Research Article

Quality, Stability, and Safety Data of Packed Red Cells and Plasma Processed by Gravity Separation Using a New Fully Integrated Hollow-Fibre Filter Device

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Background. We developed a completely closed system based on gravity separation without centrifugation steps for separation of whole blood. With this new system we compared quality and stability of the processed blood components (PRC and plasma) with respect to classical preparation. Furthermore the cost-effectiveness of this hollow fibre system was evaluated. Study Design and Methods. Whole blood collections of 15 regular blood donors were used for component preparation using the U shaped hollow fibre filter device. Results were compared to 15 whole blood preparations using centrifugation. The following parameters were evaluated: total hemoglobin, leukocyte counts, the serum concentration of total protein, lactate dehydrogenase (LDH) and potassium. Furthermore ATIII, vWF and F VIII were analyzed at different timepoints. Results. packed red cells: the data directly after separation and after 42 days of storage are in line with the guidelines of the council of Europe. Plasma. all plasma quality data are in line with the guidelines of the council of Europe for quality assurance of plasma, except for a low protein amount (factor 0.75). Conclusion. Separation of whole blood on a clinical scale in this new closed system is feasible, however the plasma protein content must be optimized.

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1. Introduction

Separation of whole blood into the different blood components is a standard procedure in transfusion medicine. This separation is either performed by centrifugation using programmable centrifuges and automated blood-component preparation systems after collecting whole blood or during the collection of blood in the case of cellular apheresis. In both cases an extensive infrastructure (central facility, expensive equipment, and well-trained staff) is required for the successful processing of the blood. Besides the necessary equipment, labor-intensive manual processes are necessary in the case of whole blood separation or careful supervision of the apheresis process in the case of cellular apheresis. We investigated if the above-mentioned separation methodologies could be replaced by a closed-system gravity filtration.

There were several attempts to replace the centrifugation and additional separation method using simple gravity filtration. The first study on the separation of whole blood to PRCs and plasma using a hollow-fibre system was performed by Sekiguchi et al. [1]. By changing the filter systems, integrating leukocyte filters and using a storage medium for extended storage, different authors were able to show that it is possible to process PRCs and plasma using gravity separation with comparable quality and stability data to those processed by classic centrifugation/apheresis methods. Nevertheless, the problem with these first attempts was the low concentration ability of the filter system with a resulting low HCT in the PRCs and a reduced plasma recovery. This low concentration ability mainly results from the relatively high flow rate through the linear filter system [2–5] preventing an effective separation into plasma and cellular components.
1. Donors.

15 donors were stochastically selected from the donated blood. All participating donors gave their informed consent.

2. Donors, Material, and Methods

2.1. Donors. 15 donors were stochastically selected from regular blood donors of the Department of Transfusion Medicine, Marienkrankenhaus, Hamburg, Germany. All donors were informed about the clinical trial and the use of the donated blood. All participating donors gave their informed consent.

2.2. Blood Collection. All blood donations were performed according to the standard guidelines for blood donations in Germany. In each donor the following parameters were measured: total hemoglobin (Hb), leukocyte count, the serum concentrations of total protein, lactate dehydrogenase (LDH), and potassium. Furthermore, the activities of the coagulation factors antithrombin III (AT III), von Willebrand factor (vWF), and factor VIII (F VIII) were analyzed. The analyzers we used for these investigations are presented in Table 1. All measurements were performed according to the recommendations of the manufacturers of the analyzers used.

After careful desinfection and successful puncture of the cubital vein we collected 450 mL blood from each donor using the integrated hollow-fibre system. During the blood collection the donation bag was placed on a standard blood-mixing device (NPBI Compomixer M2). Immediately after the blood collection the inlet tubing of the donation bag was sterile sealed.

