Manufacture of Pooled Platelets in Additive Solution and Storage in an ELX Container After an Overnight Warm Temperature Hold of Platelet-Rich Plasma

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Abstract

The processing of whole blood–derived platelet-rich plasma (PRP) to a platelet concentrate and platelet-poor plasma is currently performed within 8 hours to comply with the requirements to manufacture fresh frozen plasma. Maintaining PRP at room temperature for a longer period can have the advantage of shifting the completion of component manufacture onto day shifts. Pairs of ABO-identical prepooled platelets were manufactured by the PRP method, using the current approach with platelet storage in a CLX HP container (Pall Medical, Covina, CA) and plasma, or a novel approach with an 18- to a 24-hour room temperature hold of the PRP and the manufacture of pooled platelets in a glucose-containing additive solution (AS) and storage in a new ELX container (Pall Medical). Standard in vitro assays were performed on days 2, 5, and 7. The results showed that the AS platelets in ELX have in vitro characteristics that are equivalent or superior to those of the standard product.

Platelet concentrates (PCs) manufactured from whole blood donation (WBD) must be kept at room temperature before processing.1,2 Two schemes are in use to manufacture WBD platelets: In the United States, an initial soft-spin centrifugation of the whole blood occurs within 8 hours, producing platelet-rich plasma (PRP) as an intermediate product with a subsequent secondary hard-spin centrifugation producing platelet-rich plasma (PRP) and a platelet pellet. The platelet pellet is then resuspended in 50 to 60 mL of plasma, producing the PC. Individual PCs can be stored until the next day and pooled when the ABO type is known.3,4 An alternative approach is the buffy coat (BC) method that is commonly used in Europe.5 In this method, whole blood units are held overnight at 18°C to 20°C and then subjected to a hard spin to produce plasma, allowing the platelets to be concentrated in the BC. A subsequent soft-spin centrifugation is then performed, typically of 4 pooled BCs suspended in plasma from one of the donors or an additive solution (AS) to separate the supernatant platelets from the sedimented WBCs and RBCs. The advantage of such an approach has caused Canadian blood services to recently change from the PRP to the BC method.6

A 24-hour room temperature hold of whole blood (or intermediate processing products) can have advantages to a blood center, allowing component manufacture to occur or be completed on earlier shifts on the following day. However, holding whole blood units at room temperature for 18 to 24 hours seems to be detrimental to RBC quality, and, hence, it is preferred if RBC concentrates (RCCs) are manufactured and refrigerated within 8 hours.7 To overcome this effect on the RBC, the whole blood units can be processed within 8 hours into RBCs and PRP; the RBCs
are then refrigerated, while the PRP can be held at room temperature overnight. Such a longer room temperature hold for the PRP may optimize the platelet yield⁸ and, if AS is used, could result in the manufacture of a platelet pool similar to a BC pool in AS. However, an undesirable effect of this approach is that the longer room temperature hold could compromise the quality of the manufactured plasma, mainly with respect to factor VIII.⁹ Nevertheless, plasma manufactured using this approach would be similar to the plasma produced using a whole blood overnight hold with the manufacture of BC pools.

Recently a non–glucose-containing AS was cleared for use in the United States for storage of apheresis platelets (InterSol, Fenwal, Lake Zurich, IL).¹⁰ Previous studies have shown the importance of adequate glucose in the maintenance of platelet quality,¹¹–¹⁴ but owing to logistic difficulties in manufacturing an AS with glucose, most platelet ASs in Europe are non–glucose-containing solutions. Consequently, this requires a high level of plasma carryover (approximately 35%) as a source of glucose, in addition to the beneficial buffering effect of plasma.

The aim of this study was to compare platelet pools with minimal plasma carryover stored in a glucose-containing AS and in a new container that were manufactured by using warm overnight hold of the PRP with platelet pools manufactured and stored using the current standard method. The essence of this approach is illustrated in Figure 1.

**Materials and Methods**

**Blood Sample Collection and Initial Processing (Day 0)**

The study was approved by the institutional research board of The Miriam Hospital, Providence, RI. Samples from all donors were drawn at the Rhode Island Blood Center, Providence, and met all American Association of Blood Banks and US Food and Drug Administration (FDA) criteria for blood donation.¹ Whole blood (500 mL) was collected into a standard blood collection system using CP2D as the anticoagulant. The whole blood was centrifuged within 8 hours by a soft spin (1,900 g for 4.5 minutes) using an RC3BP Sorvall Centrifuge (ThermoFisher Scientific, Waltham, MA) to produce PRP and an RCC that was subsequently stored in AS-3. Two ABO-identical PRP units were then pooled and separated into 2 equal aliquots, yielding identical pairs of PRP.

