

Purified Granulocyte Concentrates from Buffy Coats with Extended Storage Time

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Keywords

Granulocyte concentrate · Buffy coat · Transfusion medicine · Transfusion

Abstract

Background: Granulocyte concentrates (GCs) are usually prepared by single-donor apheresis after G-CSF pretreatment and have to be transfused within 24 h after cell collection because of the rapid decrease in pH and cell survival due to high lactate production by red blood cell contamination. GCs pooled from buffy coats of whole blood donations could improve the availability of these products. Methods to reduce red blood cell and platelet contamination may improve storability. We developed a manufacturing process for pooled GCs and investigated cell viability and functionality over time. **Methods:** Six ABO blood group-identical buffy coats were pooled. Subsequently, the red blood cells spontaneously sedimented after the addition of hydroxyethyl starch. The resulting leukocyte-enriched supernatant was washed twice with saline to reduce platelets and was resuspended in ABO-identical donor plasma. The leukocyte concentrate was transferred to a platelet storage bag and stored up to 72 h at 20–24°C w/o

agitation. Cell count and viability, pH, blood gases, phagocytosis, and oxidative burst activity were monitored. **Results:** The number of red blood cells and platelets was reduced to 0.4% and 6.1% of the baseline levels. About 50% of the original present leukocytes could be extracted ($n = 76$). In the course of 72 h of storage, there were no significant changes in white blood cell counts ($p = 0.12$). The viability exceeded 98% during the entire period. The rate of granulocytes performing phagocytosis and oxidative burst remained above 95% anytime. **Conclusion:** GCs prepared from pooled buffy coats provide a precious alternative to granulocytes obtained from apheresis. Reduction of red blood cells and platelets by more than 90% extends the maximum shelf life of GCs from 24 h to 72 h. For a therapeutic dose of at least 1×10^{10} granulocytes, 15–20 buffy coats are required.

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Introduction

Bacterial and viral infections, especially sepsis, account for 20% of all deaths and are thus among the major causes of mortality worldwide [1]. Neutrophil granulocytes play

an important role in fighting bacterial and fungal infections, and diseases associated with decreased neutrophil counts increase the risk of systemic infections [2]. Patients suffering from sepsis, especially septic shock, develop a state of immune paralysis associated with increased mortality due to various cellular dysfunctions [3, 4] such as phagocytosis and clearance of bacteria by granulocytes [5–9] and macrophages [10], impaired antigen presentation, lymphopenia, and impaired T-cell activation [11, 12].

Transfusion of granulocyte concentrates (GCs) can adjunctively support the treatment of patients with sepsis, life-threatening neutropenia, or neutrophil dysfunction [13, 14]. However, although GCs have been transfused for several decades for this indication, their efficacy has not been well substantiated [2]. Furthermore, an extension of the range of indications for GCs to the area of intensive care medicine has already been approached [15]. In particular, a GC has been used in an extracorporeal therapy system for the treatment of sepsis-associated immune paralysis based on standard and purified GCs from apheresis. The extracorporeal circuit prevents cellular components from exposing the patient while harnessing the absorptive and immunomodulatory benefits of immune cells to regain immune homeostasis [16, 17].

GCs are produced by two approaches: (i) by apheresis or (ii) from a pool of buffy coats (BCs) from whole blood donations [18, 19]. Currently, in Germany, collection of GCs by apheresis is the standard approach. However, there are several disadvantages inherent to the collection and processing of GCs by apheresis [20–22]. First, donors require preconditioning with G-CSF and/or steroids before apheresis to mobilize a high number of granulocytes into the peripheral blood, which may cause adverse reactions [21, 23]. Further, preconditioning may impose a time delay of several days between an urgent request for GCs and their release in most cases. Finally, throughout granulocyte apheresis, hydroxyethyl starch (HES) (or gelatin) needs to be added directly into the cell collection system to increase the separation efficacy for granulocytes and decrease the number of red blood cells (RBCs) and platelets (PLTs) in the final product, which adversely affects the storage duration and functionality of the GCs. HES or gelatin is to some extent infused into the donor during apheresis and can induce adverse reactions in apheresis donors.

In contrast, BCs derived from whole blood donations can serve as an alternative source of granulocytes. BCs are available in every blood donation center and do not require preconditioning of donors, and GCs can be produced in large quantities, enabling rapid release in case of urgent demand [24]. In practice, however, BCs are predominantly used to prepare pooled PLT concentrates because they contain high amounts of PLTs [18], which could be a disadvantage for GC production.

Transfusion guidelines recommend that one unit of GC should contain at least 1×10^{10} granulocytes. However, higher numbers have been shown to be beneficial [25–27]. GC storage is crucial for this process. GCs have a limited shelf life and should be stored at a temperature of 20–24°C for no longer than 24 h and preferentially transfused as soon as possible after donation [26–28]. Throughout storage, apoptosis suppresses neutrophil activity, reduces neutrophil numbers, and leads to morphological changes, all of which are undesired.

Therefore, we designed a leukocyte preparation approach for GCs from pooled BCs in accordance with good manufacturing practice guidelines to enrich granulocytes and substantially remove RBCs and PLTs. The quality of these GCs was evaluated by investigation of cell counts, granulocyte viability, and cell functionality up to 72 h of storage.