2.3. Blood Separation. Before blood separation the donated whole blood unit was left to rest at ambient temperature (about 20°C) for 2 hours in order to allow phagocytosis for free bacteria. The details of the closed hollow-fibre separation system are given in Figure 2. The gravity-separation procedure is performed as follows. First the donation bag containing 63 mL CPD (1000 mL of CPD contains 3.27 g of citric acid·H2O, 26.3 g of sodium citrate·2H2O, 25.5 g of glucose·H2O, and 2.51 g of NaH2PO4·H2O) is hung on the upper hook of the main stand (a). The packed red cells (PRCs) bag (c) prefilled with extended storage medium of glucose·H2O, 1.255 g of NaH2PO4·H2O, 1.432 g of NaHPO4·H2O, 0.210 g of mannitol and 4.210 g of sodium chloride) is hung on the middle attachments both exactly on the same level. After all clamps and inline break valves have been opened the blood flows from the donation bag through the leukocyte filter (e) and subsequently into the hollow-fibre system. While the leukocyte-depleted blood is prepared for bagging some packed red cells (e) are left for leukocyte depletion. The device is connected with the blood bag through the leukocyte depletion filter (d) which prevents bacteria from entering the device. A continuous flushing of the device is performed until the device is connected with the blood bag. Then the leukocyte-depleted blood is bagged by opening the relevant valves as shown in Figure 2. The fully automated leukocyte depletion system has been developed and manufactured by Fresenius Medical Care, Germany. The system consists of two main parts: the automatic membrane separator and the blood bagging system. The automatic membrane separator is a disposable device which is connected to the leukocyte depletion filter (d) of the leukocyte depletion system of the device. The device is connected to the blood bagging system as shown in Figure 2.

2.4. Blood Separation Device.

The blood separation system was designed with hollow fibres enclosed in a plastic tubing (outside the capillaries) and red cells (within the fibres). Details of the hollow-fibre filter are given in Figure 1. This preliminary filter design was incorporated into a blood separation device and was tested by Heim [6], Stienstra et al. [7], and Hornsey et al. [8]. They concluded that whole blood separation using the hollow-fibre technique is simple and straightforward to use; however, minor changes are required to make the procedure easier to incorporate into routine use.

Based upon the practical experience of Hornsey we modified the original hollow-fibre device in an attempt to improve its performance.

In the preliminary system evaluated by Hornsey et al. the filter system and the donation bag were separate and the hollow fibre system was dry. A Pall leukocyte filter was used for leukocyte depletion. Before separation the hollow-fibre filter had to be moistened carefully with sodium chloride in order to allow efficient phagocytosis for free bacteria. The details of the closed hollow-fibre separation system are given in Figure 2. The gravity-separation procedure is performed as follows. First the donation bag containing 63 mL CPD (1000 mL of CPD contains 3.27 g of citric acid·H2O, 26.3 g of sodium citrate·2H2O, 25.5 g of glucose·H2O, and 2.51 g of NaH2PO4·H2O) is hung on the upper hook of the main stand (b). Then the packed red cells (PRCs) bag (c) prefilled with extended storage medium (100 mL PAGGS-M) (1000 mL of PAGGS-M contains 9.400 g of glucose·H2O, 1.255 g of NaH2PO4·H2O, 1.432 g of NaHPO4·H2O, 0.194 g of adenine, 0.408 g of guanosine, 10.000 g of mannitol and 4.210 g of sodium chloride) and the separation filter (d) is hung on the middle attachments both exactly on the same level. After all clamps and inline break valves have been opened the blood flows from the donation bag through the leukocyte filter (e) and subsequently into the hollow-fibre system. While the leukocyte-depleted blood

| Parameter                  | Analyzer                |
|----------------------------|-------------------------|
| Free Hemoglobin:           | Analyzer Low Hemoglobin, Hemocue |
| Hematology:                | Cell-Dyn 4000 and CellDyn 350, Abbott, USA |
| Total-Protein, Serum LDH, Serum potassium: | AU 640/AU 2700, Olympus |
| Von-Willebrand-Antigen:    | Mini-VIDAS, Biomerieux, Paris, France |
| Fibrinogen, F VIII:        | BCT, Dade Behring, Marburg, Germany |
| Residual leucocytes:       | Epics XL, Coulter, USA |