**Manufacture of Standard Platelet Pools Stored in Plasma (Days 0-1)**

One of the paired PRP products was centrifuged within 8 hours by a hard-spin centrifugation (2,350 g for 8 minutes) on day 0 (day of collection) to produce PPP and a PC. The PC was resuspended in approximately 50 to 60 mL of residual plasma and held overnight unagitated at 20°C to 24°C. Using the Acrodose PLus system (Pall Medical, Covina, CA), 4 to 6 U were pooled on day 1 (day after collection) into a transfer bag and then leukoreduced through an Acrodose PL leukocyte reduction filter (Pall Medical) into a 1.5-L CLX HP storage container [Pall Medical; the container is polyvinyl chloride with a tri(2-ethylhexyl) trimellitate plasticizer]. This pool was stored at 20°C to 24°C in an incubator (Helmer, Noblesville, IN) with horizontal agitation for up to 7 days. This product is the current standard approach for manufacturing whole blood–derived platelets and is FDA-cleared.

**Manufacture of 18- to 24-Hour Room Temperature–Held PRP-Derived Platelet Pools Stored in AS (Days 1-2)**

The second of the paired PRP products was left unagitated at room temperature for an additional 18 to 24 hours. On day 1 (the day following collection), the PRP was centrifuged by a hard-spin centrifugation to PPP and a platelet pellet that was resuspended in approximately 15 mL of plasma. Using the Acrodose PL leukocyte reduction filter (Pall Medical) into a 1.5-L CLX HP storage container [Pall Medical; the container is polyvinyl chloride with a tri(2-ethylhexyl) trimellitate plasticizer]. This pool was stored at 20°C to 24°C in an incubator (Helmer, Noblesville, IN) with horizontal agitation for up to 7 days. This product is the current standard approach for manufacturing whole blood–derived platelets and is FDA-cleared.
non-Di(2-ethylhexyl) phthalate container composed of ethylene butyl acrylate copolymer and is in current use in Europe.\textsuperscript{15} The product was stored overnight with horizontal agitation. On the next day (day 2), the pooled platelets stored in AS were leukoreduced using the Acrodose PL filter and transferred to another ELX storage container. This pool was then stored at 20°C to 24°C in the same incubator with horizontal agitation for up to 7 days. Details of this storage system have been previously published.\textsuperscript{12,13,15-17} The scheme is illustrated in Figure 2I and allows the manufacture of paired products for comparison studies. Neither the AS nor the ELX containers are cleared by the FDA for use in platelet storage at present.

**In Vitro Assays of Platelet Pools**

Samples were collected aseptically from each pair of CLX HP/plasma platelet pools and ELX/PAS-G platelet pools before leukoreduction on day 1 (prefiltration sample). Following leukoreduction (postfiltration), samples were collected from both products on days 2, 5, and 7.

The prefiltration samples were assayed for WBC count, from which the WBC yield was calculated; platelet count, from which platelet yield was calculated; mean platelet volume (MPV); and surface expression of P-selectin and observed for microscopically visible aggregates. The postfiltration samples were assayed for all of the preceding factors in addition to pH, pCO\textsubscript{2}, pO\textsubscript{2}, glucose, lactate, bicarbonate, swirling, morphologic score, extent of shape change (ESC), hypotonic shock response (HSR), and total protein.

Platelet specimens were incubated with phycoerythrin (PE)-conjugated anti-CD62p to detect surface P-selectin expression and fluorescein isothiocyanate (FITC)-conjugated anti CD42b to detect glycoprotein 1b expression, which was used to gate the platelet population. The control sample used to gate for nonspecific staining was incubated with mouse IgG-PE and IgG-FITC.

The pH, pCO\textsubscript{2}, pO\textsubscript{2}, glucose, lactate, and bicarbonate were assayed using a blood gas analyzer (GEM Premier 3000, Instrumentation Laboratory, Lexington, MA). The platelet count, MPV, and prefiltration WBC count were measured in a Horiba ABX Micros 60 hematology analyzer (GMI, Ramsey, MS). The postfiltration WBC count was measured with a flow cytometer (BD FACSCanto II, Becton Dickinson, Franklin Lakes, New Jersey).

**Figure 2I** Schematic representation of collection, processing, and preparation of platelet pools. The volume in platelet concentrates (PC) is the plasma volume expressed in milliliters. PAS-G, glucose-containing additive solution; PRP, platelet-rich plasma; WBD, whole blood donation. CLX and ELX are the containers.
Dickinson, San Jose, CA) using the BD Leucocount Reagent Kit (BD Biosciences, San Jose, CA). Surface expression of P-selectin was measured using the same flow cytometer.

