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A Review on the Recent Developments in Passive Plasma Separators and Lab-on-Chip Microfluidic Devices †

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Abstract: To ensure the country's sustainable health recovery, viruses like COVID-19 need faster detection and sampling than the rate at which they spread. Blood plasma has proven to be an important and better clinical sample for the detection and diagnosis of various medical conditions as compared to whole blood. For in situ and in vivo health monitoring, plasma can be easily processed through microfluidic Lab-on-Chip (LOC) devices without the clotting that shortens the turnaround time and using minimum amounts of sample and reagents. The present review discusses the key properties of blood plasma as a perfect sample for the microfluidic LOC devices and the importance of passive plasma separators within any kind of LOC device as an embedded unit. The passive LOC plasma separators offer rapid extraction without external forces in the form of a miniaturized automated unit. This article compares various plasma separators on the basis of plasma extraction efficiency, fabrication techniques, and separation science utilized for hemolysis-free extraction. Recent developments in the area of passive bioseparators based on microfiltration and self-driven hydrodynamic and flow-cytometric approaches are discussed in detail.

Keywords: microfluidics; Lab-on-Chip devices; passive plasma separators; self-driven extraction; additive manufacturing techniques; fused filament fabrication; material extrusion; biomedical disposable devices; 3D-printed polymers; rapid prototyping

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1. Introduction

The separation of plasma from whole blood has been the topic of sustainable research owing to its potential for the rapid and early diagnosis of critical medical conditions such as Alzheimer's disease [1], kidney damage [2], cancer [3], acute stroke [4], malaria [5], diabetes mellitus [6], and many other diseases such as viral infections; as well as the identification of the success rates of anti-tumor therapies. Plasma has now been clinically opted as a new standard analyte in the laboratory testing and diagnosis of biomarkers such as free-DNAs [7], enzymes, and other hormones.

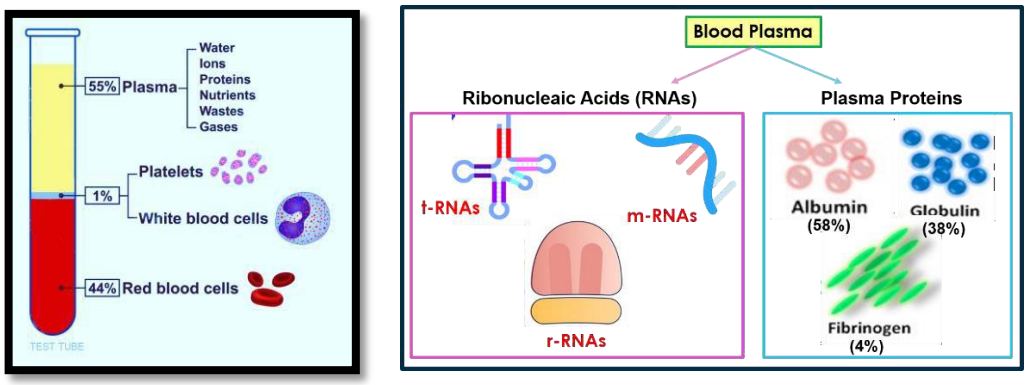
The key properties of plasma [8], such as its Newtonian behavior and its higher fluidity due to a lower viscosity and clotting profile [9], as opposed to whole blood samples (heavier and larger cells [10] other than biomarkers), support easier sample handling, preparation with a minimum number of reagents, and a free-flow operation through microfluidics with on-chip detection or Lab-on-Chip Testing (LOCT). These advantages result in short turnaround times, the low probability of false detection, and compatibility with POCT (Point-of-Care Testing). Filtering out the unwanted and interfering cells such as RBCs (red blood cells—erythrocytes), WBCs (white blood cells—leukocytes), and platelets (thrombocytes) from whole blood, supports a clog-free operation. Blood rheology, RBC clotting, and the viscoelastic physiology of whole blood [10] makes plasma separation the initial protocol for either subsequent lab-testing or chip testing. Furthermore, on-chip plasma extraction develops the base of rapid extraction extended to LOCT, μ-FT (microfluidics testing), and

µTAS (Micro Total Analysis System), to carry-out POCT successfully for the rapid and early detection of biomarkers associated with chronic diseases including HIV/AIDS (human immunodeficiency virus and acquired immune deficiency syndrome), COVID-19, and HBVs/HCVs (hepatitis B and C), etc., for both antibody (Ab)- and antigen (Ag)-based testing, where Ag-based testing is preferable for the early detection of rampant diseases.

