Blood component separation of pathogen-reduced whole blood by the PRP method produces acceptable red cells but platelet yields and function are diminished

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BACKGROUND: This study evaluated blood components processed by the platelet rich plasma (PRP) method from fresh whole blood (FWB) treated with a pathogen reduction technology (PRT). The effects of storage temperature on PRT treated platelet concentrates (PCs) were also examined.

STUDY DESIGN AND METHODS: PRT was performed using riboflavin and ultraviolet light on FWB in citrate phosphate dextrose anticoagulant. Following PRT, red blood cells (RBCs), PCs, and plasma for fresh frozen plasma (FFP), were isolated by sequential centrifugation. RBCs were stored at 4°C, FFP at −80°C, and PC at 22°C or at 4°C. Components were assayed throughout their storage times for blood gases, chemistry and CBC, hemostatic function as well as platelet (PLT) and RBC integrity.

RESULTS: Component processing following PRT resulted in a significant drop in platelet recovery. Most PRT-PC bags fell below AABB guidelines for platelet count. PRT-PC also showed a decrease in clot strength and decreased aggregometry response. Platelet caspases were activated by PRT. Storage at 4°C improved platelet function. In PRT-FFP, prothrombin time and partial thromboplastin time (PT and aPTT) were prolonged; factors V, VII, VIII, and XI, protein C, and fibrinogen were significantly decreased. Free hemoglobin was elevated two-fold in PRT-RBC.

CONCLUSION: Blood components isolated by the PRP method from PRT-treated WB result in a high percentage of PC that fail to meet AABB guidelines. FFP also shows diminished coagulation capacity. However, PRT-RBC are comparable to control-RBC. PRT-WB retains acceptable hemostatic function but alternatives to the PRP method of component separation may be more suitable.

In current practice, blood banks separate whole blood (WB) into components, subject these to transfusion transmitted disease testing and then release them for use or storage generally no sooner than 24-36 hours from collection.[1] Global events such as war or disease outbreak reveal the limitations of this process. There is no time for rigorous testing in combat zones when treating severely injured casualties and so fresh whole blood (FWB) is used when standard blood components are unavailable.[2] The recent Zika outbreak highlights the challenges of providing safe blood products in a timely manner when no viral test is commercially available.

Pathogen reduction technology (PRT) for blood products has been under development for several years.[3–6] Pathogen inactivation strategies in use include solvent detergent treatments and white or UV light irradiation in conjunction with

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photosensitizing compounds (methylene blue, amotosalen, and riboflavin).[7–9]

Previous studies determined that the Mirasol PRT which uses riboflavin in conjunction with ultraviolet (UVB/A) light to damage nucleic acids was successful in reducing bacteriological and viral contamination in platelets.[10] This PRT is in use for both plasma and platelets in several countries.[7] in vitro studies have shown that Mirasol PRT has minimal effects on the hemostatic function of WB stored under refrigeration and could conceivably prolong the utility of WB in austere conditions.[11,12] Conceivably, Mirasol-treated WB units not immediately used for transfusion as WB could be fractionated into components.

This study was initiated to provide a comprehensive analysis of the effects of Mirasol PRT treatment on WB and its subsequently separated components using the PRP method for component separation currently used in the United States.[13] We also studied the effects of platelet storage temperatures on platelet in vitro function. While room temperature storage has been shown to give the best in vivo recovery and survival of platelets,[14,15] cold stored platelets appear to have better hemostatic function as determined by aggregation and clot ultrastructure.[16–20] Therefore, the effect of 4°C storage on PRT-treated platelets was also analyzed.

Control and PRT-treated WB were examined both before and after PRT treatment. Following component separation, RBCs were stored at 4°C, plasma was flash frozen and stored at −80°C and platelets were stored at either 22°C or 4°C with agitation. Analyses for both WB and component aliquots included complete blood counts, blood chemistry and gases, and thromboelastography. RBCs were analyzed for hemolysis, and depletion of 2,3 diphosphoglycerate (2,3-DPG) and adenosine triphosphate (ATP). Plasma aliquots were analyzed for prothrombin time (PT) and activated partial thromboplastin time (aPTT), and hemostatic proteins. Platelet in vitro function was analyzed by thromboelastography, optical and electrode aggregometry, ATP release from dense granules, hypotonic shock response, extent of shape change, flow cytometry markers of activation and apoptosis, as well as ATP content. With these measured parameters, a careful comparison of the effect of PRT on blood components versus non-treated controls could be made. We hypothesized that components isolated from WB that had undergone Mirasol PRT would not differ significantly from components derived from untreated WB.