Materials and Methods

Whole Blood Donation and Preparation

Whole blood donations were collected after informed consent was obtained from the donors. Each donor fulfilled the applicable donor suitability criteria of the national and European guidelines for hemotherapy [26, 28, 29]. Whole blood (450–500 mL, containing CPD as an anticoagulant) was processed according to the manufacturing license of the blood donation service (4,000 g, 10 min, 20–24°C). Plasma, BCs, and RBCs were separated by a plasma expeller (Macopharma, Langen, Germany). BCs (62–82 mL) were stored recumbently without agitation at room temperature until processing.

BC-Derived Granulocyte Purification Procedure

All bag and tubing connections were done by sterile techniques according to industrial standards (Sterile tubing welder TSCD-II, Terumo, Leuven, Belgium). Six BCs from ABO-identical donors were connected in a row.

After the bags were massaged gently to detach the adherent leukocytes from the inner wall of the bag, the thoroughly mixed contents of these BCs were pooled together in the last BC bag of the row (pool bag). The clamps between the BC bags were closed to prevent reflux.

HES (500 mL, Infukoll HES 6% 200/0.5 KS; Serumwerk, Bernburg, Germany) was filled into a transfer bag (Compoflex; Fresenius Kabi, Bad Homburg, Germany). The HES bag was welded to the first empty BC bag of the chain, and about 50 mL of HES was transferred to the BC bag, which was rinsed thoroughly by panning and manual massage.

This step was repeated with the following BC bags by opening the tube passage, filling the next bag with the 50-mL HES cell suspension, and then closing the tube connection with a clamp to prevent reflux. Finally, the HES solution was passed into the pool bag. This operation, called “washing of the BC-bags,” was performed a second time. The pool bag was then welded from the BC chain and connected directly to the HES bag. The pool bag was filled with HES and all air bubbles were removed. Using this procedure, a total of 416 ± 13 mL BC was mixed with about 258 ± 30 mL HES.

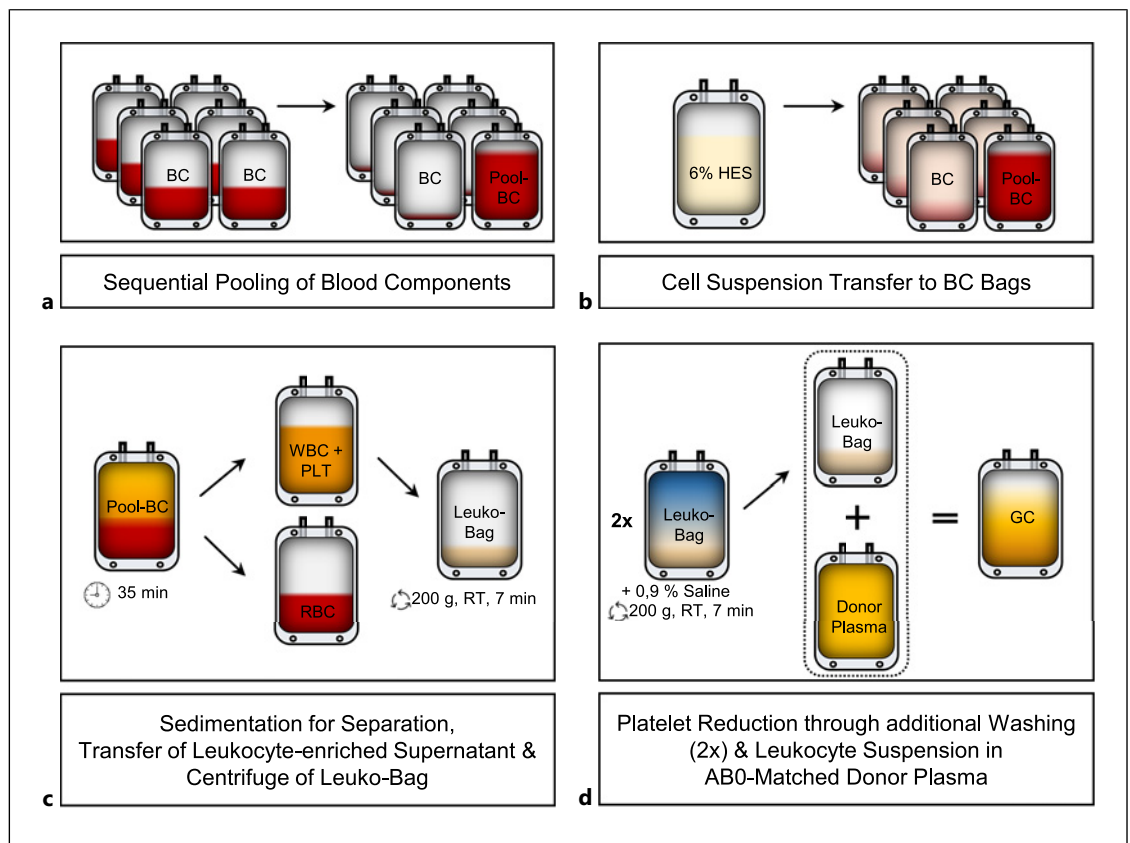


Fig. 1. a–d Manufacturing process. Schematic overview of the manufacturing process.

