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A Review on Recent Developments in Passive Plasma Separators Lab-on-Chip Microfluidics Devices

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Featured Application: On-Chip Early Detection of Protein and Non-Protein Biomarkers associated with various health conditions.

Abstract: Viruses like COVID-19 need faster detection and sampling than the rate at which they spread to ensure the country's sustainable health recovery. 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 Microfluidics Lab-On-Chip (LOC) Devices without clotting that shortens the turnaround time with a minimum sample and reagents. The presented work discusses key properties of Blood Plasma which makes it a perfect sample for microfluidics 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 miniaturized automated unit. The article compares various plasma separators on the basis of plasma extraction efficiency, fabrication techniques, and separation science utilised for haemolysis free extraction. Recent developments in the area of passive bioseparators based on microfiltration, self-driven hydrodynamic and flow cytometric approaches are discussed in detail.

Keywords: Microfluidics; Lab-on-Chip Devices; Passive Plasma Separators; Self-driven extraction; Addi-tive Manufacturing Techniques; Fused Filament Fabrication; Material Extrusion; Biomedical Disposable Devices; 3D Printed Polymers; Rapid Prototyping.

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1. Introduction

Separation of Plasma from whole blood had been the topic of sustainable research owing to its potential of rapid and early diagnosis of critical medical conditions such as Alzheimer's disease [1], kidney damage [2], Cancer [3], Acute Stoke [4], Malaria [5], Diabetes Maleates [6], and many other diseases such as viral Infections, success rates of anti-tumor therapies. Plasma is now been clinically opted as a new standard analyte in the laboratory testing and diagnosis of the biomarkers such as free-DNAs [7], enzymes and other hormones

The key properties of plasma [8] such as Newtonian behaviour, higher fluidity due to lower viscosity and clotting profile [9] as opposed to whole blood samples (heavier and larger cells [10] other than biomarkers) supports easier sample handling, preparation with minimum reagents, free-flow operation through microfluidics with on-chip detection or Lab-on-Chip Testing (LOCT). All these advantages results in short turnaround time, low probability of false detection and compatibility with the POCT (Point-of-Care Testing).

Filtering out unwanted interfering cells such as RBCs (Red Blood Cells- Erythrocytes), WBCs (White Blood Cells-Leukocytes) and Platelets (Thrombocytes) from whole

blood, is suitable for clogging-free operation. Blood Rheology, RBCs clotting and viscoelatic physiology of whole blood [10] makes plasma separation the starting protocol for either subsequent Lab-Testing or Chip Testing. Further, on-chip plasma extraction develop the base of rapid extraction extended to LOCT, μ-FT (Microfluidics Testing) and μTAS (Micro Total Analysis System), to carry-out POCT successfully for rapid and early detection of biomarkers associated with chronic diseases including HIV-AIDS (Human Immunodeficiency Virus and Acquired Immune Deficiency Syndrome), COVID-19, HBVs/HCVs (Hepatitis-B and C), etc, for both Antibody (Ab) and Antigen (Ag) based Testing, where Ag-based testing are preferable for early detection of rampant diseases.

This review paper therefore covers the importance of LOC microfluidics and microfiltration approaches for efficient and RBCs-free plasma extraction in brief. At last we concluded by discussing some of the Plasma separation devices fabricated with different subtractive, additive and replication techniques for LOCT.

2. Plasma Separation Techniques

The RBCs, WBCs and platelets are generally heavier and larger as compared to Plasma, as a result they can coagulate due to RBCs Rheology [11], sediment due to gravitational or inertial forces as shown in figure 1(a). The properties of these cells are exploited in Microfluidics-based Active Separation Techniques [12]. Generally, plasma (watery part of blood) mostly consists of smaller cells below 500nm as shown in Table 1, and key biomarkers as shown in figure 1 (b) which aids in diagnosis of various illnesses. Microfiltration-based approaches [13] utilizes cell sizes to design and optimize plasma filters to extract target biomarkers accordingly, 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].