METHODS

Blood was obtained from 20 healthy adult donors according to an approved laboratory protocol at the US Army Institute of Surgical Research and following standard blood donation guidelines. Twenty units (500 ± 50 mL) of WB were collected in commercially available bags containing 70 mL of CPD anticoagulant (Teruflex, TerumoMedical Corporation). A 25 mL sample for baseline assays was removed. PRT was performed as described previously[11] on half of the units collected (10 of 20). The remaining units served as untreated controls (CON). Briefly, each PRT unit was transferred into an illumination set (Terumo BCT), and riboflavin solution was added (35 mL of 500 M riboflavin in 0.9% NaCl; Terumo BCT). After weighing and determining hematocrit using a HematasStat II Microhematocrit Centrifuge (Separation Technology Inc, EKF Diagnostics) and ClearCrit microcapillary tubes with an inner diameter of 0.5 cm, the set was placed in the illuminator and exposed to UV light for a dose of 80 J/mL RBCs. Following PRT, the contents were drained into separation bags; a second 25 mL aliquot was taken for post-PRT processing from PRT and CON bags.

Component separation

An unused blood bag was removed and discarded from a Pall WB component set (PN:721-93) and the CON or PRT blood bag was sterile-docked onto the remaining component set containing empty CLX® bags. The blood bags were spun in a Sorvall RC 3BP+ centrifuge for 3 minutes @ 2100 RCF at room temperature with no brake. Platelet-rich plasma was expressed into a CLX®bag and allowed to rest in the dark for 30 minutes. AS-3 additive was added to the RBCs and mixed. Platelet concentrate (PC) was separated from platelet-rich plasma by centrifugation at 4427 RCF for 6 minutes, with braking. Plasma was then expressed into a second CLX®bag, leaving the PC; sterile aliquots were taken for Day 1 processing. RBCs were stored at 4°C; FFP was flash frozen and stored at −80°C; PC was stored with agitation, at 22°C (n = 5 CON, 5 PRT) and at 4°C (n = 5 CON, 4 PRT).

Baseline sample preparation

Platelet rich plasma (PRP), platelet poor plasma (PPP), and RBCs were isolated from pre- and post-treated FWB for baseline values. PRP was isolated from 4 mL WB by a centrifugation at 150 × g for 10 minutes at room temperature with no brake. The PRP layer was removed, PLT count determined, and sufficient PLT for assays removed. The remaining sample was subjected to a hard spin at 3000 × g for 10 minutes with full brake to obtain PPP for dilution of the PRP to the appropriate PLT concentration. PPP and WB-RBCs for analysis were obtained by a 3000 × g centrifugation at 4°C for 10 minutes. PPP was frozen for later analyses. WB-RBCs were assayed on Day 1. Component samples from RBC or PC were removed from blood bags by sterile connections. RBCs were analyzed on Days 1, 22, and 29. PC samples were diluted and tallied by Coulter counter; sufficient PC was set aside to achieve 250 and 300 × 10^3 PLT/μL and the remaining PC was spun at 3000 × g to achieve self-plasma for diluting PC to the appropriate concentrations. Both 22°C and 4°C stored PC were analyzed on Days 1, 3, and 5; only cold-stored PLTs were analyzed on Days 10 and 15. FFP was analyzed within 60 days of initial processing.

Assays

Figure 1 shows the overall plan of the study and the assays utilized. A complete blood count (CBC) was performed using an ADVIA 120 (Siemens Healthcare Headquarters Siemens Healthcare GmbH). For intermediate processing analyses and determinations of PLT counts for sample dilutions, a Coulter Ac-T diff2 Analyzer (Beckman Coulter, Inc.) was used. When PLT counts exceeded $700 \times 10^3/\mu L$ by Coulter CBC, samples were diluted with PBS to be in the linear range and re-counted.

Blood gases and chemistry were performed using the iSTAT cartridges: Chem 8 cartridge was used to measure glucose; CG4 cartridge determined lactate, pH, pCO_2, PO_2, HCO_3, sO_2, and base excess (CE) (Abbott Point of Care, Princeton NJ). Free hemoglobin was determined on plasma samples (both PPP and FFP) using the HemoCue® Plasma/Low Hb System (Hemocue) as a measure of RBC hemolysis and WB integrity.

