A review on microfluidic devices for separation of blood constituents

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Abstract. This paper present review of microfluidic device used for extraction of red blood cell, white blood cell, and plasma from the whole blood sample. Micro fluidic based cell separation has various advantages as it reduces sample size, faster sample processing, has more sensitivity and low device cost as compared to the conventional method of blood cell separation. Basically there are two techniques for blood separation using microfluidic device, one is called active separation and another is passive separation method. The review highlights various cell separation methods. We will also take review on microfluidic based blood cell separation techniques. Separation of blood cells helps in clinical diagnosis and therapeutic research.

1. Introduction

Now a day’s people are very concern about their health with increase of life cycle related disease. There is going to be huge demand for periodical blood test which we can carry out at home. Conventional methods are impossible to perform at home. Hence, development and characterization of easy to use microfluidic devices or lab on chip devices for blood test is much more needed.

Separation of blood cells is the first step towards the blood analysis, as every component provides different information. Anaemia disease is diagnosed by examine physiological changes in red blood cells levels. Various constituents of blood viz; white blood cell, plasma and red blood cell play various vital roles for human body. On v/v basis blood contains about 45%-50% of red cells, 1% for WBS-platelets and 55% is plasma which has yellow colored [1-2]. Each of these serves various important functions for body. Typically, the immune system largely dependent on white blood cell present in the blood. Pure blood has a density of 1040–1070 kg m−3 and a viscosity of 4–5 cP at 37 °Centigrade. The ratio of WBCs against RBCs is 1: 600–900. Blood is non–Newtonian fluid. Blood cells are extensively used in clinical diagnosis. Extracting the red blood cells from a whole blood sample gives the significant information about the pathological characteristics of breast cancer cells. Now a day complete blood count (CBC) is used everywhere as it is most common blood test. This complete blood count (CBC) test shows the overall health status of patient [3].

Mainly there are two common conventional techniques for blood cell separation, one is Centrifugation and another is filtration. These methods are expensive and require lot of time for completion, skilled labour and if not used carefully might damage cells. Due to above disadvantages researchers introduces microfluidic technique for separation of blood cell. Microfluidics is a platform
that offers significant advantages. These microfluidic techniques required less volume of blood sample also it has high response time and less expensive than the conventional methods. It is therefore not surprising that the microfluidic techniques of blood cell separation have increasing interest over the last decade. There are so many microfluidic devices which have been developed for the separation of blood cells and plasma extraction. In this review, our aim is to present the current blood cell separation techniques using microfluidic channels. Various techniques have been used for blood cells separation by microfluidic system which includes bifurcation law, geometric obstructions, membrane filtration acoustic standing wave forces, filters and cross-flow filtration. Microfluidic is an ideal technique for whole blood cell separation. Due to its very small channel size, the required amount of sample volume can be minimized. Basically, the microfluidics system for blood cells separation is categorized into passive and active separation methods [3-4]. Active separation methods require external source (forces) like magnetic force, dielectrophoretic force or acoustic force, or any combination of these forces to give blood cells a specific direction. While passive separation method simply uses physical properties of cell like their shape and size or their stiffness. This review studies various active and passive separation techniques based on their principle of separation.

Passive separation techniques doesn’t need of cell labeling process due to which they are more cost effective and suitable for separating blood cells. Passive separation techniques has many advantages like they are simple in design, continuous operation, and they are easy to fabricate. To design effective blood cell separation micro devices, which are based on hydrodynamic principal we must need to understand the theoretical mechanism of blood flow in micro channels.

2. Mechanism of blood flow separation

![Figure 1. Classification of Blood separation techniques](image-url)
There are various mechanisms for blood cells separation. They are mainly divided into two types, 1) active separation technique 2) Passive separation techniques. In active separation technique external forces are required for separation. In passive separation technique the separation takes place in designed microfluidic devised geometry by virtue of sizes of constituents of blood. These techniques are further classified into the different separation techniques. Figure 1 explain in detail classification of blood constituent’s separation techniques.

