yield for Hct = 45% (Yang and Zahn 2004) as also reported in case of T-channel geometry (Yang et al. 2006) even for whole blood. A widely used geometries in separating whole blood are T-channel and constriction–expansion. Moreover, a complex geometry, i.e., constriction–expansion geometry, has been exploited by Kersaudy-Kerhoas et al. (2010b) to obtain 99% SE for whole human blood with 5% yield. They used the dimension of entrance channel and height as 100 and 20 μm, along with the size of the contraction region as 40 × 20 μm². With the expense of such a complex geometry, they obtained SE ~ 99% for whole human blood. However, to get 100% SE of whole human blood, device must have to be improved in terms of modulation in the channel dimension, number of constriction regions, separation techniques, etc.

4.2 Comparative analysis for diluted blood (Hct < 37%)

For the better presentation of the wide range of hematocrit level of the diluted blood, separate comparative analysis plots are shown in Fig. 11a for Hct < 10%, Fig. 11b for Hct < 37%. In broad terms, for highly diluted blood (Hct < 10%), shown in Fig. 11a, almost all researcher reported SE ≥ 85% for different types of modifications in the geometries, and other flow parameters (Prabhakar et al. 2015; Tripathi et al. 2016, 2013; Jaggi et al. 2007; Tripathi et al. 2015a, b; Faivre et al. 2006; Li et al. 2020; Blattert et al. 2004; Di Carlo et al. 2008; Robinson et al. 2017). For such a highly diluted blood, the lowest SE has been reported in the literature is approximately 17% using a trifurcation geometry. The widely used geometry for separating highly diluted blood is T-channel and constriction–expansion, while the least explored geometry is Y-channel (Hymel et al. 2019). Thus, exploitation of Y-channel geometry is
an open field that can be explored in the future. Furthermore, for moderately diluted blood, \((10 \leq \text{Hct} \leq 37\%)\), it is interesting to note from Fig. 11b that bend/curved/spiral channels have not been exploited to isolate RBCs for plasma extraction. Notably, the constriction–expansion and trifurcation geometries show \(\text{SE} \sim 100\%\), while T-channel and Y-channel limit themselves up to a maximum \(\text{SE}\) of 72\% (Tripathi et al. 2013). Thus, in general term, using any of the studied geometry, \(\text{SE} \sim 100\%\) can be achieved for the highly diluted blood using some optimized flow conditions like flow rate and modulation in the geometry itself. In overall term, for the diluted blood, the least explored geometry is Y-channel, which has been exploited by Li et al. (2020) and they reported \(\text{SE}\) of 64\% at \(\text{Hct} = 10.4\%), see Fig. 11b. From Fig. 11b it is clear that many researchers reported \(100\%\) \(\text{SE}\) for diluted blood using various geometries along with different dimensions.

### 4.3 Meta-analysis of the microchannel-based geometries

This section looks to find a quantitative trend with regards to separation efficiency, \(\text{SE}\) amongst the research articles reviewed in this paper. The mean and standard deviations of the separation efficiencies concerning the observed across the different geometries have been calculated. To ensure some meaningful conclusion, these calculations have been performed only for those geometries wherein we had at least three data points. Figure 12 demonstrates the mean and standard deviation values of \(\text{SE}\) for various geometries (a) for the complete range of hematocrit (including both the diluted blood, \(\text{DB}\) and whole blood, \(\text{WB}\)), and (b) for whole blood, \(\text{WB}\) (Hct ≥ 37\%) and diluted blood, \(\text{DB}\) (Hct < 37\%), separately. Based on standard deviation values of \(\text{SE}\) for several geometries shown in Fig. 12, it can be concluded that the geometry with the least standard deviation is likely to give consistent \(\text{SE}\). Accordingly, the following sequence of the geometries is recommended in its decreasing order to give more consistent \(\text{SE}\) for the complete range of hematocrit, as \(\text{TF} > \text{CE} > \text{YC} > \text{B/C/S} > \text{TC}\), see Fig. 12a. Further, based on the calculated mean values, it can be concluded that the geometry with the highest mean values gives the best \(\text{SE}\). Looking primarily at the mean value mentioned in Fig. 12a, the following sequence is recommended with decreasing order of \(\text{SE}\), as \(\text{TF} > \text{YC} > \text{B/C/S} > \text{CE} > \text{TC}\). It can be observed that though the sequence of geometries varied for the standard deviation and the mean values, the possibility of consistent, high \(\text{SE}\) can be obtained with trifurcation geometry, irrespective of values of \(\text{Hct}\).