Thromboelastography (TEG; Haemonetics Corporation) measured WB as well as isolated PRP or PC hemostatic characteristics. Samples were activated with kaolin and parameters recorded included clotting time (R), clot formation (K), alpha angle (a-angle), maximum amplitude (MA), and clot lysis at 30 and 60 minutes (LY30 and LY60, respectively). Prior to performing the assay as recommended by the manufacturer, the sample was allowed to rest 15 minutes.

PLT function assays included hypotonic stress response (HSR) and extent of shape change (ESC) performed on baseline PRP and PC diluted to PLT counts of $300 \times 10^3/\mu L$ using the Chrono-log SPA-2000 according to manufacturer.[21] WB aggregometry was determined on a Multiplate (Diapharma) using the following reagents in a mini-cell cuvette: ADPtest (6.5 μM ADP), COLtest (3.2 μg Collagen/mL), RISTOtest (0.77 mg ristocetin/mL), TRAPtest (32 μM thrombin receptor activating peptide 6 (TRAP-6)/mL) (all from Diapharma).[22] PC and PRP were diluted to PLT counts of 250 PLT $\times 10^3/\mu L$ before measurements. Additionally, maximal ATP release from PLT dense granules was measured in response to 1 U/mL thrombin by a luminescence assay for ATP on the Chronolog -model 700 aggregometer using Chronolume reagent and thrombin reagent according to manufacturer's protocol (Chrono-log Corporation).

Flow cytometry gated PLT by side scatter and forward scatter. PLTs were confirmed by CD41 and CD42 antibody binding. PAC1 binding and CD62P (P-selectin binding) determined activated PLTs. Phosphatidylserine externalization was determined by lactadherin binding and PLT apoptosis signals of caspase 3/7, caspase 8, and caspase 9 levels were measured.

Prothrombin time (PT) and activated partial thromboplastin time (aPTT) as well as Factors V, VII, VIII, and XI, ATIII, fibrinogen and vWF, protein C, and protein S were all determined on a STA-R Evolution (Diagnostica Stago, Inc.).

Sample preparation for 2,3 DPG and ATP content measurements used a modification of the methods as described by deKorte[23] and Burger.[24] Samples were deproteinized by combining two parts sample with three parts saline on ice and mixing well (0.6 mL WB or RBCs with 0.9 mL saline; 150 μL PRP or PC with 225 μL saline). Samples were acidified by vortexing with 70% perchloric acid (60 μL and 15 μL). After >10 minutes on ice, samples were spun @ 6000 × g for 5 minutes @ 4°C. Supernatant was removed and

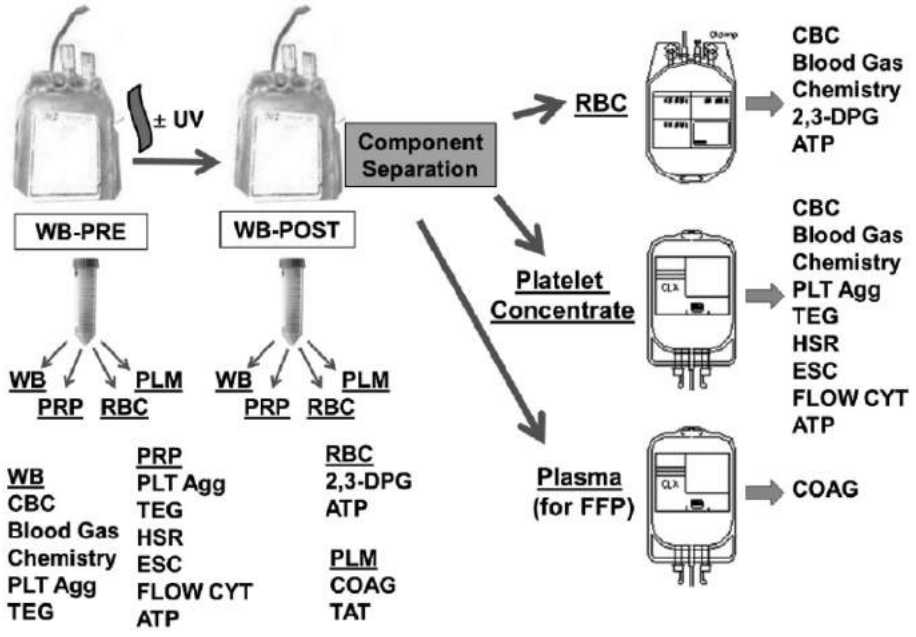

Fig. 1. Experimental schematic.

