INTEGRATED BLOOD PRETREATMENT MODULE OF DUAL FUNCTION USING ANTI-BLOOD SERUM AND ALBUMIN-ADSORPTION BEADS

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ABSTRACT

We describe herein a rapid integrated method for the separation of plasma from undiluted blood in a minute without any power supply. We generated polyclonal antibody which captures human blood cells to make cellular aggregates, which accelerates the sedimentation of blood cells even in undiluted whole blood. Additionally, the module for the reduction of plasma albumin was combined to make a pretreatment module. Double volume of plasma containing decreased albumin was harvested in a minute without any detectable blood cells form undiluted whole blood using this pretreatment module.

KEYWORDS

blood pretreatment, filter, antibody, aggregation, albumin

INTRODUCTION

Gathering plasma from whole blood, one of the most important and frequently requested pretreatments, is an inevitable bottleneck for the embodiment of field-applied diagnostic chips. Simple sedimentation of blood cells by sole gravitational force would take too long time to harvest enough plasma from whole blood (Figure 1).

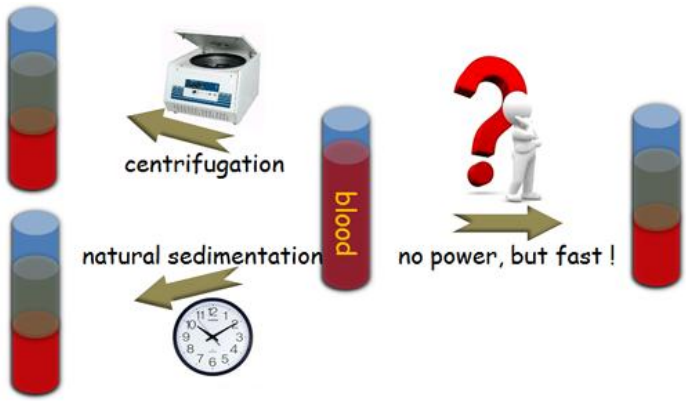

Figure 1: A fast power-free method ?

In order to speed up the sedimentation of blood cells without exogenous power supply, therefore, we introduced anti-blood cells polyclonal antibody to make cellular aggregates. The major concept of blood aggregation by anti-blood polyclonal antibody is depicted in Figure 2. Human blood cells including red blood cells and white blood cells will interact with antibody which was raised against human blood cells themselves. Because of the nature of polyclonal antibody, binding variety of antigenic determinants, the blood cells will make complex aggregates through the diverse epitopes of cells. The aggregation of blood cells will make compact lumps leaving cell-free plasma. Consequently, it is possible to use smaller amount of filter structures in order to remove blood cells from whole blood. This will reduce the time to spend and increase the amount of harvested plasma at the same time.

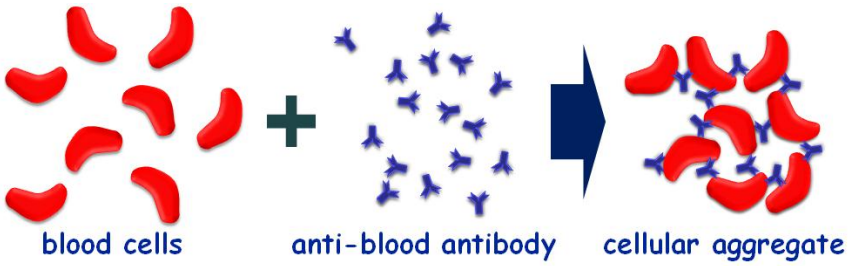

Figure 2: Aggregation of blood cells by anti-blood antibody

Additionally, removal of albumin, which covers more than 50% of plasma protein [1], by integrating albumin-adsorption beads is also a novel trial. Reduction of albumin will be helpful in detecting other tiny amount of proteins as well as in improving fluidity of plasma in microstructures. Cibacron Blue F3G-A has been widely used to remove serum albumin prior to serological assays [2]. There are many kinds of applications of this dye mainly for

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macro-scaled systems. This report may be the first application to an integrated module with micro-scale.