The pool bag was then connected to a transfer bag (CompoFlex), keeping the connection between the bags closed initially. The unit was hung up with the connecting tube at the top and left undisturbed for 35 min at $22 \pm 2^\circ\text{C}$, allowing the cells to sediment by gravity and to develop a sharp line of demarcation between the sedimented RBCs and the leukocyte-enriched supernatant. The pool bag was carefully placed in a manual plasma expresser (Medeiros, Rostock, Germany) to transfer the leukocyte-enriched supernatant to the empty transfer bag (“leuko-bag”) after the tube connection was opened. RBC sediment was discarded.

The leuko-bag was connected to an empty bag (e.g., a bag of washed BC) while the connection between the bags remained closed. The bags were then centrifuged at 200 g at $22 \pm 2^\circ\text{C}$ for 7 min (Centrifuge Roto Silenta RS, Hettich, Tuttlingen, Germany), resulting in a sediment of leukocytes and a leukocyte-enriched supernatant containing plasma, HES, and most of the PLTs. Using a plasma expresser, the leukocyte-enriched supernatant was transferred to an empty bag, which was disconnected and discarded.

Subsequently, two washing steps were performed for PLT reduction. The leuko-bag was connected to a bag of 0.9% saline (1,000 mL Macoperf, Macopharma, Langen, Germany). 30 mL of saline was added to the leuko-bag to resuspend the cell sediment prior to filling the leuko-bag with further saline to a total weight of 350 g . Subsequently, the bag was centrifuged (200 g , 7 min , $22 \pm 2^\circ\text{C}$) and the washing solution was pressed into an empty bag and discarded. This washing process was repeated once. Finally, the leukocytes were resuspended in 200 mL of ABO-matched donor plasma from one of the BC donors.

The resulting GC was transferred to a gas-permeable PLT storage bag (CompoStop F730; Fresenius Kabi, Bad Homburg, Germany), and the bubbles were removed. GCs were stored flat at RT in a gas-permeable bag without agitation for 72 h . Figure 1 provides a schematic overview of the manufacturing process.

Sampling

After 24 , 48 , and 72 h , the leukocyte concentrates have been mixed, and after discarding the first milliliter (content of the tube end) about 2 mL of the sample was taken for analysis.

Measurement of Blood Cell Counts and White Blood Cell Viability

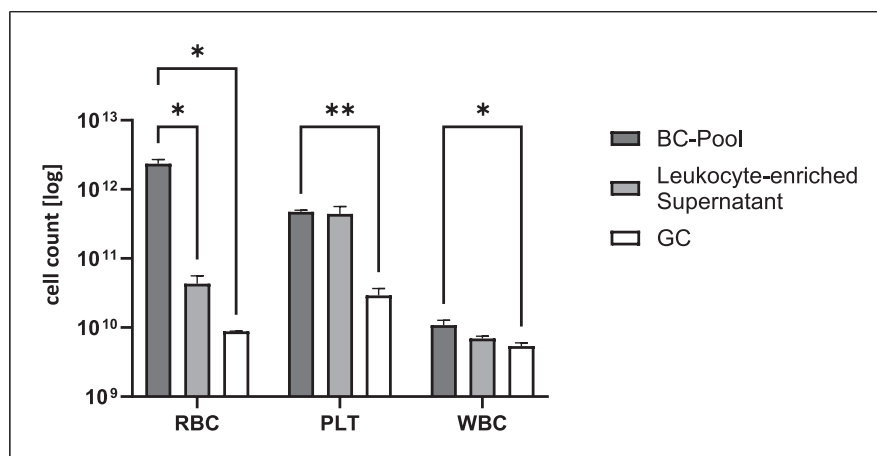
Residual PLTs, RBCs, and white blood cells (WBCs), including their differentiation, were evaluated automatically using a hematology analyzer (KX-21N, Sysmex, Norderstedt, Germany). Viability of leukocytes was determined by trypan blue exclusion test.

Evaluation of Granulocyte Function

For functional analysis, the leukocyte concentration was adjusted to 5×10^6 leukocytes/mL by adding AB plasma. Granulocyte function was analyzed in vitro with OxyBURST and phagocytosis assays using commercial Phagoburst-Kit and Phagotest-Kit (Celonis, Heidelberg, Germany), respectively.

Both tests were designed for whole blood and were used according to the manufacturer’s instructions after adaptation of the cell concentration because the granulocyte concentration in a GC is approximately 10 times higher than that in whole blood. To achieve a concentration of $5,000$ granulocytes/ μL and the same ratio of granulocytes to the stimulus (e.g., *Escherichia coli*) as in

Fig. 2. Distribution of cell types during the production steps. Red blood cell (RBC), platelet (PLT), and white blood cell (WBC) counts in pooled buffy coats (BC pool), leukocyte-enriched supernatant, and granulocyte concentrate (GC) preparations. *p* values ≤ 0.05 (*) and ≤ 0.01 (**) were considered significant. *N* = 53.



heparin-anticoagulated whole blood (4,000–10,000 granulocytes/ μ L), the samples were diluted in heparin-anticoagulated AB plasma.

Measurement of Electrolytes, pH, Glucose, Oxygen, and Carbon Dioxide Partial Pressures

pH, glucose, oxygen, and carbon dioxide partial pressures were measured using an ABL77 blood gas analyzer (Radiometer, Krefeld, Germany).

