Evaluation of Random Donor Platelets Produced from Buffy Coat Stored for 24 h at Ambient Temperature: Should This be Implemented in India?

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Abstract Whole blood derived platelets are made from platelet rich plasma (PRP) method or buffy coat (BC) method. In India majority of random donor platelets (RDPs) are prepared by PRP method. However, BC method offers the advantage of less platelet activation and fewer WBC contamination. Presently in India RDPs are prepared within 8 h of whole blood collection, whereas, in Europe this time limit is up to 24 h. Our aim was to evaluate the platelet count, WBC contamination, platelet CD62P expression, and biochemical parameters of RDPs prepared from BC within 8 h and within 24 h of collection. We prepared 40 units of RDP by the BC method from whole blood stored at room temperature within 8 h of collection (fresh BC), and another 40 units from BC stored at 22 °C for <24 h (stored BC). We analyzed the platelet counts, CD62P expression, WBC counts, glucose levels, pH, PO₂, PCO₂ in both the groups of RDPs, 24 h after respective preparation. The platelet counts from stored BC was higher in fresh BC. CD62P expression was low in stored BC compared to fresh BC. There were no differences of pH, pO₂, pCO₂ and glucose levels in fresh BC and stored BC. WBC contamination was more in fresh BC. Our study stored BC contained higher platelet counts, less WBC contamination and less platelet activation. We conclude that RDP prepared from stored BC is the better method for RDP production.

Keywords Buffy coat · Platelet count · WBC contamination · Platelet activation · CD 62P expression

Introduction

Buffy coat (BC) preparation of platelet is the most popular method of preparation of random donor platelets (RDP) in countries such as Canada, UK and Europe. In Europe, there is an approximately 50:50 split between the use of BC- and apheresis-derived platelet concentrates (PC) [1]. In Canada (Quebec exempted), 70 % of platelets are derived from whole blood donation. Denmark, Finland, and the Netherlands prepare 85–95 % of their concentrates by the BC method [2]. These countries demonstrate that a national platelet supply can be derived almost entirely from collected whole blood rather than relying upon apheresis PC production [1, 2]. A special kind of bag which has top and bottom outlets are used to prepare the platelet concentrate in this technique. Whole blood collected into the main bag is subjected to heavy spin and semi-automated expresser is used to expel out platelet poor plasma from the top port and red blood cells from the bottom port. 30 mL of RBC and 30 mL of plasma remains in the main bag with majority of platelets and WBCs. The main bag is subjected to re-centrifuge in soft spin and platelet rich plasma is expelled out and collected from the top port, leaving BC and red blood cells in the main bag.

In the United Kingdom and continental Europe, four to six buffy coats are combined, re-suspended in plasma from a male donor or in a platelet additive solution, and soft centrifuged to remove red cells and white cells [3]. Platelets can be prepared using this method from WB stored at room temperature (not <20 °C) for up to 24 h [4]. Advantages of the BC method are that there is less

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activation of platelets than in the PRP method because in the BC method, platelets are cushioned against red cells during the hard spin. Plasma units prepared from BC-depleted units have approximately 41 mL more plasma than plasma units prepared by the PRP method. Platelets prepared by the PRP method result in 21 % of the plasma and 19 % of the platelets remaining with the red cells. Therefore, the hematocrit of the packed RBCs produced with the PRP method is lower (51 %) than in the BC method (60 %). BC depletion also results in a 13 % loss of the donated red cells [5]. In PRP method of platelet preparation PRP is subjected to hard spin leading to formation of platelet pellet. Pelleting make close contact of platelets which make them activate temporarily. In contrast BC method gives the luxury of cushion to platelets among RBC during the hard spin. Therefore platelets are less activated than PRP method [6].

Platelet activation during processing and throughout storage is accompanied by surface expression of sequestered granular membrane proteins (P-selectin and CD63) and conformational changes of the fibrinogen receptor, GP-IIb/IIIa [7]. P-selectin (also known as CD62P, GMP-140, and PADGEM) is an integral membrane protein found in α -granules that becomes expressed on the surface of activated platelets after granule release [8].

In India when RDP is prepared by the BC method, it is done so, almost entirely from the whole blood stored at room temperature, for less than 8 h as per DGHS guidelines [9].