Furthermore, looking at whether dilution of blood affects these aforementioned conclusions, the same analysis based on the mean values of \(\text{SE}\) for the whole blood (Hct ≥ 37\%) suggests that the sequence of geometries as \(\text{TF} > \text{CF} > \text{TC}\). On the other hand, to get a more consistent \(\text{SE}\) for Hct ≥ 37\%, one should choose the geometries in the following sequence as \(\text{TF} > \text{TC} > \text{CE}\), see Fig. 12b. In particular, for the diluted blood (Hct < 37\%) the following for decreasing order of the geometries basis calculated mean values is observed as \(\text{B/S/C} > \text{TF} > \text{CE} > \text{YC} > \text{TC}\), while \(\text{TF} > \text{B/S/C} > \text{CE} > \text{TC} > \text{YC}\) suggests the achievement of less spread-out \(\text{SE}\). In general terms, regardless of the hematocrit level, trifurcation (TF) geometry seems to be the most appropriate for producing more consistent \(\text{SE}\), regardless of the range of hematocrit levels. It is also interesting to note that for whole blood, however, TC and CE geometries show

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**Fig. 12** Comparison of the various geometries using meta-analysis performed herein a for complete range of hematocrits, b separate analysis for whole blood, \(\text{WB}\) (Hct ≥ 37\%) and diluted blood, \(\text{DB}\) (Hct < 37\%)
approximately equal standard deviations; still, a significant change in the mean values can be observed, Fig. 12(b).

Further, the “median” as a representative of the 50th percentile of the data set for SE noted in this review article has also been calculated. Table 6 represents the median values for different geometries for the complete range of Hct’s and for diluted blood (Hct < 37%). Notably, the median values for Hct < 37% are pretty close to the values found for the complete range of Hct’s. The same analysis particularly for Hct ≥ 37% predicts the median values as 42, 75.97, and 95.61 for TC, CE, and TF, respectively. However, regardless of Hct’s, the lowest median value has been noted for TC geometry. The median values have been compared herein against the mean to see if any changes in the sequence of geometry-wise SE can be seen between the two. Interestingly, the sequence of the geometries basis the median values are seen to be different than the obtained sequence basis mean/average values of SE, regardless of Hct’s, see Fig. 12 and Table 6. Further, one important thing to note basis the data available for the Y-channel is that YC was able to consistently achieve 100% SE more frequently. For Hct < 37%, this becomes more apparent, wherein the mean SE for YC is 88.0 but the median indicates SE as 100. Thus, the meta-analysis performed in this study for the microchannel-channel-based geometries utilized to separate blood and plasma suggests that TF and YC appear to be promising designs for achieving high SE.

### 5 Summary and outlook

The present review article demonstrates the microfluidic geometries involved in effective blood plasma separation, such as T-channel, Y-channel, hybrid geometry, etc. Based on the available data, a detailed comparative analysis between the various geometries has been attempted. The key conclusion from present extensive literature study are as follows:

1. The present review article compares various hemodynamic blood plasma separation geometries, based on separation efficiency, SE and hematocrit, Hct, in three categories such as (1) highly diluted blood, i.e., Hct < 10%, (2) moderately diluted blood, i.e., 10 ≤ Hct ≤ 37 (3) whole blood, i.e., Hct ≥ 37%. From the comparative study, it can be concluded that obtaining both high SE and yield, simultaneously is very challenging as it requires a precise control over flow instabilities (i.e., high-pressure fluctuation), flow rate, specific channel dimensions, etc. (Haque et al. 2020, 2021). Certain limitations have been noted for channel-based separation devices, in terms of their application in niche areas as in clinical field, biological field, etc., which requires the devices to separate blood with high purity, high yield, and biological validation.