Measurement of Lactate Concentration and Lactate Dehydrogenase Activity

Lactate concentration and lactate dehydrogenase (LDH) activity were measured using Cobas Mira Plus CC (Roche, Ludwigsbuurg, Germany).

Statistical Analysis

Statistical analysis was performed using IBM SPSS Statistics (version 27, Chicago, IL, USA) and GraphPad Prism 9 (GraphPad Software Inc., San Diego, USA). Determination of the corresponding parameters was carried out in the BC pool, in the leukocyte-enriched supernatant, in the GC after preparation, and during storage up to 72 h. The results are expressed as mean $\pm$ standard deviation. The analytical evaluation was performed using univariate single-factor analysis of variance (ANOVA). If the *F* test was significant, the Fisher/least significant difference test was performed. According to the distribution of data (using the Shapiro-Wilk test), the Wilcoxon test was used for two dependent samples for continuous variables. The Kruskal-Wallis test was used to test the difference between multiple independent samples with the non-normal underlying population distribution, and appropriate post hoc tests were applied if necessary. The Friedman test was used when data arose from more than two related samples. Statistical significance was set at *p* < 0.05.

Results

Reduction of Contaminating Cells during Production Steps

A total of 76 BCs were used for all experiments. For each experiment, five to six BC bags from donors of the same blood type were combined, resulting in a total volume of 416 ± 13 mL for each BC pool. A total volume

of 258 ± 30 mL HES 200 per BC pool was added sterilely. After 35 min sedimentation, in the leukocyte-enriched supernatant the number of RBCs was $4.31 \times 10^{10} \pm 1.29 \times 10^{10}$ compared to $2.36 \times 10^{12} \pm 0.33 \times 10^{12}$ in the BC pool, the number of PLTs $4.41 \times 10^{11} \pm 1.24 \times 10^{11}$ versus $4.73 \times 10^{11} \pm 0.25 \times 10^{11}$ in the BC pool, and the number of WBCs $6.97 \times 10^9 \pm 0.53 \times 10^9$ versus $1.08 \times 10^{10} \pm 0.19 \times 10^{10}$ in the BC pool (Fig. 2). RBCs were reduced by more than 98% (*p* = 0.012), but PLTs only by 7% (*p* = 0.89). Two subsequent washing steps reduced the RBC content to 0.4% and the PLT content to 6.1% of the original RBC and PLT concentrations, respectively (*p* = 0.012 for RBC; *p* = 0.001 for PLT). However, the yield of WBCs was $5.38 \times 10^9 \pm 0.62 \times 10^9$ cells/pool, which corresponds to 49.8% of the initial leukocyte count in the BC pool (Fig. 2). Finally, the leukocytes were resuspended in 200 mL of ABO-matched donor plasma, resulting in a total volume of 230 ± 25 mL per unit. During subsequent storage, no change in the total WBC content nor in one of the major WBC subsets was observed (Fig. 3).

Granulocyte Viability and Function

The percentage of granulocytes that performed phagocytosis of FITC-labeled *E. coli* accounted for $96.9 \pm 2.1\%$ in the BC pool and remained $>90\%$ at all time points (72 h: $97.3 \pm 0.9\%$) (Fig. 4a). The percentage of granulocytes that performed *E. coli*-induced oxidative burst was $94.6 \pm 2.7\%$ in the BC and remained stable until 72 h of storage ($95.1 \pm 3.1\%$) (Fig. 4b). Viability was $99.8 \pm 0.2\%$ in the BC pool and showed a consistent trajectory for up to 72 h ($98.7 \pm 0.2\%$) for the whole observation period (Fig. 4c).

Glucose Consumption and Lactate Generation

Glucose levels in BCs as well as in the GC after preparation were measured: 19.1 ± 1.2 mmol/L and 20.8 ± 2.2 mmol/L, respectively. During storage, glucose levels decreased to 18.6 ± 1.1 mmol/L after 72 h (*p* = 0.015)