The aim of our study was to evaluate the platelet count, WBC contamination, platelet activation and other biochemical parameters of platelet concentrate prepared from BC within 8 h and within 24 h of collection, respectively, in order to assess and compare the two methods.

Materials and Method

40 units of RDP prepared from fresh BC separated from whole blood within 8 h of collection (fresh BC) and 40 units of RDP prepared from BC stored at 22 °C, overnight (stored BC) were studied in an unpaired study design.

80 units of whole blood were collected into quadruple bag (Fenwal, Lake Zurich, IL 60047, USA) and subjected to high spin separation within 8 h of collection. Semi-automated plasma expresser (Optipress—Fenwal, Lake Zurich, IL 60047, USA) was used to express out the platelet poor plasma from the top port of the bag and red blood cells were expressed out from the bottom port leaving BC and platelets in the primary collection bag. Out of them 40 units were subjected to rest for 1 h and then centrifuged soft spin. Platelets were expressed out from the top port using semi-automated plasma expresser, leaving

the BC and RBC in the primary collection bag. The other 40 units were left undisturbed overnight at 22 °C and RDPs were prepared after soft spin centrifugation on the following day. Samples were taken on 24 h of preparation of platelets and day 5 of blood collection in both the groups.

Our primary objective was platelet count in the RDP and secondary objectives were WBC count, pH, glucose, pO₂, pCO₂ and platelet surface CD62P expression. All in vitro assays were undertaken according to the established validated method. Platelet counts and WBC counts were determined by using automated cell counter (Sysmax, Wakinohama-Kaigandori, Chuo-ku, Kobe, 651-0073, Japan) in the blood bank. In vitro glucose values were determined by the biochemistry laboratory of the hospital. [National accreditation board of laboratories (NABL) accredited lab]. pO₂, pCO₂ and PH were determined by using blood gas analyzer (Roshe, Carolina Inc. Old Marion Highway, Florence, SC, USA). Platelet surface CD62P expression were measured by using flow cytometry (FACS Calibur, BD Biosciences, Qume Drive, San Jose, California, USA). Two samples from each platelet units (50 μ L) were taken and one sample was stained by 10 μ L monoclonal mouse antibodies and phycoerythrin according to the manufacture's instruction. Second sample was analyzed without staining. First unstained sample was analyzed, followed by the stained sample. 6,000 events per second were counted in both samples by the flowcytometer. Result graphs of both samples were superimposed and results were obtained as a percentage of activation.

Statistical analysis were done by using IBM SPSS statistical software version 21 and *p* value <0.05 were considered as statistically significant.

Randomly selected platelet samples were sent for bacteriological culture to the microbiology department of the hospital.

Results

At 24 h After Preparation

Fresh BC and Stored BC showed mean platelet counts of $5.7 \times 10^{10} \pm 1.57$ and $6.32 \times 10^{10} \pm 1.18$ per unit, respectively. Among the fresh BC, 31 (77.5 %) out of 40 units contained more than 6×10^{10} platelets per unit whereas in stored BC only 36(90 %) units out of 40 units contained more than 6×10^{10} platelets per unit. Mean WBC contamination in fresh BC was $5.88 \times 10^6 \pm 2.85$ and stored BC was $5.55 \times 10^6 \pm 1.53$ per unit respectively. Fresh BC, 31 units and stored BC 35 units out of 40 units each, contained $<5.5 \times 10^6$ WBC respectively. Mean CD62P expression in fresh BC and stored BC showed $33.35 \% \pm 13.75$ and $19.5 \% \pm 14.45$ respectively. Range

Table 1 Mean values of each variables of platelet concentrate of 24 h after of preparation

Variables	Fresh BC	Stored BC
Platelets $\times 10^{10}$ per unit	5.7 ± 1.57	6.32 ± 1.18
WBC $\times 10^6$ per unit	5.88 ± 2.85	5.55 ± 1.53
CD62P expression %	33.35 ± 13.75	19.5 ± 14.45
Glucose mmol/L	389 ± 67.91	360 ± 62.18
pO ₂ (kPa)	17.12 ± 4.1	16.5 ± 3.12
pCO ₂ (kPa)	7.7 ± 3.07	7.7 ± 3.07
pH	6.99 ± 0.23	6.91 ± 0.21

CD62P expression of platelet in fresh BC was 14–55 % and in stored BC, it was 4–44 %. Mean plasma glucose levels in fresh BC was $389 \text{ mmol/L} \pm 67.91$ and stored BC was $360 \text{ mmol/L} \pm 62.18$. Mean pH of the fresh BC was 6.99 ± 0.23 where as in stored BC was 6.91 ± 0.21 . Mean pCO₂ of both fresh BC and stored BC was $7.7 \text{ kPa} \pm 3$. Mean pO₂ of the fresh BC was $17.12 \text{ kPa} \pm 4.1$ and stored BC $16.5 \text{ kPa} \pm 3.12$ (Table 1).