Glucose consumption and lactate generation were calculated by using the platelet yield on day 2 and the concentration difference in these analytes between days 2 and 7. The results were expressed as nmol/10^{12} platelets per day.

Swirling was measured by using visual observation of the pooled platelets. A scoring system of 0 to 3 was used in which 0 indicates no swirling and 3 indicates the highest swirling. The morphologic score was assessed by observation of 100 platelets using phase contrast microscopy, and the morphologic features were scored as follows: disks × 4, spheres × 2, dendrites × 1, and balloons × 0; scores were added to achieve a score of 0 to 400.

The ESC and HSR were assessed in a model 540 aggregometer (Chrono-log, Havertown, PA), the ESC using photometric detection of the increase in optical density of platelet suspension in response to adenosine diphosphate and the HSR measured by the photometric change in the refractive index of the platelet suspension after exposure to a hypotonic environment.

Total protein was measured on day 2 in the platelet supernatant using a chemistry analyzer (UniCel DxC 800 Synchron Clinical System, Beckman Coulter, Brea, CA). The percentage plasma concentration in the ELX/PAS-G platelet pool was calculated by using the following formula: [ELX Total Protein (mg/dL)/CLX Total Protein (mg/dL)] × 100.

### Statistical Analysis

All data were entered into a statistical software application (Epistat, Richardson, TX). Products were characterized by description statistics and comparisons performed by using paired t tests. Statistical significance was arbitrarily defined as a P value of less than .01 owing to the large number of comparisons.

### Results

We manufactured 12 paired pools of ABO-identical PCs. Of the pools, 6 were pools of 4 PCs and 6 were pools of 6 PCs. Of these 12 paired pools, 3 were blood group A and 9 were blood group O.

### Paired Platelet Pools

Table I shows the prefiltration characteristics and the effects of leukoreduction. Prefiltration products seem equivalent except in volumes because the AS has a fixed volume of 225 mL. Postfiltration, there was a 3 to 4 log reduction in the WBC count, and all products met current FDA and Council of Europe specifications and standards for leukoreduced components. No difference was present in the platelet count or platelet yield between the 2 products postfiltration. An increase in the surface expression of P-selectin was observed postfiltration, as might be expected, without any difference between the product pairs.

Table II shows in vitro platelet quality data for days 2, 5, and 7. On day 2, the platelet yields of both products were similar, but the pH of the ELX/PAS-G was lower, likely reflecting the low pH of the AS. The lower lactate level in the ELX/PAS-G can be attributed to dilution of the plasma by the AS. Day 5 ELX/PAS-G platelet pools showed an increase in pH compared with day 2, presumably caused by the buffering capacity of the NaHCO_3 tablets. The ELX/PAS-G platelet pools showed superior swirling, morphologic scores, and ESC relative to the CLX HP/plasma pools. There was an increase observed in platelet yield on day 5 compared with day 2 in the CLX HP/plasma pools. We believe that this effect is likely an artifact due to the presence of microparticles because the MPV was decreased and because the platelet yield returned approximately to day 2 levels by day 7. Differences in pO_2 and pCO_2 were also evident, most likely attributable to the better gas exchange characteristics of the ELX container. The
day 7 data continued to show superior storage characteristics of the ELX/PAS-G product in comparison with the CLX HP/plasma pools, with most measures of platelet quality significantly better in the ELX/PAS-G pools (Table 2). The day 7 ELX/PAS-G pools showed better maintenance of pH, even compared with the day 5 CLX HP/plasma (P < .01). Surface expression of P-selectin increased throughout the 7-day storage period, without any difference observed between the 2 product types.

As illustrated in Figure 3A, there was lower glucose consumption and lactate generation in the ELX/PAS-G product compared with CLX HP/plasma (mean ± SD, 2.2 ± 0.6 vs 3.7 ± 1 mmol of glucose consumption per 10¹² platelets per day, P < .01; and 2.7 ± 0.3 vs 4.9 ± 1.5 mmol of lactate generation per 10¹² platelets per day, P < .01, respectively). Thus, on average 1.85 mmol (2.72) of glucose catabolism is accounted for by glycolysis in the ELX container compared with 2.45 mmol (4.92) in the CLX-HP container. This leaves 0.35 mmol of glucose (2.2-1.85) unaccounted for in the ELX container and 1.35 mmol (3.7-2.45) unaccounted for in the CLX-HP container, reflecting glucose metabolism through other pathways, such as the hexose monophosphate shunt. However, the lower lactate generation and lower lactate levels indicate better aerobic metabolism in the ELX container on days 5 and 7 (Table 2).
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the ELX/PAS-G (3.96 vs 2.41 mmol glucose consumption per 10^12 platelets per day, \( P = .05 \); and 6.32 vs 2.98 mmol lactate generation per 10^12 platelets per day, \( P < .01 \), respectively). These data show that 80% of the glucose in this subset was consumed through glycolysis in the CLX HP/plasma pools, resulting in higher lactate production and, therefore, lowering of the pH. The corresponding platelet pools stored in the ELX/PAS-G system had lower glucose consumption (\( P < .01 \)), with only 59% of glucose consumption explained by glycolysis, resulting in less lactate generation and, therefore, a higher pH. All platelet pools showed some improvement in pH when stored in the ELX/PAS-G system, but this subset showed the greatest improvement, suggesting that this AS-container system may maximally benefit a subset of platelet donations that store poorly in vitro.