Table 1. Physiology of Whole Blood

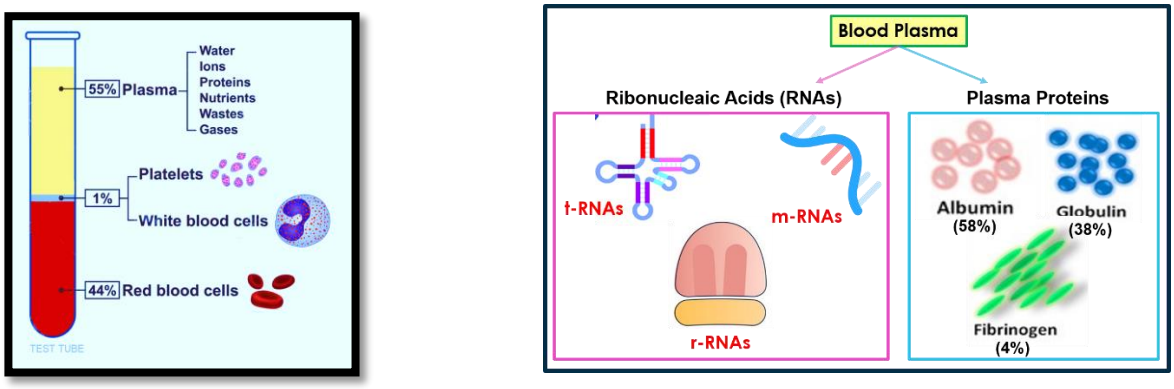
Whole Blood Cells	Cell Type	Value
Above 500nm	RBCs	6-8 μm
(Whole Blood Cells)	WBCs	10-18μm
	Bacterias	0.5-5μm
Below 500nm	RNAs, Proteins and	2nm (t-RNA)
(Plasma Cells)	Viruses	100-200nm (mRNA)
		3.8X15nm (Albumin)
		10-35nm (Globulins)
		50-140nm (SARs-CoV-2)
		HIV (100nm)
		HBV, HCV (40-80nm)
		CHIK-V (70nm)

*Ribonucleic Acid

Table 2. Comparison of Whole Blood and Plasma Characteristics

Property	Whole Blood	Blood Plasma
Fluid Type	Non-Newtonian	Newtonian
Specific Gravity	1.052-1.056	1.022-1.026
Dynamic Viscosity	3.5-5 cP@ γ>200s^{-1}	1.2-1.3 cP
Fluid Density	1125 kg/m^3	1025 kg/m^3
Cells size range	2-8 μm	20nm to 140nm

*γ is shear rate at normal temperature



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

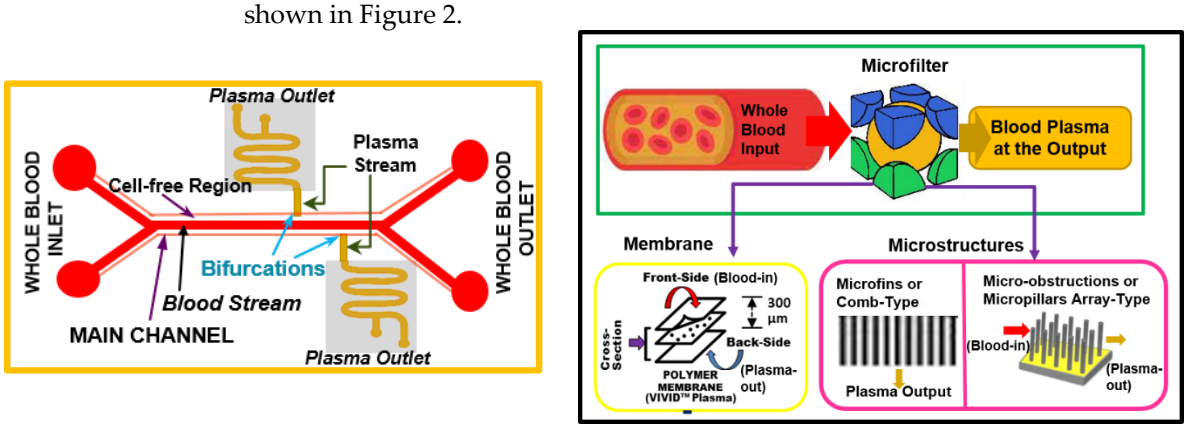
Figure 1. (a) Components of Human Whole Blood depicting RBCs settling at the bottom of test-tube due to Gravitational-assisted sedimentation; (b) Constituents of Blood Plasma depicting volumetric percentage of Plasma Proteins and various RNAs.