At 5th Day of Storage

Fresh BC and Stored BC showed that mean platelet counts at the 5th day of storage were $5.5 \times 10^{10} \pm 1.4$ and $5.83 \times 10^{10} \pm 1.05$ per unit, respectively. Mean WBC contamination in fresh BC after 5 days of storage was $5.33 \times 10^6 \pm 2.69$ and stored BC was $4.93 \times 10^6 \pm 0.59$ per unit respectively. CD62P expression in fresh BC and stored BC at the end of the 5 day storage showed that $38.47 \% \pm 13.18$ and $22.3 \% \pm 13.86$ respectively. Mean plasma glucose levels in fresh BC was $379.5 \text{ mmol/L} \pm 56.48$ and stored BC was $348.35 \text{ mmol/L} \pm 53.13$. Mean pH of the fresh BC was 6.93 ± 0.27 where as in stored BC was 6.85 ± 0.25 . Mean pCO₂ of fresh BC and stored BC were $8.55 \text{ kPa} \pm 2.72$ and 8.4 ± 2.58 respectively. Mean pO₂ of the fresh BC was $16.6 \text{ kPa} \pm 3.46$ and stored BC $15.37 \text{ kPa} \pm 2.82$. Microbiological cultures of randomly selected samples of both fresh BC and stored BC were found to be sterile.

Discussion

In our study we studied the effect of ambient temperature on platelet concentrate which was prepared from overnight hold BC, and results were compared with platelet concentrate prepared from fresh BC within 8 h of collection. It was found that the stored BC contained comparatively higher platelet counts, and were less activated compared to the fresh BC prepared platelet concentrate.

Table 2 CD62P expression (%) in fresh BC and stored BC in day 1 and day 5 of storage

	Fresh BC		Stored BC		<i>p</i>
	Mean	Range	Mean	Range	
Day 1 (%)	33.35 ± 13.74	13–60	19.5 ± 14.44	4–44	16.17
Day 5 (%)	38.48 ± 13.18	17–73	22.3 ± 13.86	5–49	13.35
<i>p</i>	0.04		0.19		

Lower platelet count in the fresh BC may be due to relatively less resting period of BC before preparation of platelets. Platelets within the 8 h of collection, are still in a relative stage of aggregation and activation due to the centrifugation they were subjected to, while being prepared. Due to these possibilities platelets were trapped among the concentrated WBCs and could not come to the plasma layer during soft centrifugation. On the other hand stored BC, have the advantage of relatively more resting period during which period the aggregation and activation of platelets would reduce, which will eventually lead to more and more platelet release into the plasma layer in soft second centrifugation of platelet production. In our study 10.87 % more platelets were concentrated in stored BC compared to fresh BC. Dijkstra-Tiekstra et al. [10] explained in their study that the overnight platelet concentrate (PC) showed higher PLT count (approx. $460 \times 10^9/\text{PC}$ vs. approx. $310 \times 10^9/\text{PC}$) compared to that of fresh BC derived PC. This finding was comparable with our results.

Mean WBC contamination of fresh BC and stored BC was 5.88 ± 2.85 and 5.55 ± 1.53 WBC $\times 10^6$ per unit respectively. 5.94 % more WBC s were contaminated in fresh BC compered to stored BC. Overnight exposure to room temperature (20–24 °C) lead to lysis of the WBCs, and, overnight undisturbed standing may have settled down the WBCs in the stored BC. Therefore stored BC contained less WBC s compared to fresh BC.

