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# Highly Portable Continuous Plasma Separator for Whole Blood

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## Abstract

Plasmapheresis for separating plasma from whole blood is important in therapy of immune disease and in treatments of various diseases. This paper discusses a new method to develop a highly portable, slow flow rate plasmapheresis device for continuous therapeutic treatments of patients. The plasmapheresis device, composed of blood flowing tubes and pump, allows a closed, sterile continuous process, which will be applicable to therapeutic usage. The new separation process is based on hydrodynamic effects and the gravitational force. It is very simple and gentle process which can minimize mechanical damage to the cells and also minimize clotting and coagulation problems of blood cells. The separation method provides a unique apparatus with few moving parts and low pressure drop that is very different in operating principle from existing techniques such as centrifugation and membrane ultrafiltration. The new method makes possible development of a small plasmapheresis device which will result in an easily operated pheresis system. It should be less expensive than existing methods, and also operate with less cost in hospital or home. Such a pheresis device would make possible continuous or more frequent, e.g. daily, treatment of patients with immune diseases. It will also minimize hypotension of patients during treatment.

**Keywords**: Plasma separation, blood plasma, plasmapheresis, immune disease, field flow fractionation, FFF, gravitational FFF, GFFF.

## Contents

# 1. Introduction

Plasmapheresis is a separation process to remove plasma from whole blood. Plasmapheresis treatments are usually applied to patients who have failed in other standard therapies. Plasmapheresis is very important in therapy of immune disease and is widely used in the treatment of various diseases, such as myasthenia gravis, Goodpasture's syndrome, Guillain Barre syndrome, macroglobulinemia, hyperglobulinemia, thrombotic thrombocytopenic purpura, chronic inflammatory demyelinating polyneuropathy, deteriorating life-threatening rheumatoid vasculitis, and deteriorating life-threatening lupus erythematosus [1].

Principal methods of existing plasmapheresis has been a high speed spinning centrifugation or an ultrafiltration using membranes to separate cellular components from plasma [2]. A continuous centrifugation is composed of a bowl or ring-shaped blood chamber spinning at approximately 1500 rpm. Blood is continuously fed from the patient into one end of the chamber, and plasma and blood cells are separately recovered from the other end. In the ultrafiltration method of plasmapheresis, blood is pumped through semi-permeable membranes of hollow fibers or plates. These membranes have a specific pore size to cut-off cells and certain molecules. Typically, cellular components such as red cells, white cells and platelets are retained, and immunoglobulins are passed through.

The separated blood cells may be returned to the patient with plasma replacement fluids; albumin, or fresh frozen plasma [3]. According to clinical needs with regard to blood diseases, additional treatments of several sorbent systems are used for relatively specific in absorption of classes of antibodies, including the Staph Protein A column [4]-[7]

We intend to develop a highly portable, slow flow rate plasmapheresis device for continuous therapeutic treatments of patients. In most present plasmapheresis treatments, the plasmapheresis removes approximately 2 liters of plasma and replaces by donated plasma protein during a 2 to 5 hour period. However, the benefits of slow continuous plasmapheresis therapies should be considered [2], [8], [9]. Slow treatment could reduce various risks such as unexpected clinical complications due to rapid fluid replacement, effectiveness of immuno-suppressive agents, hypotension caused by the kallikrein content of donated plasma, and hepatitis accompanying large volumes of donated plasma and albumin [10]. Also the removed globulin could just be collected and discarded since the body would recreate new globulins during the slow process. A slow therapy could reduce treatment cost by reducing expensive donated plasma protein [2], [9].

Plasmapheresis treatment is not painful, but it requires repeated and prolonged procedures. Furthermore, present plasmapheresis therapies have significant limitation such as the high cost of the equipment, complexity of equipment operation, anticoagulation problems, and the high cost of replacement fluid.