This review paper, therefore, covers the importance of LOC microfluidics' and microfiltration approaches for efficient and RBC-free plasma extraction in brief. We conclude with a discussion on selected plasma separation devices fabricated with different subtractive, additive, and replicative techniques for LOCT.

2. Plasma Separation Techniques

RBCs, WBCs, and platelets are generally heavier and larger compared to plasma, as a result they can coagulate due to the RBCs' rheology [11] or the sediment due to the gravitational or inertial forces as shown in Figure 1a. The properties of these cells are exploited in the microfluidics-based active separation techniques [12]. Generally, plasma (the watery part of blood) mostly consists of smaller cells below 500 nm as shown in Table 1, and key biomarkers, as shown in Figure 1b, which aid in the diagnosis of various illnesses. Microfiltration-based approaches [13] utilize the cell size to design and optimize plasma filters to extract target biomarkers accordingly; while, on the other hand, the hydrodynamics properties of plasma, which are better than the whole blood as shown in Table 2, are exploited for microfluidics-based plasma separation [14].



(**a**) (**b**)

Figure 1. (**a**) The components of human whole blood depicting the RBCs settling at the bottom of a test-tube due to the gravitationally assisted sedimentation; (**b**) the constituents of blood plasma depicting the volumetric percentage of plasma proteins and various RNAs.

Table 1. The Physiology of Whole Blood.

Whole Blood Cells	Cell Type	Value
Above 500 nm (Whole Blood Cells)	RBCs	6–8 µm
	WBCs	10–18 µm
	Bacteria	0.5–5 µm
Below 500 nm (Plasma Cells)	RNAs, Proteins and Viruses	2 nm (t-RNA *)
		100–200 nm (mRNA *)
		3.8 × 15 nm (Albumin)
		10–35 nm (Globulins)
		50–140 nm (SARS-CoV-2)
		HIV (100 nm)
		HBV, HCV (40–80 nm)
		CHIK-V (70nm)

* Ribonucleic Acid.

Table 2. A Comparison of Whole Blood and Plasma Characteristics.

Property	Whole Blood	Blood Plasma
Fluid TypeSpecific Gravity	Non-Newtonian1.052–1.056	Newtonian 1.022–1.026
Dynamic Viscosity	3.5–5 cP@ γ * > 200 s^{-1}	1.2–1.3 cP
Fluid Density	1125 kg/m^3	1025 kg/m^3
Cell size range	2–8 µm	20 nm to 140 nm

* γ is shear rate at normal temperature.

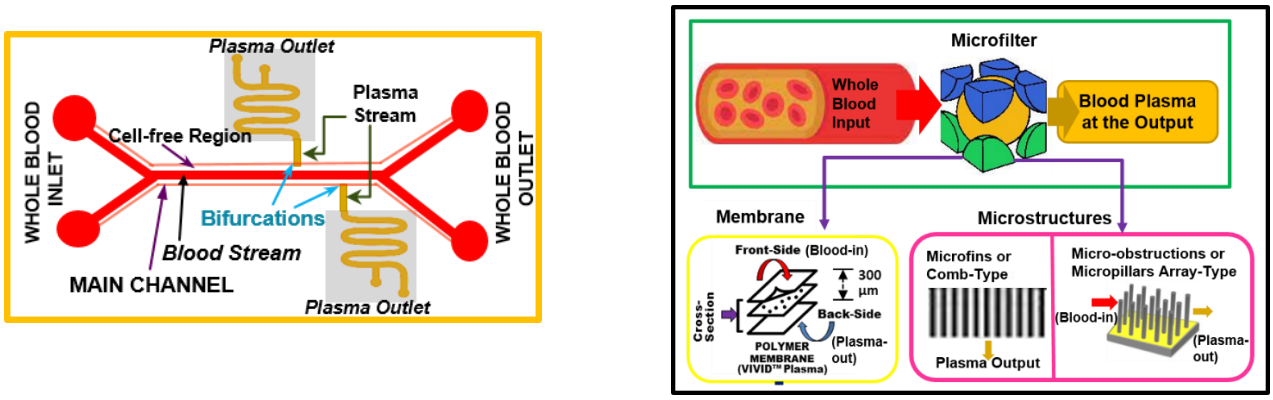
2.1. Force-Driven Active Plasma Separation