CD62P expression in the 24 h after the production, was $33.35 \pm 13.74 \%$ in fresh BC and $19.5 \pm 14.44 \%$ in stored BC respectively. Thus in fresh BC, 13.85 % more platelets were activated, compared to that of stored BC. However *p* value was not statistically significant. At the end of the 5th day of storage both fresh and stored BC platelets expressed CD62P more than their respective 1st day values. In fresh BC 5.13 % more platelets expressed CD62P after 5 days of storage when compared to that of fresh BC in 1st day. This difference was statistically significant. In stored BC 2.8 % more platelets were activated when 1st day and 5th day were compared, and this difference was not statistically significant. Comparison of both fresh and stored BC at the 5th day of storage has shown that fresh BC contained 11.18 % CD62P expressed platelets than in the stored BC. (Table 2)

High expression of CD62P of platelets in fresh BC appear to be due to shorter resting period compared to stored BC. Longer resting period would result in disaggregation as well as less activation of the platelets. However the platelets expressed CD62P more and more during the storage due to reduced pH.

We analyzed metabolic parameters of the platelets, such as glucose pO_2 pCO_2 and pH after 24 h and 5th day of storage. Amount of glucose (mmol/L) in fresh BC was 389 ± 67.91 and in stored BC was 360 ± 62.18 . WBCs and platelets stored overnight in room temperature, metabolized glucose and produced lactic acid as a byproduct. This was the reason of reduced amount of glucose in stored BC. Due to increased amount of lactic acid production in stored BC, pH has reduced to 6.91 compared to fresh BC (6.99). However pH was within the quality control parameters of BC platelets. pO_2 and pCO_2 values of both the products were almost similar. However none of the metabolic parameters between two groups were statistically significant even after 5 days of storage. Dijkstra-Tiekstra et al. [10] found that the overnight PC higher pCO_2 , and lactate concentration and lower pH, pO_2 , glucose concentration, CD62P expression (until day 5). We did not measure the lactate concentration but rest of the findings are comparable with our results. Another study done by Dijkstra-Tiekstra et al. study the lactate, pCO_2 , and hypotonic shock response pH, glucose, pO_2 , and CD62P expression swirling effect, white blood cell count, annexin V binding, or aggregation between overnight and fresh BC derived PC. They found that significant difference in lactate, pCO_2 , hypotonic shock response, pH, glucose, pO_2 , and CD62P expression between the fresh and overnight held PC. In our study we found that findings were not statistically significant [11]. Another similar study done by Lu et al. [12] revealed that the platelet yield in PCs prepared from an overnight-hold WB sample was significantly higher, while CD62P expression and annexin V binding were lower ($p < 0.05$). These findings were comparable with our results.

Platelets experience a progressive decline in function accompanied by characteristic morphologic changes. Studies document up to 20 % loss of platelet recovery through 5 days of storage. In our study 12.75 % loss of platelet was observed in fresh BC at the 5th day of storage, whereas the amount loss in the stored BC at the end of storage was 6.82 % [8]. Activation of platelets was the main reason for platelet loss during storage. Less activated platelet in stored BC showed that much less amount loss during storage in our study. Loss of WBC of fresh BC was 15 % whereas in stored BC 30 % after 5 days of storage. Exposure to room temperature for longer time in stored BC resulted in lysis of WBC more than fresh BC. This has an additional advantage of less number of WBC contamination in stored BC. However the effects of cytokines, released into the platelet

concentrate due to lysis of WBC has to be studied further. An added advantage of stored BC is that when WBCs are in contact with prospective bacterial contaminants for a longer period of time, as is the case with stored BC, then the chance of increased pathogen destruction due to phagocytosis is prevalent [13].

However keeping BC for 24 h before preparation of platelets, reduce the storage time of platelets for 24 h is a drawback of the study.

Conclusion

Our study concluded that stored BC contained higher platelet counts, less WBC contamination and less platelet activation. Platelets can be prepared from stored BC, during business hours of following day. This would provide for better supervision, ensuring a better product. This would benefit blood banks where the preparation of components are delayed due to longer transportation time either from satellite blood banks or blood donation camps, which is a common problem in India. Also fewer RDPs are discarded due to low platelet counts, as this method recovers comparatively more platelets. Less number of units requires to be transfused to the patients, since the quality per unit is better, which eventually leads to patients exposure to fewer donors. We conclude that RDP prepared from BC method stored at room temperature for 24 h, is a better method for production of RDPs.

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