It is desired to make plasmapheresis treatment much easier and less expensive. If a small portable, wearable plasmapheresis device is available and is used comfortably in patient's daily life, the above-mentioned problems could be significantly minimized. However, a small portable wearable plasmapheresis device would be very difficult to

make utilizing present components such as centrifugal bowl or ultrafiltration membranes, because of the power requirements of these devices, and the need for complete anticoagulation of the blood passing through these devices.

Our new plasma separation method will make possible development of a small, portable, slow-flow-rate plasmapheresis device. It will be easily operated and should be less expensive than existing methods. Such a pheresis device would make possible more frequent, e.g. daily, treatment of patients.

The new plasmapheresis device is based on hydrodynamic effects and the gravitational force. The device concept is similar to the Field Flow Fractionation (FFF) method [11]. In general FFF is composed of a separation flow cell with an applied field such as gravitational, thermal, electric or magnetic field with a pump and an analytical device [12]. The concept of FFF was introduced in the mid 1960s [11]. Since then it has been widely extended to analytical instruments and separation technologies in various fields, such as characterization and purifications of macromolecular, colloidal, particulate materials and biological cells [12], [13]. One of the techniques for cell sorting by mass, size or density have been called gravitational FFF (GFFF) which is operated with the earth gravity of 1 g. It has been applied for blood cell sorting mainly for analytical purposes [14]-[16]. Our plasma separation device is conceptually similar to GFFF, however the operation is quite different. Our plasmapheresis device continuously separates plasma from blood containing highly concentrated cells for prolonged time.

The proposed plasmapheresis device is mainly composed of blood flowing through tubing and blood circulation pumps. The separation is a closed, sterile continuous flow process, which will be applicable to clinical usage. Except for the pump, there is no moving part such as a high speed-spinning chamber of a centrifugation machine, and can be operated with relatively low-pressure drop unlike a conventional membrane separator. Therefore, our proposed plasmapheresis device can be very small and portable, and will be wearable since the operation energy requirement can be minimized.

Our plasmapheresis machine operates at the blood flow rate of 1 - 3 mL/min. The separation process is based on the gravitational force, and it is very gentle, minimizing or eliminating mechanical damage of blood components. It can minimize contamination, clotting, and coagulation problems of blood cells during separation. Usage of anti-coagulant can be minimized. Blood cellular components separated from plasma could be returned to the patient directly after treatment. For plasma treatment sorbent systems mentioned earlier for absorption of antibodies could be included in a wearable and nighttime pheresis systems.

Compared with existing commercial entities such as continuous centrifugation or an ultrafiltration method using permeable membranes, the new proposed device is very simple and small. It will allow developing an inexpensive, highly portable wearable plasmapheresis device which will be useful for slow continuous therapeutic treatments of patients [2].

If a small wearable plasmapheresis device is available in therapy and is accepted for continuous, daily treatments during working or during night, it will have significant benefits in plasmapheresis therapy as follows [2], [9]:

1. Minimizes various symptoms such as weakness, malaise, or paresthesia.
2. Reduce possible bleeding or hypocalcemia since doses of anticoagulants such as heparin can be minimized.
3. Prevent the "rebound" effect accompanying immuno-suppression.
4. Prevent risks of hepatitis and hypotension by reducing or eliminating the usage of donated plasma or albumin.
5. Reduce treatment costs by minimizing the usage of expensive human protein.
6. Does not need a large size intravenous needle.

An intravenous slow plasma separation system was investigated for the development of a mobile, wearable ultrafiltration plasmapheresis system [8], [9]. The device used hollow fibers to separate blood cells from whole blood at the flow rate of 3 mL/min. It could separate platelets as well as red and white cells from plasma. However, it was reported that the separator required a backflush cleaning process every 5 minuets periodically to maintain hollow-fiber efficiency [8]. We also intend to develop a slow plasma separator. Our method will provide a much simpler device, and have less operation problems. Our method is not desired to separate platelets and blood components very well other than red blood cells. If we need to remove blood components such as platelets, we can combine our method with a hollow-fiber separator. After separating red blood cells with our new separator, platelets and specific other blood components such as immunoglobulin molecules can be efficiently separated with a membrane separator.