EXPERIMENT

Human whole blood cells were used as antigen in order to generate polyclonal antibody which binds human blood cells. Human whole blood was drawn from volunteer's fingertips. The drawn blood was immediately washed with PBS (phosphate-buffered saline, pH 7.4) for 3 times. The washed blood cells were fixed by 2 % (v/v) of formaldehyde and 0.2 % (v/v) of glutaraldehyde. The fixed cells were washed again with PBS for 3 times to remove the fixing solution. Female C57/BL6 mice, 5 weeks old, were intraperitoneally immunized with the prepared human blood cells [3]. Each inoculum contained human blood cells from 10 μL of original whole blood, which corresponds to about 5×10^7 cells. Immunizations were performed 5 times at 3 week intervals. Blood was collected from each mouse two weeks after the final immunization. The drawn whole blood was let at room temperature for sufficient clogging. Serum which contained anti-human blood polyclonal antibody was harvested after centrifugation at 15,000 g for 20 min at 4℃. Aliquots of the harvested anti-human blood serum were frozen at -20℃ until usages.

Pretreatment module could be integrated into any kind of platforms. In order to exclude additional platform development and to concentrate on the verification of integrated function of pretreatments, we adopted previously introduced blood filter module as a demonstrative platform. The detailed fabrication of magnetically actuated filter module was previously described [4].

RESULTS

Harvested anti-human blood serum was tested by simple mixing. 5 μL of undiluted human whole blood was mixed with equal volume of anti-human blood serum. As shown in Figure 3, there was instantaneous total aggregation although control serum, pre-immune serum, showed no aggregation (data not shown). The two kinds of sera can make aggregation until being diluted to 5 to 10 folds (Figure 4).

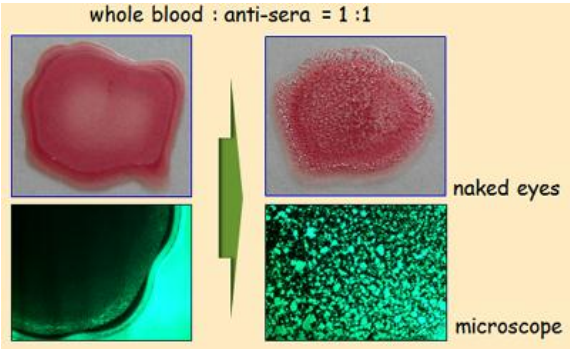


Figure 3: Instant (< 5 sec.) aggregation of blood cells).

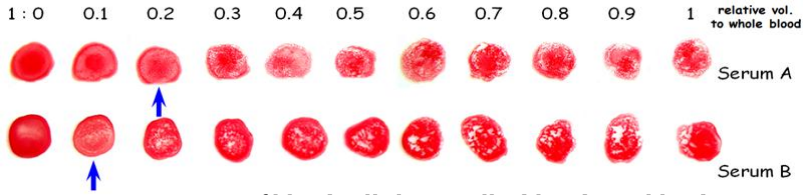


Figure 4: Aggregation of blood cells by serially diluted anti-blood sera. The dilution limits of aggregation are noted by arrows.

In order to investigate any effect on the sedimentation of blood cells, anti-human blood serum was mixed 2.4 times volume of undiluted human whole blood (Figure 5). On mixing, there was visible aggregation of blood cells, and the blood aggregate showed increased sedimentation velocity 5 times compared with control groups.