2.1. Hydrodynamic technique
This study presents most of the blood cells separation techniques based on microfluidic. In study it is found that most method separates only one type of cell component by extraction or rejecting. Study optimizes the design of bifurcation channel, they also added trapping units, the author introduces new techniques through which it can simultaneously separates blood components by a continuous-flow whole blood processing microfluidic device. This is working on the principle of hydrodynamic [3]. Hydrodynamics filtering does not require any external force for working of a device hence hydrodynamic filtering is widely used. As compared to other separation technique, Hydrodynamic technique has simple design. There are two types of side channel in this device and both are at angle of 60 degree to the main channel (see Fig. 2). The device also contains series of white blood cells trapping units based on hydrodynamic principle. The total time required for overall process is just 20 minutes and it requires only 6μL of whole blood sample [3].

![Figure 2. Entrance of the plasma zone and RBC zone [3].](image)

![Figure 3. Microfluidic device [3].](image)

Figure 3 shows microfluidic device which consists of two inlets one is for blood sample inlet and another is a buffer inlet. As the blood sample enters from inlet and passes through the bifurcation region i.e. the orange colour zone, plasma and red blood cells were trapped into plasma zone and red blood cell zone, respectively. Then White blood cells will flow towards the main channel and will trap in the white blood cell zone. As you can see in the figure 3 red particle are RBCs and green particle in the plasma zone are packed beads.

The author aim is to examine the microfluidic device which can simultaneously extract red blood cells, plasma and white blood cell, when only 6 μL of whole blood sample is loaded into the device in just 20 minutes. By this microfluidic device all three cells of blood can be extracted simultaneously without any interference.
In the figure 4 the green line indicates plasma zone, white and yellow lines represents RBC and WBC zone respectively [3]. The author has successfully developed the microfluidic device which can simultaneously separate plasma, red blood cell and white blood cell. This experiment has two main important features, 1) plasma and red blood cells are separately trapped in the side channels of the bifurcation region i.e. orange zone. And we can use the extracted red blood cells for blood test; also the extracted plasma has low haemolysis effect. Second important feature is that, in the design of device the author added some hydrodynamically based white blood cells trapping units in a series form to main channel. In just 20 minutes near about 1800 white blood cell can be trap in that main channel. These trapped white blood cells will be helpful for various blood tests.

2.2. Microfiltration

Microfiltration is one of the important passive separation methods; this is used in micro devices for filtration. The sample volume required for this process is very small. This technique is further classified as cross flow filtration or dead end technique. The fluid i.e. blood sample flows directly into the micro filters are considered as dead end technique. While in cross flow technique blood separation take place tangentially to flow direction. The scientists have come across with the micro filters which have a pore size of 14 μm and it contains near about 14400 holes. This technique is mainly used for plasma separation, as plasma cells are very small in size. But this technique has some disadvantages as it has low flow rate and its fabrication is complex.

2.3. Sedimentation

Sedimentation technique is based on the differences in density of cells and plasma; it is also one of the passive separation techniques. Its main function is to separate plasma from the whole blood sample. Due to the density difference blood cells sediment at the bottom and layer of plasma comes up at top from which we can easily extract plasma. It is an old technique and now days no one prefer this technique because it takes lot of time for processing and also extraction rate is very low.

3. Materials and fabrication

Selection of material and fabrication technique for the microfluidic devices is one of the major issues. Initially, glass and silicon were the materials used for fabrication. But these materials have some disadvantages like high cost, brittle nature, complex fabrication techniques. Due to these disadvantages scientist introduced alternative material which is based on polymer called polydimethylsiloxane (PDMS). The reason behind choosing this material is low cost, easy to fabricate device, elasticity and transparency. PDMS is used widely especially those who working in biomedical. Many microfluidic devices are fabricated using a standard soft lithography [14-18].

4. Techniques of blood separation
This section presents review of literature on separation of constituents of blood. Table1 presents comprehensive review of different mechanisms presented by researchers for separation of blood constituents.