The proposed plasma separator will make possible development of a highly portable mobile plasmapheresis device. It should be less expensive and can be used during working or at night. Our preliminary experimental results of the proposed method are discussed in the following section.

## 2. Preliminary Studies

### 2.1. Method of Plasma Separation

The proposed plasmapheresis device is composed of a separation column and blood flow pumps. The separation column has a long flow channel of a thin tube of a rectangular-cross-section with one-inlet and multiple outlets.

An experimental setup to demonstrate the proposed plasmapheresis device was composed of a separation column of a thin tube of a rectangular-cross-section with one-inlet and three-outlets as shown in Fig. 1. The separation channel of our test device was 4.8 m long and was wound in a groove on an 82 mm diameter Plexiglas tube surface. The tube axis was kept vertically. The cross-section of the flow channel was 1.96 mm x 0.61 mm.

The operation principle of plasma separation is very simple. Blood is pumped through the separation channel. During traveling in the tube, blood cells are settling in the flow channel by the gravitational force, and a plasma rich phase is formed at the top of the flow channel. A blood cell rich phase is gradually established at the bottom of the flow channel during flowing and recovered from the bottom outlet (outlet #1 in Fig. 1). Plasma is recovered from the top outlet (#3 in Fig. 1). The separation process can be operated continuously. The middling from the outlet #2 in Fig. 1 can be returned to the inlet and is recycled.

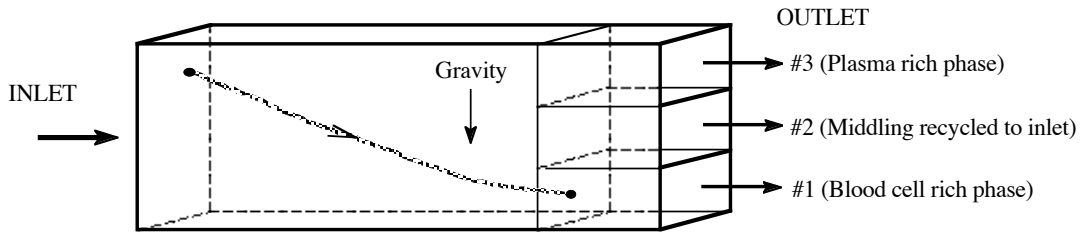


Fig. 1 Schematic showing a concept of a gravitational channel separation process having a rectangular-cross-section flow cell with one inlet and three outlets.

2.2. Experimental results and observations of Plasma Separation

Fig. 2 shows red blood cell concentrations (hematocrit: Hct %) of the outlet products for the inlet feeds of whole blood (Hct 44%) and Hct 33% blood diluted with plasma. The blood samples were fed through the 4.8 m separator at room temperature of 22°C. The flow rate was 0.3 mL/min. As seen in the figure, plasma rich blood of Hct 33% was obtained from whole blood at the outlet #3. When the feeding blood was Hct 33%, Hct 10% blood was obtained from the outlet #3. At the double speed of the flow rate (0.6 mL/min), blood products obtained by the same separator are shown for the various hematocrit blood samples of whole blood (Hct 44%), 21%, 17% and 9% in Fig. 3. The #3 outlet product from the blood sample of Hct 17% was Hct 0.8% plasma. From Hct 9% blood, both #2 and #3 outlet products were Hct 0.5% plasma. In this case the plasma production rate of each channel was 0.2 mL/min.