250 μL each were neutralized with 14 μL 5 N potassium carbonate and stored at −80°C. On the day of assay, the samples were thawed on ice, vortexed, and centrifuged to pellet the perchlorate precipitate. The cleared supernatant was assayed.

Measurement of 2,3 DPG in WB and RBCs used the ROCHE 2,3-Diphosphoglycerate Assay (Cat No 10 148 334 00, Roche, from Sigma Aldrich) following the general procedure as described by the manufacturer. This assay was modified for use in a 96-well flat bottom polystyrene plate and a SPECTRAmax plate reader using the SOFTmax PRO software with path length correction in comparison to a reference cuvette. The concentration of 2,3 DPG is measured by determining the conversion to phosphoglycerate by phosphoglycerate mutase (PGM). An initial reaction is performed to quench any other substrates in the assay mixture. Volumes were scaled back 10-fold from the recommended volumes for use in the 96-well plate; 10 μL sample was mixed with 210 μL of Solution Mix A containing ATP, NADH, phosphoglycerate kinase, glyceraldehyde-3-phosphate dehydrogenase, triosephosphate isomerase, and glycerol-3-phosphate dehydrogenase in a triethanolamine buffer. After 5 minutes at room temperature, the plate was read at 340 nM with path check correction. A volume of 4 μl of a solution containing PGM and glycolate-2 phosphate was added, the reaction incubated at room temperature, and the plate again read at 340 nM with path check correction. The change in absorbance is used with the extinction coefficient for 2,3 DPG to determine the initial concentration of 2,3 DPG, as described by the manufacturer. Using the CBC determination of Hgb, a value of μmol 2,3 DPG/g Hgb can be obtained. Linearity of the assay was confirmed by serial dilution of control samples.

ATP levels were determined in deproteinized samples prepared as described above. Samples were diluted 10-fold in PBS; 100 μL of sample was mixed with 100 μL of CellTiter-GLO Luminescent Cell Viability Assay (Cat No 10 148 334 001, Promega) and processed as described by manufacturer.

Differences between treatment groups were evaluated with repeated measures analysis of variance (RM-ANOVA) with analysis of individual effects (time, PRT treatment, and temperature storage) to determine significance, which was set at $p < 0.05$.

RESULTS

Multiple measures assessed processed sample quality over time (Table 1). Lactate, a by-product of anaerobic respiration, increased significantly in both RBC and PC with storage. There was no difference between PRT and CON in RBC; the increase in lactate in PC was less in PRT-PC, especially if cold stored (4C-PRT PC). Glucose, analyzed as a measure of metabolic activity, was slightly but significantly decreased by PRT in WB, 5.0% ± 0.9% compared to CON. This slight but significant decrease compared to CON samples was similarly seen in RBC throughout storage and escalated resulting in reduced glucose levels in RBC over time of 35.1% and 45.5% for CON RBCs and PRT RBCs, respectively. For PC, there was no significant difference between CON PC and PRT PC; both showed decreased glucose levels. Cold storage of 4C-CON

TABLE 1. Glucose, lactate, and pH. Data are average ± SEM

		WB		RBC		
		PRE	POST	D1	D22	D29
Glucose	CON	383 ± 8	382 ± 10.9	535 ± 7.7	375 ± 16.4*	347 ± 13.1*
	PRT		364 ± 3.5*	504 ± 3.6 †	321 ± 9.5*†	292 ± 6.8*†
Lactate	CON	1.69 ± 0.17	2.60 ± 0.36	2.21 ± 0.18	16.6 ± 0.83*	18.8 ± 0.72*
	PRT		2.41 ± 0.18*	2.08 ± 0.16	18.2 ± 0.58*	19.8 ± 0.44*
pH	CON	7.12 ± 0.01	7.11 ± 0.02	6.83 ± 0.02	6.50 ± 0.02*	6.42 ± 0.01*
	PRT		7.14 ± 0.01	6.83 ± 0.01	6.42 ± 0.01*†	6.40 ± 0.00*
		WB		RBC		
		D1	D3	D5	D10	D15
Glucose	CON	401 ± 13.3	343 ± 9.8*	291 ± 15*		
	PRT	373 ± 3.4	333 ± 10.7*	280 ± 26.9*		
	4C-CON		397 ± 27.2	355 ± 17.9	342 ± 30.1	291 ± 33.5*
	4C-PRT		365 ± 4.8	386 ± 38.9	329 ± 6*	308 ± 8.33*
Lactate	CON	3.17 ± 0.30	6.36 ± 0.79*	11.13 ± 1.23*		
	PRT	2.67 ± 0.16	6.17 ± 1.46*	8.89 ± 1.76*		
	4C-CON		5.27 ± 0.4*	6.21 ± 0.69*	10.53 ± 1.29*	13.52 ± 2.2*
	4C-PRT		3.89 ± 0.38*†	4.79 ± 0.38*	6.8 ± 0.48*†	8.58 ± 0.6*
pH	CON	7.31 ± 0.02	7.56 ± 0.07*	7.52 ± 0.09		
	PRT	7.38 ± 0.03	7.55 ± 0.09	7.48 ± 0.17		
	4C-CON		7.36 ± 0.04	7.46 ± 0.06	7.47 ± 0.09	7.48 ± 0.16
	4C-PRT		7.39 ± 0.03*	7.50 ± 0.03*	7.64 ± 0.05*	7.82 ± 0.09*