Table 2: Donors, initial values.

| N = 15 | Hb (g/dL) | Leukocytes (10^9/µL) | Total Protein (g/L) | LDH (U/L) | K⁺ (mmol/L) | AT III (% o. N.) | vWF-Antigen (% o. N.) | F VIII (% o. N.) |
|--------|-----------|----------------------|---------------------|-----------|-------------|------------------|----------------------|------------------|
| Mean ± SD | 13.8 ± 1.1 | 5.2 ± 1.5 | 70 ± 4 | 176 ± 42 | 4.1 ± 0.2 | 104 ± 11 | 86 ± 24 | 98 ± 28 |
| Range  | 11.2–15.2 | 3.5–8.5  | 62–77  | 122–290 | 3.7–4.7 | 93–119 | 58–118 | 57–149 |
Table 3

(a) Packed red cells directly after preparation.

| Parameter | Mean ± SD | Range |
|-----------|-----------|-------|
| Volume (mL) | 346 ± 19 | 326–402 |
| Hb Concentration (g/dL) | 17.4 ± 1.2 | 14.3–19.0 |
| Hb/Unit (g) | 60 ± 6 | 47–73 |
| Hct (%) | 54 ± 4 | 43–59 |
| Residual Leukocytes/Unit (×10⁶) | 0.03 ± 0.00 | 0.03–0.04 |
| Residual Protein/Unit (g) | 3.3 ± 0.8 | 2.0–4.9 |
| K⁺/Unit (mmol/L) | 1.8 ± 0.2 | 1.4–2.0 |
| K⁺/Unit (mmol) | 0.28 ± 0.04 | 0.21–0.32 |
| Free Hb (g/L) | <0.03 | <0.03 |
| LDH in Supernatant (U/L) | 346 ± 19 | 25–73 |

(b) Packed red cells after 42 days of storage

| Parameter | Mean ± SD | Range |
|-----------|-----------|-------|
| Free Hb (g/L) | 1.21 ± 0.33 | 0.8–1.7 |
| Lysis rate | 0.69 ± 0.16 | 0.5–0.9 |

Table 4: Requirements for PRC.

| Parameters | Requirement | Observations |
|------------|-------------|--------------|
| Haemoglobin im PRC | ≥40 g/Unit | 47–75 g/Unit |
| Residual Leukocytes in PRC | <1 × 10⁶/Unit | <0.05 × 10⁶/Unit |
| Visual Examination | No Leakage | No Leakage |
| | No Agglutination | No Agglutination |
| | No Change of Color | No Change of Color |

Figure 1: Figures 1(a) and 1(b) show the hollow-fibre filter in more detail. Each hollow-fibre filter is made of 600 capillary membranes, produced from polyethersulfon with a thickness of 100 µm ± 25 µm. The capillaries are U-shaped (Figure 1(a)) fixed within the synthetic filter cylinder (Figure 1(b)) and moistened with a sodium chloride solution. The capillaries have a pore size of 0.5 µm ± 0.1 µm. The total length of the capillary amounts to 175.5 m with a diameter of 300 µm ± 40 µm. The hydrostatic pressure of the donation bag fixed above the filter is enough to press the erythrocytes into the erythrocytes bag. During the separation process the plasma penetrates the capillary membranes, is sampled in the filter cylinder and flows down to the plasma bag. Because of the cells’ volume the erythrocytes are not able to penetrate the membrane and flow along the U-shaped capillary to the upper exit in the erythrocytes bag hung at the same height and prefilled with the 100 mL PAGGS-M additive solution.