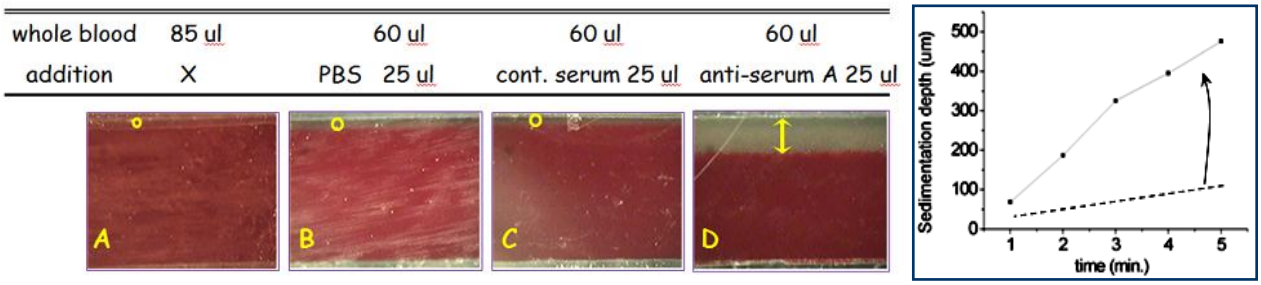


Figure 5: Rapid sedimentation of blood cells by anti-blood serum. Whole blood was mixed with PBS (B), control serum (C), or anti-blood serum (D). Yellowish plasma layers are noted by circles (A, B, and C) or an arrow (D). Dotted line in the graph means the sedimentation of untreated whole blood. The sedimentation velocity was calculated to increase 5 times.

We integrated this anti-blood serum into previously reported filter unit [4] with additional albumin-adsorption beads, Cibacron Blue F3GA. Figure 6 shows a completed module with schematic explanations.

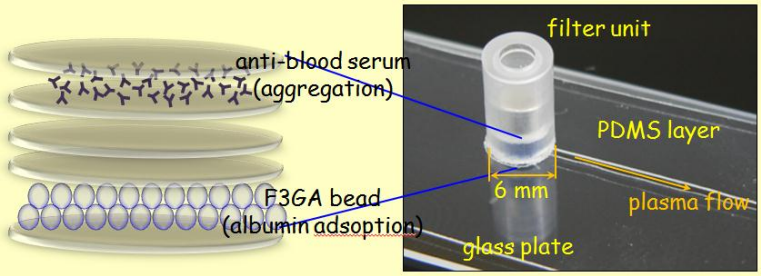
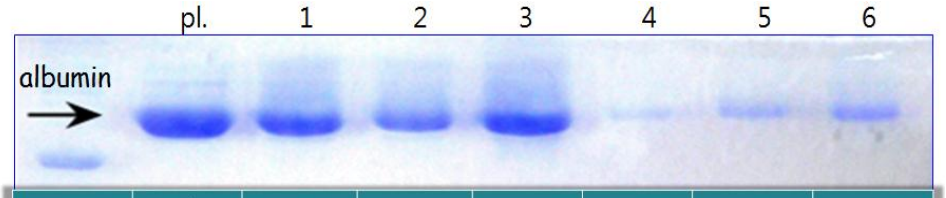


Figure 6: Integrated pretreatment module

20 μL of undiluted human whole blood was applied into the top of the filter unit. After one minute, the inner module was manually or magnetically pushed down to squeeze out plasma. Table 1 summarizes the functional comparisons of filter module with different components of units along with SDS-PAGE. Results of lane 3 and lane 6 show increased harvest of plasma compared with that of lane 2 and lane 5, respectively. The blood cells at the beginning of filter module seemed to have aggregated to make compact clot resulting in the increased harvest of plasma. Application of control serum (pre-immune serum) did not affect the plasma volume. In addition, more than 80% of albumin was reduced according to the analysis using ImageJ [5].

Table 1: Comparison of the integrated units



	pl.	1	2	3	4	5	6
Function	**plasma**	**filter only**	**cont. serum**	**anti-blood**	**Δ albumin**	**cont. serum + Δ albumin**	**anti-blood + Δ albumin**
Filter (ea)	-	2	2	2	3	2	**2**
Serum	-	-	**cont. serum**	**anti-blood**	-	**cont. serum**	**anti-blood**
Bead	-	X	X	X	O	O	**O**
Δ albumin	**-**	**no**	**no**	**no**	**yes**	**yes**	**Yes**
[1]recoverd plasma	**-**	**3 ul**	**2.8 ul**	**5.5 ul**	**8 ul**	**7.3 ul**	**10 ul**
[2]resident blood cells	**-**	**-/+**	**-/+**	**-**	**-/+**	**-/+**	**-**

[1]from 20 ul of undiluted whole blood,
[2] +/- : less then 1 % of whole blood, - : no detectable cells

This pretreatment module is made of simple and cheap but novel components. Its working time is only one minute with excellent dual performances for undiluted whole blood. The simple platform of this module will make it easy to be integrated into other applications.

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