Plasma is extracted conventionally from the whole blood contained inside the centrifuge, or a sedimentation chamber of compact discs as per Stoke's Law, by employing centrifugation, electromechanically, at a high rotational velocity (3800 rpm), to release pure plasma at the output [15]. The advancement from CD-based microfluidics towards slanted-spiral microchannels and multiplexed slanted-spirals [16] has assisted ultra-fast rapid extraction, improving the flow rates from 1.5 mL/min to 24 mL/min.

Microfluidics-based cell sorting techniques through activated cell sorting (ACS) exploit flow cytometry via magnetic (MACS) [17], dielectrophoresis [18], and acoustic [19] forces for the microscale extraction of plasma based on cell properties—such as supermagnetic (RBCs) and paramagnetic (WBCs), cell-interaction with the fluid, and cell shape, size, stiffness, and weight, etc., to guide the target cells towards the dedicated direction or position within 25 min of operation with almost 100% purity and label-free detection.

2.2. Self-Driven Passive Plasma Separation

Self-driven passive plasma separation, also referred to as passive cell-sorting techniques, makes use of the internal fluid properties and physical sizes of the cells for the self-separation process rather than the external forces. A schematic of various self-driven passive mechanisms for plasma separation is shown in Figure 2.



(**a**) (**b**)

Figure 2. A schematic showing the operating principal of microfluidics and microfiltration-based plasma separation: (**a**) microfluidics-based self-driven passive plasma separation through the cell-free layer and bifurcations; (**b**) microfiltration-based self-driven passive plasma separation through membrane-assisted and microstructure-assisted plasma filtration.

Microfluidics-based approaches make use of the Newtonian characteristics of blood plasma and hydrodynamic effects. The plasma is extracted via a capillary force-driven followability assisted hydrophobic µ-fluidic channel. The unwanted cells are separated through the hydrophilic or main channel. Microfiltration employs passive cell sorting-based approaches, which operate according to the size-selection trapping of larger blood cells and the microfiltration of plasma either through microstructures or through microporous separation membranes.

Some of the passive cell sorting techniques based on flow cytometry are gravitation-assisted [20], sedimentation [21], deterministic lateral displacement [22], pinched-flow fractionation [23], and biomimetic separation methods [24]. Passive cell sorting through microstructures is based on various filter designs with pores and nano-fibers [25], and comb-like [26] and mesh-type [27] structures. Microporous separation membranes and micro-obstructions oriented for different filter modes such as cross-flow filtration [28], dead-end filtration [29], and tangential-flow filtration [30]—pertaining to blood flow—provides the liberty for the designers to optimize new and better passive plasma separators.

3. Passive Lab-on-Chip Plasma Separation

The field of LOC plasma separators is a recent phenomenon that has cleverly integrated the principles of separation science, flow cytometry, plasma physiology, and blood rheology, to fabricate a rapid, compact, and POC device compatible with LOC architecture. A brief comparison between them on the basis of fabrication technology, device structure, extraction efficiency, and separation technology is depicted in Table 3.

Table 3. A comparison between the passive microfluidic Lab-on-Chip plasma separators.