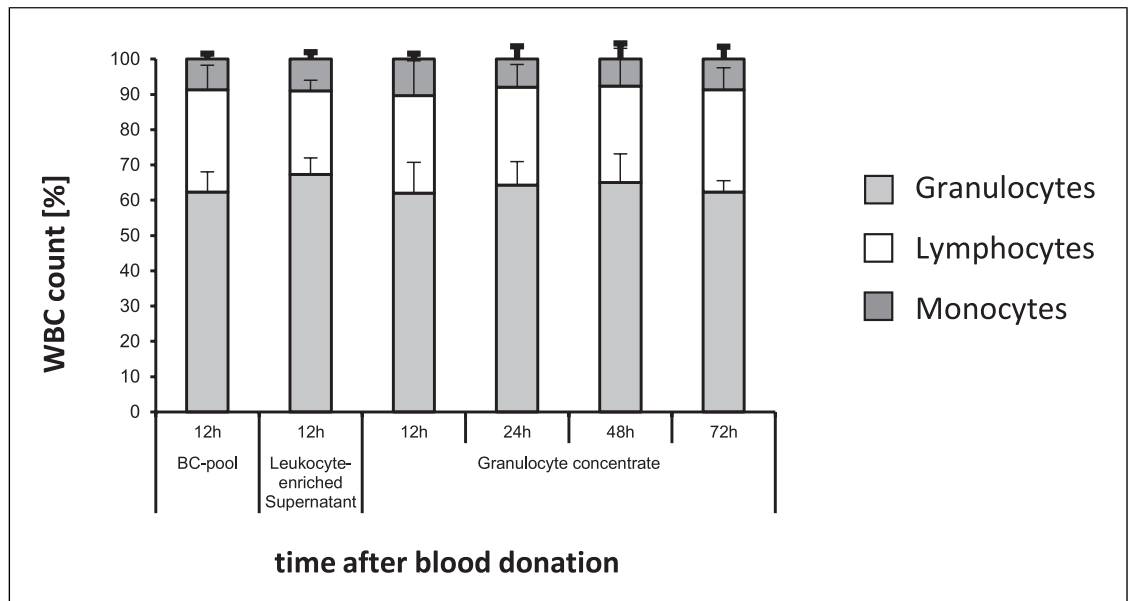


Fig. 3. Quantification of WBC subpopulations. Percentage distribution of WBC subpopulation contained in each preparation; Leukocyte concentrate during storage until 72 h at room temperature ($N = 23$); no difference between WBC populations.

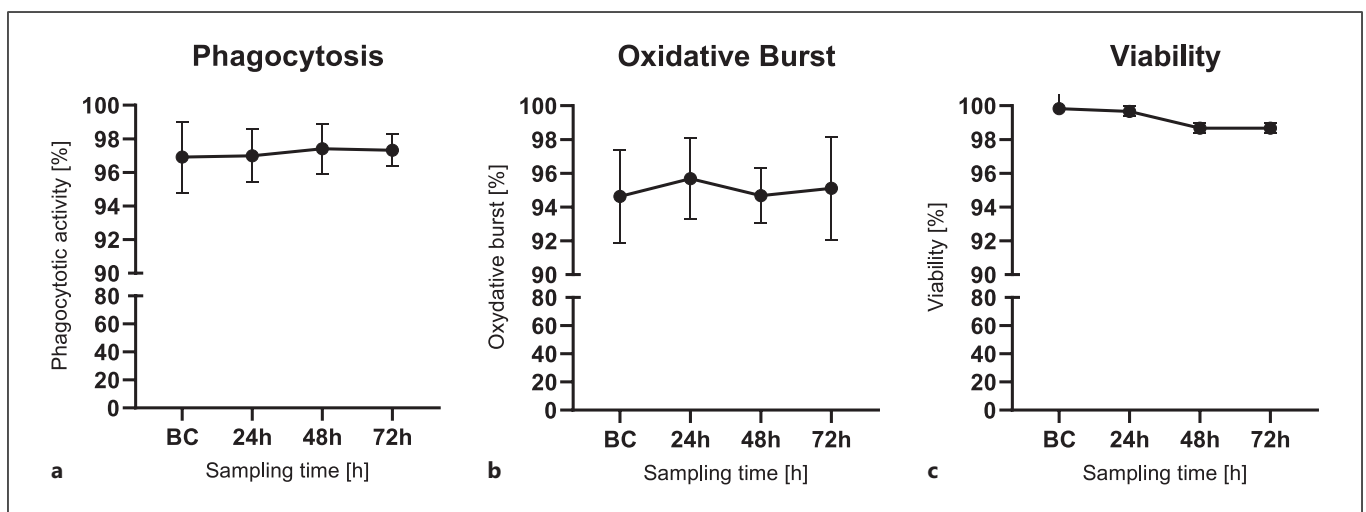


Fig. 4. Functional granulocyte activity of purified GC. Functional granulocyte activity of purified GC at defined observation time points. **a** Phagocytic activity. **b** Oxidative burst activity. **c** Viability. No statistically significant differences in cellular functionality were detected during the study period of 72 h of storage.

(Fig. 5a). The significant changes over the storage period are of minor clinical importance. In the BCs, lactate levels were 5.5 ± 1.5 mmol/L. In the fresh GC, lactate levels equaled 4.7 ± 1.1 mmol/L and increased during storage to 7.2 ± 0.7 mmol/L after 72 h ($p = 0.010$) (Fig. 5b).

pH Values

At baseline, the mean pH of the GC was 7.2 ± 0.07 immediately after manufacturing and showed a significant increase after 24 h up to 7.5 ± 0.06 and stable values up to 72 h of storage (7.5 ± 0.12 ; $p = 0.009$) (Fig. 5c).

Impact on LDH Activity

Figure 5d shows LDH activity as an indicator of cell damage, which in turn allowed conclusions to be drawn on the viability of the granulocytes. LDH values were within the physiological range (<225 U/L) and were stable during the experiment (Fig. 5d).

Discussion

This study describes a method for preparing GCs from whole blood donation-derived BCs. These GCs contain less than 1% of RBCs and less than 6% of PLTs