* Indicates $p < 0.05$ versus D1 CON.
† Indicates $p < 0.05$ for CON versus PRT at indicated time and temperature.

and 4C-PRT PC slowed the glucose utilization. pH decreased in RBCs equally for CON and PRT samples. The pH in stored 4C-PRT PC was significantly increased versus Day 1 CON PC throughout storage while pH in 4C-CON PC was not significantly different from Day 1 CON PC through Day 15.

Focusing on the individual components, a decrease in PLT count greater than expected by dilution was evident from PRP isolation. This decrease in PLT recovery was exacerbated by PC processing from the WB. On average, the PRT PC did not meet the AABB mandated value of 5.5×10^{10} PLT for transfusion with only 3.7×10^{10}/per unit for PRT while control units averaged 8.9×10^{10}/ per unit. (Fig. 2A, B). Despite some aggregation issues, 8 of 10 control bags were above 5.5×10^{10} PLTs per bag while only 2 of 9 bags met this requirement from the PRT treated WB. Hemostatic function as analyzed via thromboelastography, while still within normal ranges, was significantly changed for PRT-treated samples with increases in time for clot initiation (R) and clot formation (k) and decreases in clot strength (MA, Fig. 2C). This held true for the initial WB analyses, the PRP analyses

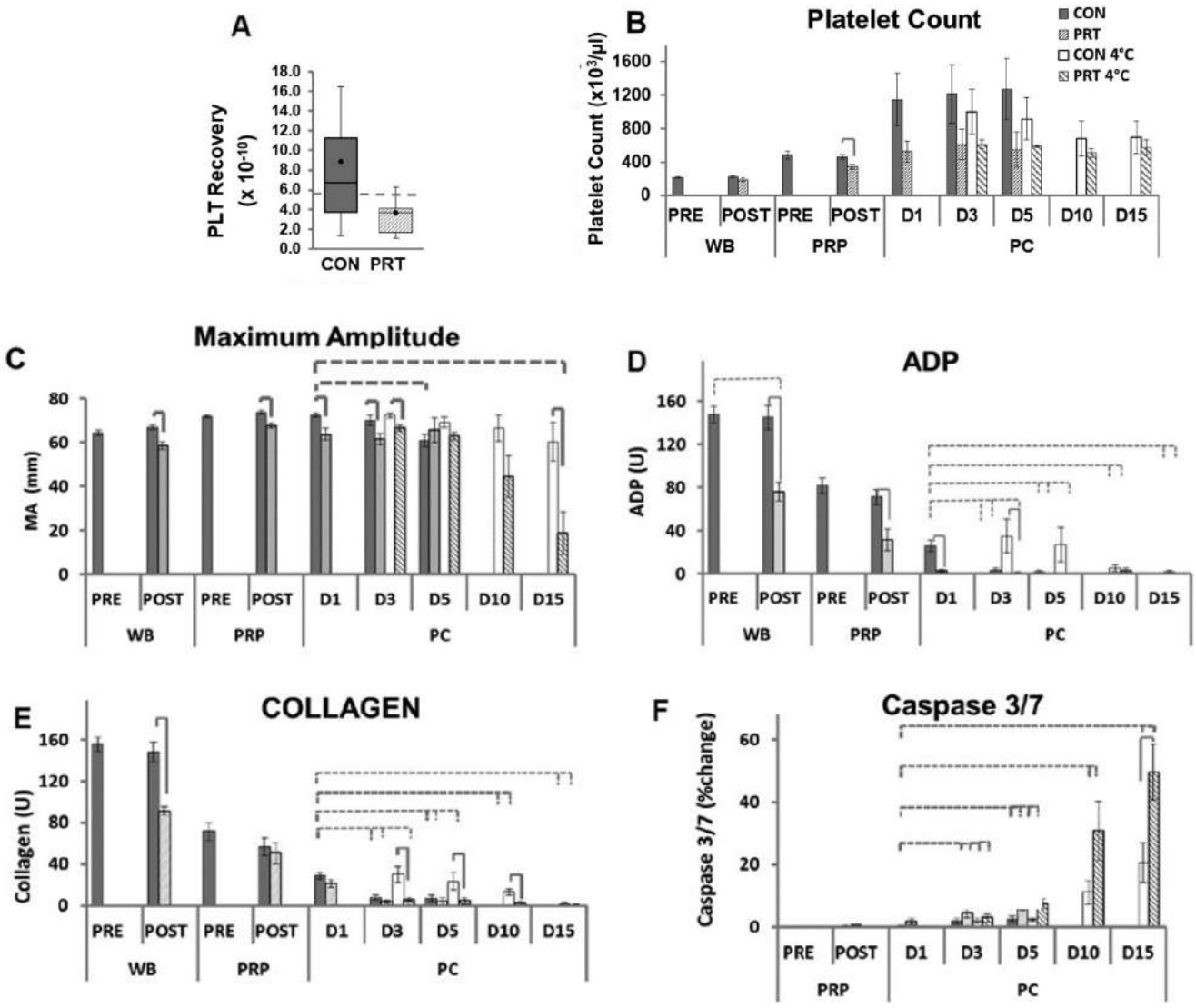

Fig. 2. Platelet Concentrates (PCs): Recovery. Component processing following PRT resulted in a decrease in platelet recovery (Fig. 2A, B). Most PRT-PC bags fell below AABB guidelines for total platelets (depicted by dashed line in Fig. 2A). Hemostatic function. PRT-PC also showed a minimal, but significant decreased MA (TEG) (Fig. 2C) and decreased aggregometry to all agonists (ADP and Collagen; Figs. 2D, E). While PRT-PC differed significantly at D1 from CON-PC, by D3 differences were less profound. Storage at 4°C improved platelet function for PRT-PC-4 compared to CON-PC or PRT-PC at 22°C. Apoptosis. Apoptotic caspases 3, 7, 8, and 9 were differentially elevated at all time points in the PRT samples. Data for caspases 3 and 7 are shown in Fig. 2F. Data in Panels 2B-I are averages ± SEM. Significance ($p < 0.05$) between initial controls and later samples is shown by a dashed bar; significance ($p < 0.05$) between CON and PRT matched samples is shown by a solid bar.