Fabrication Technology	Plasma Separator /Researcher/Year	Device Structure	Separation Technology	Efficiency/Analyte
Standard SU-8 Photolithography followed by PDMS Soft Lithography	On-chip whole blood plasma separator based on microfiltration, sedimentation and wetting contrast. Park et al. [31] (2015)	Patterning of PDMS to form a micropillar array employing soft lithography on the UV-developed and etched SU-8 mold for retarded flow and microfiltration. Patterning of glass via etching for developing micro-channels for plasma collection.	Retarded flow-assisted sedimentation and filtration of RBCs and WBCs, through array, while free flow wetting of plasma through the ethanol-treated microchannel.	16 nL out of 15 µL of whole blood. Experimental model solution filtering out 4.5 µm of PS beads.
	Self-driven filter-based blood plasma separator microfluidic chip for point-of-care testing. Madadi et al. [32] (2015)	The clogging delay caused by RBCs in a hydrophilic PDMS channel and the symmetric out-of-plane cross-flow filtration microchannel integrated micropillars (MIMPs), exploited to maximize the extracted plasma from undiluted blood.	Separation science, fluid dynamics, and blood rheology. Shear force acting on the main channel while the capillary forces are exerted on the plasma collection channel.	Extracted 0.1 µL of plasma from 5 µL of blood TSH qualitative testing employing diagnostic kit.
SLA 3D Printing with a clear and colorless 3D printing material (Accura ClearVue™)	A self-pressure-driven blood plasma separation device for POC diagnosis. Kim et al. [33] (2022)	The separation device consists of a barrel and a plunger. The barrel holds the diluted whole blood sample. The plunger holds the glass fiber filter. Multiple LFA strip holder/house cover provided to hold rapid diagnostic kits.	Set of seals, self-pressurize the flow through the pored-matrix, binding the erythrocytes on the filter surface readily extracting plasma.	Multiple assay diagnostics 1. HIV Ab 2. HBVs Ag 3. HBVs Ab 4. HCV Ag

Table 3. *Cont.*

Fabrication Technology	Plasma Separator /Researcher/Year	Device Structure	Separation Technology	Efficiency/Analyte
CNC/CAD-CAM Micromachining of PMMA	High-efficiency plasma separator	Cup-shaped primary separation chamber (outer) loaded with anti-RBC-soaked acetate fiber	Acetate fiber matrix allows RBCs' immunocapture.	Extracted 100 µL of Hemolysis-free 100%
	Based on immunocapture and filtration. Su et al. [34] (2020)	pillar matrix (inner) and holds the blood sample. The final purification (bottom) chamber holds the separation membrane (VIVID™ GX), and connects the primary chamber and the plasma collection outlet.	GX- membrane allows size selection trapping of WBCs and platelets.	Pure plasma from 400 µL of whole blood sample. Quantitative PCR HBV testing. Non-protein biomarker glucose recovery rate is 100%± 0.73%
Hybrid Technology	Capillary flow of blood in a micro-channel with differential wetting for blood plasma separation and on-chip glucose detection. Maria et al. [35] 2016	DLP 3D printing, SU-8 photolithography followed with PDMS soft lithography. The main PDMS microchannels designed to be hydrophilic near the inlet side, and hydrophobic near the detection window.	Capillary-driven PDMS channel with dual wettability nature acts as a self-filter for plasma extraction exploiting differences in the viscosity and dynamic fluid velocities of the blood and plasma.	450 nL pf plasma was extracted. Plasma recovery efficiency was 22.5%. on-chip detection of glucose.

4. Conclusions

In vivo monitoring of diseases is a critical issue, especially in the severe stages of an infection. LOC plasma separators can improve the survival rate of patients by achieving an early and rapid diagnosis. We have briefly reviewed the prominent techniques and devices for the passive microfluidic LOC plasma separators developed to date; however, while detailed elaboration is beyond the scope of this paper, the presented information sequentially covers their key aspects in terms of the fabrication technology, extraction efficiency, and the detected analytes.

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Abbreviations

DNA	Deoxyribonucleic acid
COVID 19	Corona Virus Disease 2019
SARS-CoV2	Severe Acute Respiratory Syndrome Coronavirus2
CHIKV	Chickengunya Virus
PDMS	Polydimethylsiloxane
PMMA	Polymethylmethacrylate
DLP	Digital Light processing
SLA	Stereolithography
SU 8	Epoxy based Negative Photoresist

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