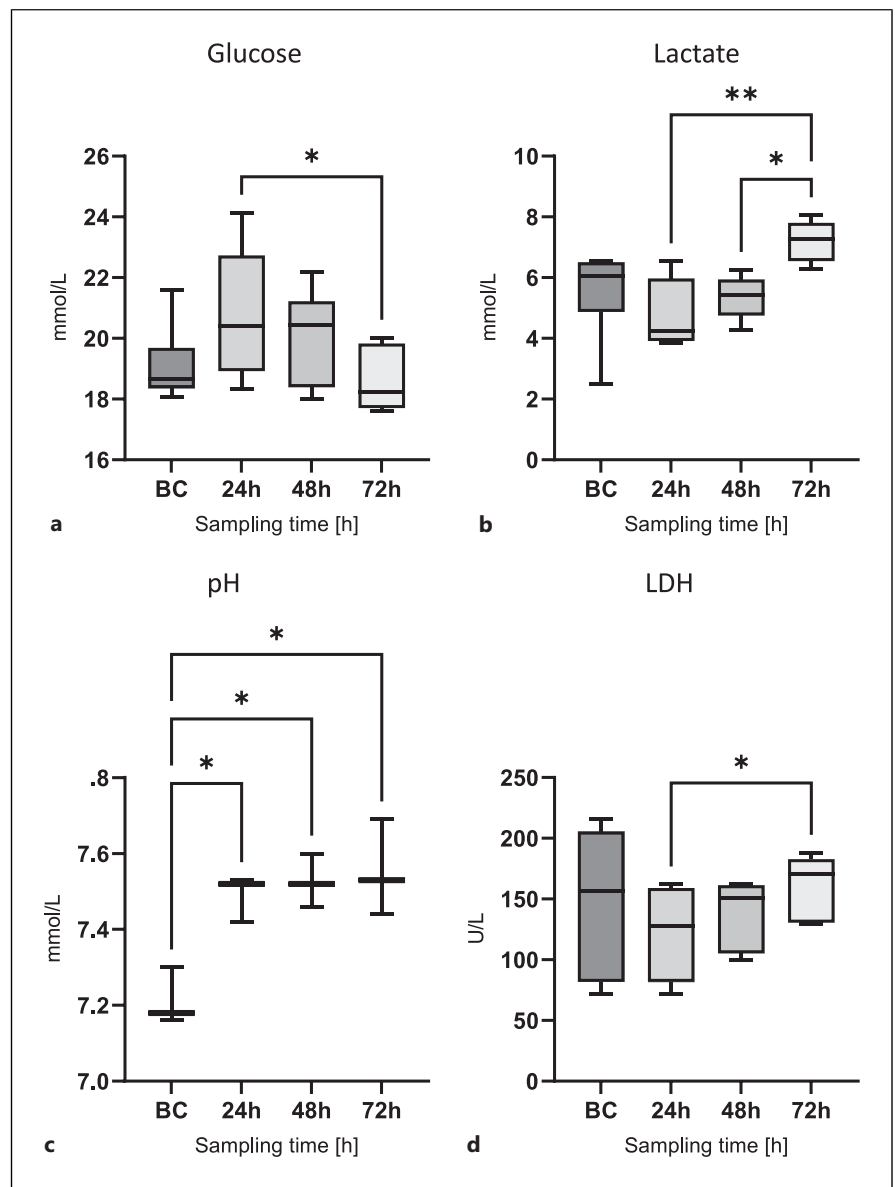


Fig. 5. Cellular metabolism: pH, glucose, lactate, and LDH activity. **a** Glucose concentration. **b** Lactate concentration. **c** pH value. **d** LDH activity was analyzed as an indication for cell damage. Compared to the reference value (<225 U/L), measured values are within the physiologic range and stable during the experiments. Therefore, no relevant cell damage was observed. *p* values ≤0.05 (*) and ≤0.01 (**) were considered significant.

compared to pooled BCs, while ~50% of leukocytes remain in the product. The final preparation contains approximately 3.4×10^9 granulocytes in 200 mL of ABO-compatible plasma, which can be stored at a temperature of 20–24°C for at least 72 h without significant loss of cellular function. In particular, the oxidative burst and phagocytosis ability remained above 90% of the baseline values of the fresh GC, even after 72 h of storage. The processing and storage procedure complies with the current GMP guidelines in a closed bag system with comparatively low time and cost expenditures. We provide a proof-of-principle concept that BCs could be used as an alternative to apheresis-derived GCs as a way to overcome potentially unacceptable risks for apheresis donors.

Regarding the capacity of the bags, an optimal yield could be achieved with pools of six BCs. Since a dose of at

least 1×10^{10} granulocytes is required for clinically meaningful use, approximately 15–20 BCs would need to be pooled [29].

In Germany in 2021, about 3.7 million whole blood donations have been performed, of which about 1.1 million BCs are used for the preparation of 275,000 pooled PLT productions [30]. Approx. 1–2 million BCs are discarded each year in Germany and could be used for purified pooled GCs. However, we see the need for improvements in granulocyte purification to open up the perspective to produce purified pooled GCs by single blood donation centers. The quantitative dimensions of these results warrant a more detailed discussion. However, the optimal dosing of BC-derived GCs needs to be addressed in clinical studies. Further refinement of the described methodology and additional clinical research is imperative

to advance our understanding and optimize the application of neutrophil transfusions in medical practice.

An important aspect to consider is the tendency of granulocytes to adhere to extraneous material [31–33]. This may explain why, after the initial sampling from the BC bags, the leukocytes in the leukocyte-enriched supernatant were dominated by the lymphocyte number. To prepare the samples, the granulocytes adhering to the bag surface were solubilized by rinsing the BC bags with the HES solution. Thus, a granulocyte content of approximately 70% in the leukocyte-enriched supernatant could be obtained. This will be a source of optimization to reduce granulocyte loss through this phenomenon.