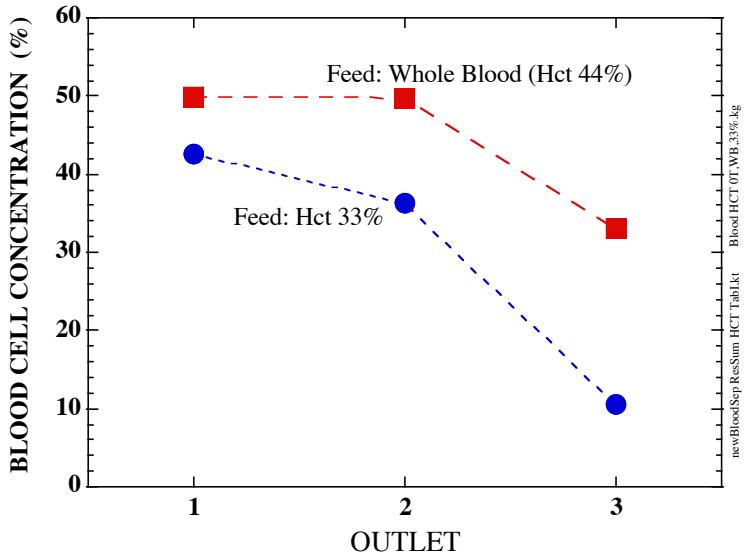


Fig. 2 Red blood cell concentrations (Hct %) of the products obtained from three outlets of a 4.8 m separator for the feeds of whole blood (hematocrit 44%) and hematocrit 33% diluted blood with plasma at the inlet flow rate of 0.3 mL/min.

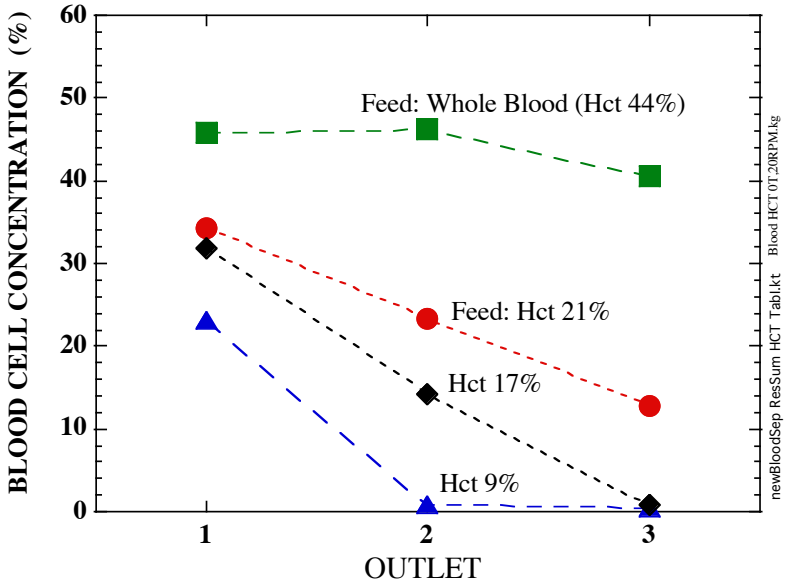


Fig. 3 Red blood cell concentrations (Hct %) of products obtained from three outlets of a 4.8 m separator for the feeds of whole blood (hematocrit 44%), hematocrit 21%, 17% and 9% blood samples at the inlet flow rate of 0.6 mL/min.

## 2.3. Multiple Stage Continuous Flow Operation

Key elements of separation designs are the flow channel size and the flow speed. To make a plasmapheresis device for whole blood, a multiple stage operation will be required. Two-stage operation using two three-outlet separators was used for this demonstration test. The separation setup is shown in Fig. 4. Fig. 5 shows the block diagram of the two-stage system. The first stage was a 4.8 m separator, and the second was 3.6 m. These two stages were connected with an open reservoir. However they can be directly connected if the pump flow speeds are adjusted properly. The cross-section dimension of both flow channels was 1.96 mm x 0.61 mm. Inlet flow rate of both separators was 0.3 mL/min. The two-stage separator used here was not optimized, but it produced plasma of Hct 10% from whole blood of Hct 44% at the net production rate of 0.03 mL/min. Figure 3 suggests that a three-stage system by adding one more stage to the device of Fig. 2 would produces very clean plasma (less than Hct 0.5%) from whole blood at the net flow rate of 0.02 mL/min. Using an optimized system it will be possible to increase the process speed to more than 1 mL/min as will be discussed later.