**Table1. Review of various mechanisms for separation of constituents of blood.**

| Author            | Active / Passive | Mechanism | Injected sample | Sample volume | Desired target | Performance |
|-------------------|------------------|-----------|-----------------|---------------|----------------|-------------|
| Da-Han Kuan [3]   | Passive          | Hydrodynamic | Whole blood     | 0.3 μL/min    | Plasma, RBC, WBC | Low hemolysed plasma, 1200–1800 trapped WBC |
| Myoungg on Kim[6] | Passive          | Hydrodynamic | Whole blood     | 0.33 μL/min   | WBC            | 96.9% RBC purity 97.2% WBC recovery rate. |
| John A Davis[7]   | Passive          | Hydrodynamic | Whole blood     | 0.4 μL/min    | Plasma         | 100% plasma recovery rate 100% cell removal rate |
| Ivan K. Dimov[8]  | Passive          | Sedimentation | Whole blood     | 5 μL in 10 min | Plasma         | 99.9–100% blood cell retention |
| Hye-Kyoung Seo [9]| Active Passive   | Magnetic + Hydrodynamic | 1000X RBC dilution using PBS | 1000 μL/min | WBC, RBC | 86.8% RBC separation efficiency 29.1% WBC separation efficiency |
| Mahdi Mohammadi et al [10] | Active/Passive | Dielectrophoretic + Hydrodynamic | Whole blood mix with 1:1 heparin sodium | 2 μL in 7 min | Plasma | 100 nL plasma with 99% purity |
| Yuchao Chen[11]   | Active           | Acoustic   | Whole blood     | 1000 μL/min   | Platelet       | >85% Platelet recovery rate >80% RBC/ WBC removal Rate |
### 4.1. Comparative analysis of passive blood separation techniques

This section presents comparison of three main passive blood separation techniques. Further section discusses their advantages and disadvantages in the Table 2. From the Table 2 we can observe that techniques based on sedimentation or microfiltration required very low amount of sample volume. But these techniques have so many challenges and disadvantages.

**Table 2. Comparison of various methods of blood flow separation techniques**

| Method              | Principle                                      | Advantage                      | Disadvantage                        |
|---------------------|------------------------------------------------|---------------------------------|-------------------------------------|
| Hydrodynamic        | Biophysical effects, geometric                  | Continuous output and High throughput | Sample volume Counteracting yield and separation |
| Microfiltration     | Differences in cell size and shape,             | Sample volume required is very small | It has low flow rate and fabrication is complex |
| Sedimentation       | Difference in density of cells and plasma       | Volume require is 1–10 µL       | Time consuming, extraction rate is very low |

| Ki-Ho Han[13]       | Active Magnetic                                 | 10X diluted whole blood using sodium hydroxulfit e. | RBC, WBC separation efficiency 97.4% RBC separation efficiency |
|---------------------|------------------------------------------------|--------------------------------------------------|---------------------------------------------------------------|
| P. Dow et[19]       | Active Acoustic                                 | Spiked bacteria in PBS diluted whole blood to 20%Hct | >85% RBC removal rate, And 45–60% Bacteria yield |
| DowSung Yang[20-21] | Passive Hydrodynamic                            | Whole blood                                    | 100% plasma purity plasma Extraction |
5. conclusion
The review has focused on various blood cells separation techniques. We mainly review different passive blood separation methods like hydrodynamic and microfiltration. Passive separation techniques have high potential to give solutions, when the difficulty arises during blood separation. Due to use of microfluidic devices the sample volume decreases by default. Among all passive blood separation techniques, the hydrodynamic technique for blood separation is reliable. We have also seen a device which only can simultaneously trap red blood cells, plasma and white blood cells by using passive hydrodynamic separation technique. Polydimethylsiloxane (PDMS) is extensively used for fabrication of many microfluidic devices. Many challenges which arise in sedimentation and filtration are solved by using hydrodynamic technique.

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