In 2008, Bashir's method involved pooling 10 BCs, adding a PLT additive solution, centrifuging the mixture, and transferring the granulocyte-rich layer to a PLT storage bag with plasma addition. Our method applied in the present study resulted in a product with a lower hematocrit of 0.3% compared to Bashir's method, which obtained a hematocrit of 21%. In addition, our method resulted in a tenfold lower PLT content than Bashir's method. After 72 h of storage, our method resulted in a higher percentage of vital granulocytes, with 98.7% being viable in the final product compared to 84% for Bashir's method [18].

The number of neutrophils in the final product was higher in Bashir's method with 8.8×10^9 compared to 3.4×10^9 extracted from the leukocyte preparation in our method, whereas we used fewer BCs for the manufacturing process. Moreover, our method showed a higher rate of phagocytic activity by granulocytes, with a constant rate of 97%, whereas phagocytosis decreased from 94 to 92% in the Bashir method. In addition, our method showed a higher rate of reactive oxygen species production by granulocytes, with a constant rate of 95%, whereas oxidative burst activity decreased from 87 to 81% in the Bashir method [18].

Another innovation of the method presented here relates to the washing of leukocytes, which serves the purpose of PLT depletion, as well as HES removal. HES removal was intended to avoid additional stimulation of leukocytes and to minimize the risk of allergic reactions and other side effects of HES [34]. PLT depletion is necessary for better storage of leukocyte suspensions because PLTs should be stored with uniform agitation [28, 35], whereas resting storage is recommended for granulocytes [28]. Keating et al. [36] also reported the formation of PLT-leukocyte aggregates during storage of non-leukocyte-depleted RBC concentrates. These aggregates appear to be associated with increased leukocyte apoptosis and procoagulant activity. Although these processes are preceded by PLT activation, and HES 200 is thought to have a more inhibitory effect on PLT function

[37], the risk of PLT-leukocyte aggregate formation should be circumvented by depleting PLTs. The very low RBC content in our purified preparations (less than 5×10^9 , which corresponds to less than 0.5 mL of packed RBCs) precludes meaningful ABO compatibility testing. This was confirmed by the AABB guidelines. Herein, for example, ABO testing is waived for PLT concentrates if the total RBC volume is less than 2 mL [27]. This may allow ABO-independent transfusions and thus significantly expand the pool of potential donors. This is also consistent with the work of Bryant et al. who transfused 66 ABO-incompatible GCs (RBC volume 1.6–8.2 mL) with even higher RBC content, with no post-transfusion reactions or signs of hemolysis [38].

We show that 72 h of storage is feasible for BC-derived GCs. Schwanke et al. [39, 40] investigated granulocyte function after 72 h of storage in standard GC obtained by apheresis. These were tested either undiluted or diluted 1:4 in autologous plasma. In the undiluted preparation, no oxidative burst and only 15% phagocytic activity were detectable after 72 h. Similar to our results, 1:4 dilution with plasma showed only a slight decrease in oxidative burst and phagocytosis activities after 72 h. In another study by Schwanke et al. [39, 40], similar results were obtained for GCs diluted 1:8 with autologous plasma. Mochizuki et al. [41] investigated the influence of donor pretreatment on granulocyte function after 72 h of storage using the bag separation method. An untreated control sample, composed of a BC, was used for comparison. During storage (resting, RT), there was a successive loss of 10% of granulocytes, while in our observation, counts remained consistent, with similar viability (Mochizuki et al. [41]: 96 vs. Klinkmann et al. [15]: 99%). Oxidative burst was stable at a high level, similar to our findings, whereas our cells showed a significantly higher phagocytosis rate (Mochizuki et al. [41]: 83 vs. Klinkmann et al. [15]: 97%). Using another innovative purification concept in which standard GC was collected by granulocytapheresis, we succeeded in developing a closed system procedure compatible with standard blood bank technologies to remove 98% of RBCs and 96% of PLTs and enrich the GC. Purification extends the maximum storage time of purified GCs from 24 to 72 h with high viability and functionality [15].

The range of indications for GCs can be extended to the field of intensive care medicine, particularly extracorporeal therapy of sepsis-associated immune paralysis, using purified BCs. The use of leukocytes to treat sepsis in an extracorporeal setting was proposed by Mitzner et al. [42]. Regarding cellular immunocompetence, functional impairment of neutrophils and monocytes and lymphopenia have been linked to increased mortality in advanced stages of sepsis [4–12, 43, 44]. The use of donor granulocytes to treat critically ill sepsis patients in an