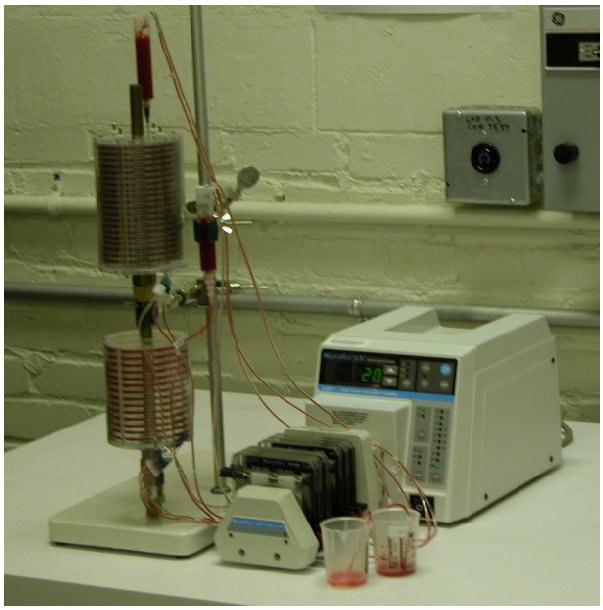

Fig. 4 Demonstration of two stage continuous operation for plasma separation from whole blood by using existing flow cells and the pump. Those components can be made much smaller for a portable device.

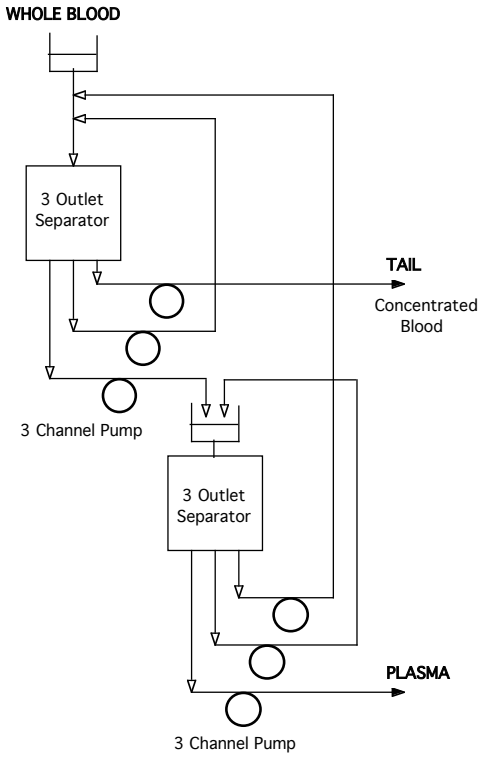


Fig. 5 Block diagram of a two-stage separation system used for the demonstration shown in Fig. 4.

2.4. Pulsed Flow Operation

We have found that the plasma phase is pumped preferentially rather than the blood cell rich phase in the flow channel when pumped after the plasma phase is established.

This might be due to lower viscosity and lower shear force for the plasma phase of lower blood cell concentrations. It is well known that the hydrodynamic behavior of blood is very complicated [17]. Blood viscosity significantly increases with increasing hematocrit of blood cell concentration, and viscosity of plasma strongly depends on shear rate [17], [18]. The phase boundary of plasma is also known to be stimulated with a polymolecular layer in the presence of high molecular weight proteins [17].

In our test operation using a 0.5 m long tubing of the rectangular-cross-section (1.96 mm x 0.61 mm), 0.1 mL clean plasma (Hct less than 1%) has been obtained after a 10 min holding for blood cell sedimentation. This phenomenon can be useful to develop a unique plasma separation method using a "pulsed" flow operation instead of continuous flow, as discussed in the following section of 5.2.