2.4. Cost-Effectiveness. To compare the presented method of blood separation by gravity with the classic method using whole blood and centrifugation as to its cost-effectiveness, we analyzed the hands-on time of each work step in both days of storage the frozen plasma was thawed and F VIII activity was measured again.
Table 5

(a) Plasma directly after preparation.

|          | N = 15 | Volume (mL) | Total Protein Concentration (g/L) | Total Protein/Unit (g) | Residual Leukocytes/Unit (×10⁶) | Fibrinogen (mg/dL) | Fibrinogen/Unit (mg) | AT III (% o. N.) | vWF-Antigen (% o. N.) | F VIII (% o. N.) |
|----------|--------|-------------|----------------------------------|------------------------|---------------------------------|-------------------|---------------------|--------------------|------------------------|-----------------|
| Mean ± SD| 234 ± 12| 51 ± 4      | 12.0 ± 1.2                       | 0.02 ± 0.00            | 191 ± 41                        | 450 ± 109         | 82 ± 9              | 82 ± 27            | 82 ± 27                |
| Range    | 214–253| 44–58       | 9.7–14.6                         | 0.02–0.03              | 138–264                        | 304–663           | 67–95              | 52–119             | 46–115                |

(b) Plasma F VIII after 366 days of storage.

|          | N = 15 | F VIII (% o. N.) | % of activity before storage |
|----------|--------|------------------|-----------------------------|
| Mean ± SD| 57 ± 21| 78 ± 5           |                             |
| Range    | 35–97  | 69–88            |                             |

Table 6: Plasma electrophoresis directly after preparation.

|          | Results | Reference values |
|----------|---------|------------------|
| Total protein | 58 g/L | 60–80 g/L |
| Albumin  | 57%   | 55–67% |
| Alpha 1 Globulin | 2.7% | 2.1–3.7% |
| Alpha 2 Globulin | 9.4% | 8.0–14.0% |
| Beta Globulins | 10.8% | 8.0–13.0% |
| Gamma    | 20.1% | 9.0–20.0% |

3. Results

3.1. Donor Parameters. The results of the donors’ blood parameters are given in Table 2.

3.2. Packed Red Cells. The quality data of the processed PRCs directly after separation are given in Table 3(a). After 42 days of storage the content of free Hb was 1.2 g/L (range: 0.8 g/L ± 1.7 g/L), which corresponds to a mean lysis rate of 0.7% (range: 0.5%–0.9%) (Table 3(b)). All values are in line with the guidelines of the Council of Europe [9] for the quality of PRCs (Table 4). The HCT of the PRC directly after separation can be calculated from the mean total volume (346 mL) and the mean HCT (0.54) of the PRCs and the knowledge of the added SAG-M amount according the formula 346 mL × 0.54/(346 mL–100 mL) = 0.76. The donor’s original HCT of 0.43 is diluted by 63ml CPDA in the primary bag to an HCT of 0.37(450 × 0.43)/(450 + 63)). Based on these values the calculated concentration ability of the filter is 76/37 = 2.05.

3.3. Plasma. The quality data of the plasma preparations are given in Table 5(a). The protein content is reduced by a factor of about 0.75 compared to the values of the initial donor blood (Table 1). The plasma activity of antihrombin III, von Willebrand factor, and factor VIII correlates to the protein content reduction and was approximately 0.8. The residual leukocyte count in the plasma was at the lower detection limit (below 2000/unit) and the plasma contained no measurable free hemoglobin. The factor VIII activity after one year of storage was 0.57 with a range of 0.35–0.97 (Table 5(b)). This corresponds to 0.78 of the activity before storage (range: 0.69–0.88). Serum electrophoresis of randomly selected plasma shows a normal protein fraction distribution within the reference values (Table 6). All plasma quality data are in line with the guidelines of the Council of Europe.
Europe for the quality assurance of plasma (Table 7), except for the low protein amount.

3.4. Cost-Effectiveness. The analysis and comparison of the different steps of both ways of whole blood processing are given in Table 8 with the time necessary for a certain step given in minutes. This table shows that the processing of whole blood into PRCs and plasma takes a total of 70 minutes with the centrifugation/separation method and 57 minutes with the gravity-separation method. Both the centrifugation process and the gravity-separation process themselves are independent of a technologist and are times in which the technologists can perform other tasks. We therefore postulate that a technologist is needed for 50 minutes using the classic method and only 12 minutes using the gravity-separation method.