Discussion

More than 13 million WBDs are collected in the United States annually,\(^23\) and different schemes are available to manufacture blood components from these collections. There has been a trend in the United States to disregard the manufacture of PCs and toward the manufacture of apheresis-derived platelets to meet community needs.\(^24\) The driving force for this trend has been concerns about an increased risk of disease transmission, particularly bacterial sepsis, due to the multidonor nature of pooled platelets and the logistic difficulties in testing single PCs for bacterial contamination. While the concern is realistic and the relative risk of bacterial sepsis may be higher, this risk needs to be balanced against other risks such as transfusion-related acute lung injury (TRALI) and ABO-mismatched hemolysis, which are reported to be higher with high-plasma-volume components such as apheresis-derived platelets.\(^25-27\) Although a recent meta-analysis by Vamvakas\(^28\) dismissed the higher risk of TRALI in apheresis platelets compared with pooled platelets as unproven, Schrezenmeier and Seifried\(^29\) concluded that there were conflicting data about the relative risks of TRALI compared with BC-derived PCs (both high-plasma-volume components) and that in risk analysis they were equivalent, but a clear preference toward pooled PCs was expressed when considering several factors, including donor safety and adverse effects. Therefore, the overall risk of WBD pooled platelets vs apheresis-derived platelets remains controversial.\(^25-29\)

This scheme shows a new approach to the manufacture of whole blood–derived pooled platelets. In this report, there are 2 variables: the AS and the different containers. Although ideally separating each variable would be interesting in deciphering the relative benefit of the AS to the novel container, we believe that the ELX container is largely responsible for the improved pH by promoting better aerobic metabolism in the AS-stored platelets, but, in addition, the AS supplies additional buffering capacity and ensures adequate glucose for more prolonged storage. The more important advantage of the AS is the reduction in residual plasma carryover, which has been shown to improve safety by reducing allergic reactions in transfusion recipients.\(^30-32\) Thus, the benefit of the container to the AS is complementary, improving potency and safety, respectively.

This scheme for the manufacture of pooled WBD PCs may be practical, particularly for small blood centers. Such centers receive their WBDs later in the day, and, hence, the manufacture of an RCC in AS and an intermediate PRP may be performed on the day of collection. On the following day, when infectious disease testing and ABO grouping has been completed, further processing can be continued as described in Figure 2; this could alter the manufacturing logistics and
effectively encourage the manufacture of platelet pools from such centers. Our report shows that such platelets have similar platelet yields and, when stored in the ELX container with PAS-G, produce a pooled platelet product with superior in vitro measures compared with plasma stored platelets. The day 7 product seems similar to the current day 5 pools stored in plasma, and, hence, 7-day storage may be reasonable, allowing that a point-of-issue test can be performed to test for bacterial contamination. The minimal plasma carryover (15 mL) can be expected to reduce or attenuate allergic reactions, and TRALI. The currently approved non–glucose-containing AS for use in apheresis platelets will still result in a high-plasma-volume product because only two thirds of the plasma will be removed. This will leave a residual 80 to 120 mL of plasma from 1 donor, continuing to put recipients at risk for TRALI.

The plasma manufactured using this approach is not fresh frozen plasma but plasma kept at room temperature for up to 24 hours before freezing. This product (FP24RT) is currently unapproved. The characteristics of this plasma have been described in a previous report and showed similarity to fresh frozen plasma, except for a 15% to 20% reduction of factor VIII. It will be necessary for this plasma to be approved as a transfusible product or as source plasma for further manufacturing. Otherwise, as a discarded product, it would be wasteful, and the cost of this manufacturing scheme would likely be prohibitive.

Validation of the quality of the platelets manufactured using this scheme will ideally require radiolabeled studies as single PCs in autologous recipients or radiolabeled allelogenic platelet pools in transfusion recipients.

This proposed manufacturing and storage scheme has practical logistic advantages and, subject to FDA clearance, may be particularly beneficial for smaller blood centers or hospital blood banks with limited staffing.

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