## 3. Feasibility and Scale-up

3.1 Continuous Flow Operation

Feasibility of the continuous flow operation method will be discussed on the basis of the experimental results shown in Figs. 2 and 3 obtained using the 4.8 m separator of the fine rectangular cross-section tube of 1.96 mm x 0.61 mm. There are two basic operation methods: 1. Direct plasma separation from whole blood, and 2. Plasma separation diluted with replacement plasma and then separate plasma since a plasma separation from whole blood is more difficult than that from lower hematocrit blood as seen in Figs. 2 and 3.

3.1.1 Direct plasma separation from whole blood

Whole blood is separated with a two-stage separator which is composed of a three-outlet and a two-outlet separator. Fig. 6 shows the block diagram of the two-stage system. Each outlet of the first stage is pumped at 0.1 mL/min. The one outlet of the first stage is directly connected to the second stage. Each outlet of the second stage is pumped at 0.05 mL/min. One outlet (middling) of the first stage and one outlet of the second stage will be return to the inlet of the first stage. Concentrated blood cells are recovered from one of the outlet of the first stage. In this multi-stage separator the plasma can be separated at 0.05 mL/min. Blood will be treated at 0.15 mL/min. If we scale up this method to 10 times by using the flow channel (for example 1.96 mm x 6.1 mm) of 10 times cross-section, the blood treatment rate will be 1.5 mL/min and treated plasma will be obtained at 0.5 mL/min. The scale-up can be also achieved by a parallel operation of multiple units.

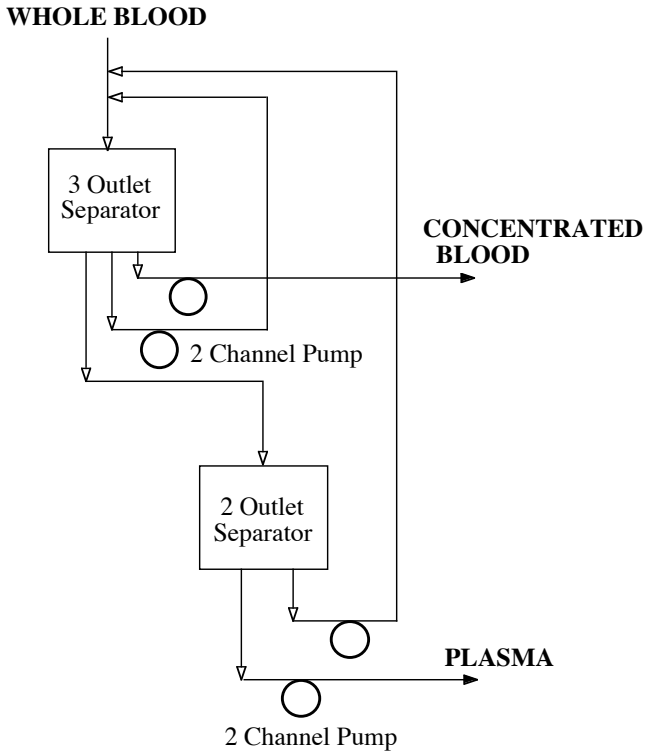


Fig. 6 A plasma separation system using a two-stage continuous separator composed of a three-outlet and a two-outlet separators.

3.1.2 Plasma separation diluted with replacement plasma

Whole blood is first diluted with replacement plasma before plasmapheresis treatments. Fig. 7 shows a one-stage separator of two outlets with two outlets. If the whole blood (Hct 44%) diluted to Hct 17% is treated at each pump flow rate of 0.3 mL/min, the resultant plasma from one of the outlet will be estimated to be the hematocrit less than 1% from Fig. 3. In this operation blood will be treated at 0.3 mL/min, and the plasma product can be obtained at 0.1 mL/min (since both pumps are operated at 0.3 mL/min.). Original blood is diluted, therefore the net plasma treatment rate will be 0.1 mL/min (0.3 mL/min x 36%). If we scale up this method 10 times by using 10 times cross-section of the flow channel, the blood treatment rate will be 3 mL/min and treated plasma will be obtained at 1 mL/min.