4. Discussion

Our study demonstrates that whole blood can be separated efficiently into its components using a gravity-driven completely closed hollow-fibre system. The quality and the stability of the PRCs and plasma thus processed are comparable to the quality and the stability of blood components processed in the classic way and correspond to the German and European guidelines for the preparation, use and quality assurance of blood components. However, the protein concentration of the processed plasma is still too low. The reasons for this protein loss are not completely understood. We speculate on an interaction of the plasma protein with the capillary membranes of the hollow fibre (polyethersulphone) causing absorption of plasma protein. Taking into account that the total free length of the hollow fibre is 175.5 m, minimal absorption could lead to a measurable difference in plasma protein. A second mechanism could be by activation of the coagulation system (platelet activation?) due to contact with the artificial surface of the hollow fibre. Again this activation of the coagulation system might be minimal, but the total surface of the hollow fibre system could make the difference. We plan to work on these aspects by chemically modifying the surface of the hollow fibres to inhibit adsorption or activation. The hollow-fibre filter blood-separation device eliminates the need for either centrifugation or automated separation steps during the processing of whole blood into red cells and plasma components. A source of electrical power is not required and the closed system can be used anywhere, irrespective of environmental conditions. This means that an integral system for safe blood collection and processing is also within reach of under-resourced countries. However, other applications are focussed upon as well. Blood collections obtained at remote blood drives can be processed during the actual blood drive locally without the need for a speedy

### Table 7: Requirements for plasma preparations.

| Parameters                              | Requirement           | Observations       |
|-----------------------------------------|-----------------------|--------------------|
| Volume fluctuation of plasma preparations | $\pm 10\%$          | $\pm 7.8\%$       |
| Residual leukocytes in plasma           | $< 1 \times 10^6$/Unit | $< 0.04 \times 10^6$/Unit |
| Visual examination                      | No agglutination      | No agglutination   |
|                                         | No change of color    | Clear, unclouded, amber coloured plasma |

### Table 8: Comparison of the mean processing times of the classical separation method and gravity separation.

| Process activity                                      | Centrifugation | Gravity Separation |
|-------------------------------------------------------|---------------|-------------------|
| Transport of the blood bags to the lab                 | 2 min         | 2 min             |
| Preparation of the leucocytes depletion                | 4 min         |                   |
| Leucocytes depletion                                  | 15 min        |                   |
| Preparation of the centrifugation step (cooling)      | 5 min         |                   |
| Centrifugation                                         | 20 min        |                   |
| Preparation of the blood separator                    | 4 min         |                   |
| Separation of the whole blood to PRC and Plasma       | 12 min        |                   |
| Documentation                                          | 3 min         |                   |
| Preparation of the PRC and Plasma for storage         | 5 min         |                   |
| Preparation of the gravity Separation filter system   | 4 min         |                   |
| Gravity separation                                     | 45 min        |                   |
| Documentation                                          | 3 min         |                   |
| Preparation of the PRC and Plasma for storage         | 5 min         |                   |
| Sum                                                    | 70 min        | 57 min            |
| Minus centrifugation, respectively. gravity separation time (technician is free to perform other things) | 20 min        | 45 min            |
| Time technician is required for the processing         | 50 min        | 12 min            |
return to a central facility. The handling of the system is easy and handy in practice. The net working time for processing the blood components is lower compared to the centrifugation method, only requiring a small number of staff and no large, specialized machines. Only a heat melting clamp or clamping tongs for closing the tubes, a tubing roller and the usual cooling and freezing equipment are necessary for the production of the preparations from donor blood described above.

The system has been in clinical use in different countries (Turkey, South Africa, and Mozambique, among others) for 2 years. To the authors’ best knowledge no adverse effects related to the separation system have been observed.

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