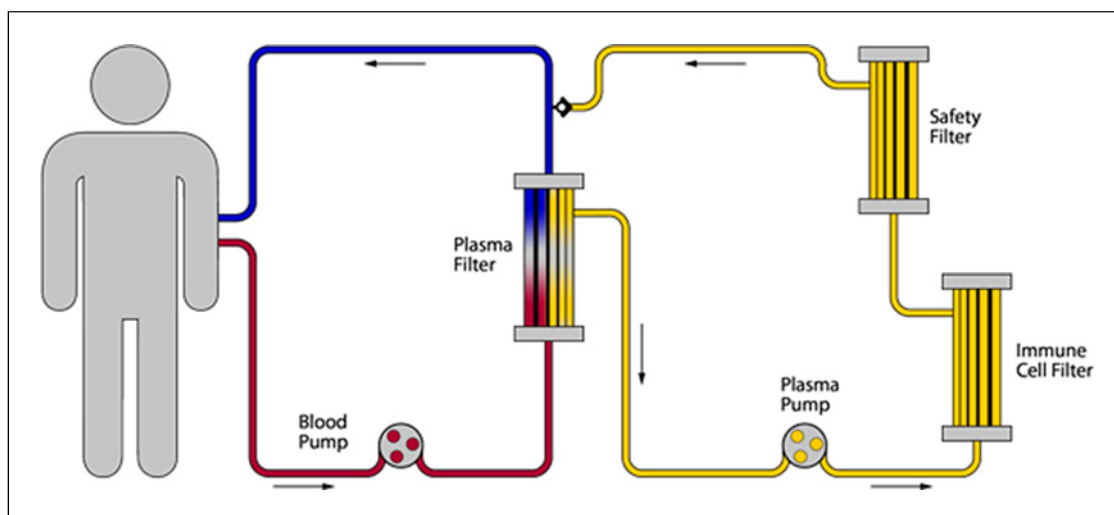


Fig. 6. Extracorporeal immune cell therapy. Extracorporeal immune cell therapy is a plasma treatment technology. Plasma is continuously filtered from the patient's extracorporeal bloodstream and transferred to a closed "cell circuit" where the patient's plasma is brought into direct contact with therapeutically active immune cells from a human donor (i.e., the GC). The schematic illustrates the optimized "one-way" immune cell perfusion method using purified GCs. The plasma filter CC2 serves as a redundant safety filter.

extracorporeal setting have been investigated both pre-clinically and in pilot clinical trials [45–49]. This therapeutic approach has already yielded promising results for its potential improvement of established sepsis therapies [47, 48]. As such, the immune cell perfusion therapy consists of a plasma separation unit and an extracorporeal circuit containing purified GCs (Fig. 6). Because all immune cells are fully retained extracorporeally and not transfused into the patient, irradiation is not required to prevent GvH reactions. Avoiding irradiation may better preserve granulocyte function [50, 51] and immunomodulatory function of lymphocytes in extracorporeal applications. However, Schwanke et al.'s [39] findings suggest the preservation of neutrophil function in irradiated plasma-diluted preparations stored for 72 h, prompting consideration for initial and late irradiation in cases where purified preparations are intended for direct transfusion. No radiation tests were carried out during the development of our method, so further studies are needed to evaluate the clinical use of the purified preparations.

The development of the extracorporeal therapy of sepsis-associated immune paralysis using purified BCs culminated in the initiation of an RCT (NCT05442710), which began in July 2022 and is currently actively enrolling patients. Purified GCs from granulocytapheresis [15] at a dose of $1.2\text{--}2.5 \times 10^{10}$ granulocytes per day are being used clinically in this extracorporeal immune cell plasma perfusion therapy.

This is a striking example demonstrating the potential for further clinical expansion of the indications for the use of GCs. To create infrastructural conditions for clinical

use, it is essential to make the best possible use of the various sources, in addition to the quality of the preparations.

Conclusion

To date, the clinical applicability and usability of GCs have been limited owing to the rapid deterioration in quality caused by an autolytic process of the granulocytes and high levels of lactate production from large amounts of RBCs. This led to a low pH within the first 24 h. However, a new purification process has been developed to remove impurities, such as RBCs, thrombocytes, and sedimentation agents, resulting in better storage conditions for leukocytes and fewer side effects for recipients. This purification process increases the shelf life of GCs, while maintaining cell functionality, making it more usable in clinical practice. Additionally, the reduced RBC content could allow for the ABO-independent use of purified GCs, enabling the immediate use of GCs without relying on a specific recipient.

Overall, the development of a closed blood bag system and the production of GCs from pooled BCs have significant potential to solve the problem of storage of cellular blood products and could have important implications for the treatment of sepsis. The preceding quantitative investigation highlights the necessity for a thorough investigation in this domain. It is crucial to enhance our comprehension and refine the methodology, alongside continuous clinical research, to optimize the

utilization of neutrophil transfusions. Subsequent research endeavors are warranted to substantiate the efficacy of GC therapy in diverse clinical settings.

Statement of Ethics

Whole blood donations were collected after informed consent was obtained from the donors. Each donor fulfilled the applicable donor suitability criteria of the national and European guidelines for hemotherapy. The study was approved by the Local Ethics Committee of the University of Greifswald (Reg. No.: BB014/14).

Conflict of Interest Statement

Fanny Doss, Sandra Doss, Steffen Mitzner, and Jens Altrichter are employees or shareholders of Artcline GmbH. Kathleen Selleng received personal fees from Aspen Germany, travel support from Sobi, and research funding from Immucor and Grifols, outside the submitted work. All others have no conflict of interest to declare.

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Author Contributions

Gerd Klinkmann, Fanny Doss, Jens Altrichter, and Steffen Mitzner: concept, data collection, data interpretation, and drafting and critical revision of this article. Antje Schwarz, Susanne Reichert, and Kathleen Selleng: GC preparation, performance of the in vitro experiments, data collection, data interpretation, and critical revision of this article. Sandra Doss, Daniel A. Reuter, and Thomas Thiele: data interpretation and critical revision of this article.

Data Availability Statement

All data generated or analyzed during this study are included in this article. Further inquiries can be directed to the corresponding author.

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