3.1. Force Driven Active Plasma Separation

Plasma is extracted conventionally by employing centrifugation, electromechanically, at a high rotational velocity (3800 rpm) from the whole blood contained inside the centrifuges or a sedimentation chamber of the compact discs per the Stroke's Law, to release pure plasma at the output [15]. The advancement from CD-based microfluidics towards slanted-spiral microchannel and multiplexed slanted-spirals [16] assists 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 Accoustic [19] forces for microscale extraction of Plasma based on cell properties like supermagnetic (RBCs), paramagnetic (WBCs), cell-interaction with the fluid, cell shape, size, stiffness, weight, etc. to guide the target cells towards the dedicated direction or position within 2-5 minutes of operation with almost 100% purity and label-free detection.

3.2. Self-driven Passive Plasma Separation

Also sometimes referred to as Passive Cell-Sorting Techniques make use of internal fluid properties and physical sizes of the cells, itself for self-separation rather than external forces. The schematic of various Self-Driven Passive Mechanism for Plasma Separation is shown in Figure 2.



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

Figure 2. Schematic showing operating principal of Microfluidics and Microfiltration-based Plasma Separation: (**a**) Microfluidics-based Self-Driven Passive Plasma Separators through Cell-free Layer and Bifurcations; (**b**) Microfiltration-based Self-Driven Passive Plasma Separation through Membrane-Assisted and Microstructures-Assisted Plasma Filtration

Microfluidics-based approaches makes use of the Newtonian characteristics of blood plasma and hydrodynamic effects. The plasma is extracted via capillary force driven followability assisted hydrophobic μ-fluidic channel. The unwanted cells are separated through hydrophilic or main channel. Microfiltration employ Passive Cell Sorting based approaches by size selection trapping of larger blood cells and 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 are based on various filter designs with pores and nano-fibers [25], 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], w.r.t. blood flow, provides liberty to the designers to optimize new and better Passive Plasma Separators.

3. Passive Lab-on-Chip Plasma Separation

The field of LOC Plasma separators is quite new, that smartly integrated the principles of separation science, flow cytometry, plasma physiology, blood rheology, together to fabricate a rapid, compact and POC device compatible to LOC architecture. A brief comparison among them on the basis of fabrication technology, device structure, extraction efficiency and separation technology is depicted in Table 3.

Table 3. Comparison among Passive Microfluidics Lab-On-Chip Plasma Separators

Fabrication Technology	Plasma Separator /Researcher /Year	Device Structure	Separation Technology	Efficiency/ Analyte
Standard SU-8 Photolithography followed by PDMS Solf-Lithography	On-chip whole blood plasma separator based on microfiltration, sedimentation and wetting contrast. Park et.al [31] (2015)	Patterning of PDMS to form Micropillar array employing Soft-Lithography on the UV developed and Etched SU-8 Mold for retarded flow and microfiltration. Patterning of Glass via etching to developing microchannels 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.	16nl out of 15μl of whole blood. Experimental Model solution filtering out 4.5μm 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 act on the main channel while the capillary forces exerted on the plasma collection channel.	Extracted 0.1μl of plasma from 5μl 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. Barrel holds the diluted whole blood sample. 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
CNC/ CAD-CAM Micromachining of bulk PMMA	High-Efficiency Plasma Separator	Cup-shaped primary separation chamber (outer) loaded with Anti-RBC soaked acetate fiber	Acetate fiber matrix allow 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), connects the primary chamber and the plasma collection outlet.	GX- Membrane allow size selection trapping of WBCs and Platelets.	Pure Plasma from 400µl 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 microchannel 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 at near the detection window.	Capillary driven PDMS channel with dual wettability nature act as a self-filter for plasma extraction exploiting differences in the viscosity and dynamic fluid velocities of the Blood and Plasma.	450nl pf plasma was extracted. Plasma recovery efficiency was 22.5%. On-Chip Detection of Glucose.

4. Conclusions

In-vivo monitoring, is critical issue, especially in case of severe stage of infections. LOC Plasma separators can assist to improve the survival rate of such patient with early and rapid diagnosis. We have reviewed the best known techniques and passive Microfluidics LOC Plasma Separators developed so far in brief, as detail elaboration is beyond the scope this paper. However, presented information covers all aspects in terms of fabrication technology, extraction efficiency, and detected analytes in a sequential manner.

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