2. From this review, it is sufficient to say that the modification in the existing geometries in terms of cascading, modulation of the principal/daughter channel dimensions, choice of inclination angle, encompassing a constriction–expansion, including a series of parallel plasma channels, including a bend and curved channels, a combination of converging and bending channels, etc., plays a vital role in enhancing the blood plasma separation (Blattert et al. 2004; Yang et al. (2006); Sollier et al. 2009; Kersaudy-Kerhoas et al. 2010a, b; Prabhakar et al. 2015). A researcher can propose it by combining any of two different geometries intended towards approaching the objective of a ~ 100% SE and high yield for Hct ≥ 37%.

3. The specific questions raised in the introduction section have been answered in detail in the paper. Few key points have been summarized concerning each question: (a) The T-channel, TC, and constriction expansion, CE, geometries have been explored widely regardless of the range of hematocrit level. On the other hand, Y- and curved channels are seen to be the least explored geometries for the complete range of the hematocrit (please refer to Figs. 10, 11a, b for more detail). (b) Through the critical review, it has been noted that the choice of flow rate plays a significant role in the blood plasma separation process. Several researchers have used a wide range of flow rate up to ~ 1200 μL/min according to geometry, channel dimension, hematocrit, etc. An optimum flow rate between 20 and 80 μL/min has shown optimal performance for low hematocrit levels, i.e., Hct ≤ 9.0%, specific to the acoustophoretic separation (Gonzalez et al. 2020). A discussion on the optimum flow rate ratio between the two branches of bifurcating channel has been found in the literature. Several researchers found a flow rate ratio of 6:1 or greater (~8:1) between the two bifurcating branches to be the optimum to achieve better separation efficiency. (c) The bend, spiral, and curved channel-based geometries have not been explored yet for the moderate range of hematocrit level 10 < Hct < 37%.

### Table 6 Summary of the median values calculated for the available SE data

| Geometry | For complete range of Hct’s | For diluted blood, Hct < 37% |
|----------|-----------------------------|------------------------------|
| TC       | 78.50                       | 80.00                        |
| B/C/S    | 90.00                       | 99.00                        |
| CE       | 93.02                       | 93.75                        |
| TF       | 96.75                       | 96.75                        |
| YC       | 100.00                      | 100.00                       |

Data available for the
Also, for the whole blood (Hct \( \geq 37\% \)), the bend and spiral geometries are still to be fully explored. (d) In general, the constriction–expansion geometry is the most exploited geometry, which showed a better performance in separating the blood and plasma regardless of hematocrit level.

4. Although it is difficult to comment on which one is the best geometry to get the highest SE, as each has its advantages and limitations. Still, with the help of meta-statistical analysis, it has been noted that the trifurcation geometry serves best to give a consistent SE either for Hct \( \geq 37\% \) or for Hct < 37\%. Also, specifically for Hct \( \geq 37\% \), the statistical analysis suggests that again trifurcation serves best to give the highest SE on average. In comparison, for Hct < 37\%, bend/spiral/curved geometries seem to be preferable.

5. The present work, focuses specifically on blood plasma separation; thus, the details related to different exploited geometries in separating the biomarker, such as the separation of microorganisms, proteins, molecules, circulating nucleic acid, etc., have been avoided, and minimal information has been included. However, this indicates future scope of this work on those microfluidic structures that separates biomarkers efficiently. Notably, lab-on-a-chip microfluidic devices have been getting attention for separating biological cells and their analysis, therapeutics, and treatment in the past two decades. Further advancement in this field can lead to a microfluidic device as a lab-on-a-cell and organ-on-a-chip for the identification of a particular biological cell and biomarkers along with its characterization, clinical translation, etc. [Clausell-Tormos et al. (2008); Sollier et al. 2009; Kersaudy-Kerhoas and Sollier 2013; Minas and Catarino 2015; Zhang and Radisic (2017); Catarino et al. 2017].

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Declarations

Conflict of interest The authors declare that there are no conflicts of interest with the work submitted in this review paper.

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