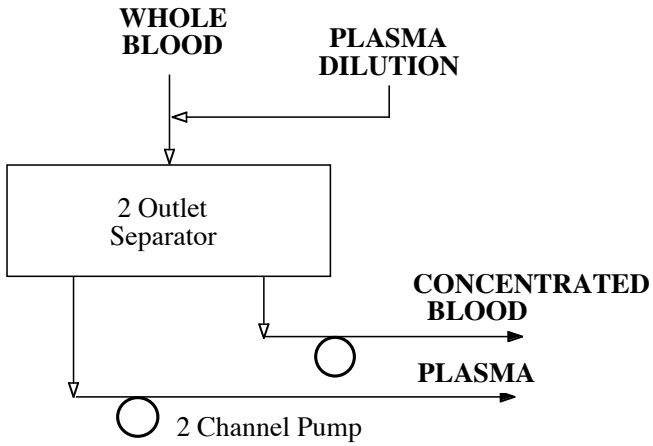


Fig. 7 A plasma separation system with plasma dilution using a one-stage separator with two outlets.

3. 2 Pulsed Flow Operation

A system block diagram of a basic pulsed operation separator is shown in Fig. 8. The flow pumps connected separately to each outlet are operated intermittently (on and off). During the off cycle (holding cycle) of all pumps, the blood cells sediment downward and the plasma phase forms at the top of the channel. Then the pump for plasma is operated to recover preferentially only the plasma rich phase. After recovering the plasma, the pump is stopped and then the other pump for the blood cell outlet is turned on to collect the blood cells. The pump operation will be controlled by a densitometer which determines blood cell concentration in plasma. At the end of this cycle, new whole blood to be treated is introduced into the separation channel, followed by the holding cycle of precipitation. Plasma treatment capacity will be increased by an optimization of the flow cell sizes including length and operation cycle intervals. The pulsed method requires only one outlet and can be operated by one pulsed pump with a splitter (Fig. 9) or by two pulsed pumps (Fig. 10). The pulsed operation method will make the device smaller and also minimize electric power consumption.

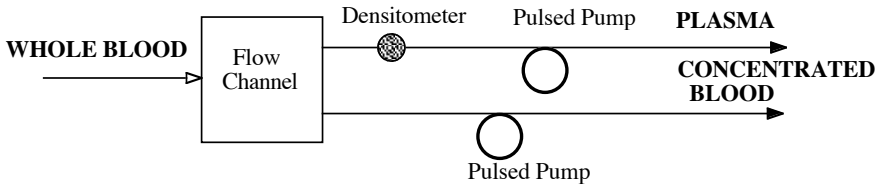


Fig. 8 Block diagram of a basic pulsed operation separator.

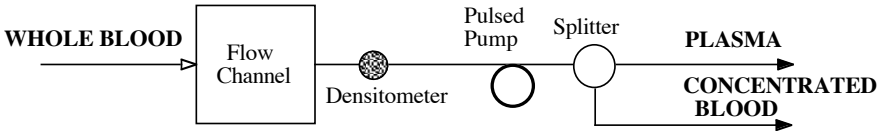


Fig. 9 Block diagram of a pulsed pump separator operated by one pump.

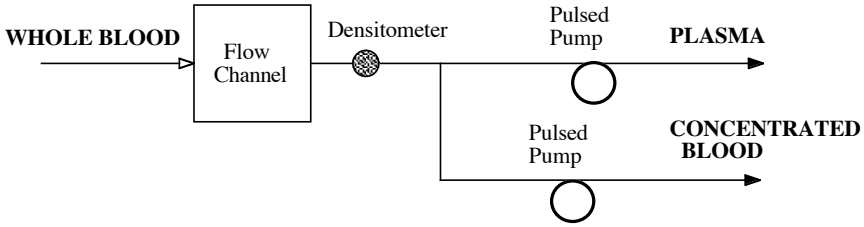


Fig. 10 Block diagram of a one-outlet pulsed pump separator operated by two pumps without a splitter.

As mentioned earlier, a 0.5 m long tubing of the 1.96 mm x 0.61 mm rectangular-cross produced 0.1 mL clean plasma (Hct less than 1%) after 10 min sedimentation at stopped flow. This means a plasma separation rate of 0.01 mL/min. By scaling-up this method by 7.5 times the cross-section of the flow channel and double the length (1 m), the plasma separation rate is equivalent to 0.15 mL/min with the blood treatment rate of 1.5 mL/min. This method will be improved much more by optimizing the flow cell dimensions and length, and the sedimentation time. Furthermore, multiple units of the separation cells can be operated in parallel. One cell can be processed while holding the other unit. By using two-unit parallel operation, plasma will be treated at 0.3 mL/min with blood treatment rate at more than 3 mL/min.

### 3.3. Plasmapheresis System Concept

A schematic representation of a plasmapheresis system is shown in Fig. 11. Blood cells would be separated by passing through the separation channel. Plasma and cell rich blood would be recovered from the separator outlets of “Plasma” and “Blood Cells”,

respectively. The plasma could then be discarded or treated. Blood components separated from plasma could be returned to the patient directly with the treated plasma and/or with a plasma replacement. The system could allow for single needle operation.

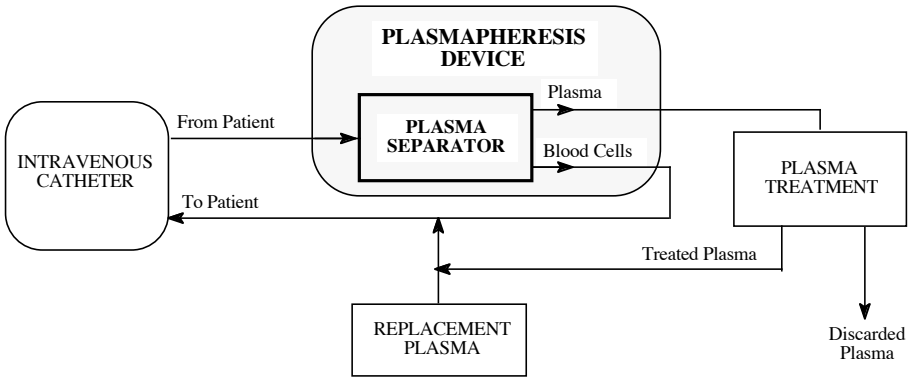


Fig. 11 Schematic representation of a plasmapheresis system to simulate continuous therapy.

Further feasibility and system evaluation of the proposed continuous separator will require system operation tests over a prolonged period of time. A plasmapheresis system will be further improved with adapting computer control systems for flow speeds and valve operation sequences. In system tests using human blood and animal blood, the following concerns should be investigated.

1. Anti-coagulant usage. How much can it be minimized?
2. Protein deposition and clots on the separator inner surface.
3. Mobility of the device. How much vibration and change of orientation can be allowed?
4. Conformability and safety as a portable device?

## 4. Conclusions

A portable, slow flow rate plasmapheresis device seems to be feasible to make a plasma separation for slow, continuous therapeutic treatment at the flow rate of 1 – 3 mL/min for continuous therapeutic treatments of patients. The plasmapheresis device continuously separates plasma from whole blood in a simple, inexpensive method using hydrodynamic effects and the gravitational force which is conceptually similar to Field Flow Fractionation. Both continuous flow and pulsed flow operation methods of the plasmapheresis device were discussed. Pulsed flow operation will allow flexibility of the designs. A hybrid device using both continuous and pulsed methods will give a better system for a highly portable small mobile plasmapheresis device. Further investigations of feasibility and benefit of the plasmapheresis device will be desired for therapeutic applications.

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