and the PC analyses over time, no matter the storage temperature. By Day 15, while cold-stored 4C-CON PC were still within normal ranges, cold-stored 4C-PRT PC fell out of range. In assays of PLT function, impedance aggregometry revealed a decreased response of WB-post versus WB-pre to all agonists tested: ADP and Collagen (Fig. 2D, E) and TRAP, ASPI, and ristocetin (data not shown) in the WB analyses. These decreases were also observed in both 22C- and 4C-PRT PC. Only cold-stored 4C-CON PC maintained appreciable PLT aggregation in response to agonist through Day 10. No significant difference was seen between CON and PRT PC by extent of shape change throughout the study period at either storage temperature (data not shown). Cold-stored PC responses were significantly decreased from 22C-CON PC by Day 5 in the hypotonic shock response assay for both 4C-CON PC and 4C-PRT PC samples (data not shown). Cold stored 4C-PRT PC responses were further decreased from cold-stored 4C-CON PC. The release of ATP from dense granules in response to agonist (Chronolog assay with Chronolume luminescent reagent, data not shown) was maintained in the cold-stored 4C-CON PC but largely lost in the 4C-PRT PC. Assays of total ATP content revealed that by Day 15 cold-stored 4C-CON PC were 48.1% ±9.6% of d1, while 4C-PRT PC were 7.5% ± 2.25%. Conversely, while aggregation responses and clotting responses decreased with PRT, PLT activation and apoptotic indices increased and were exacerbated by cold storage. GpIIb/IIIa complex binding (CD41a) was maintained throughout all storage conditions only falling to 93% positive for both 4C-CON and 4C-PRT cold-stored PC at Day 15. Gp1b binding (CD42) fell significantly with storage time to 71.8% (4C-CON PC) and 54.8% (4C-PRT PC) for cold-stored PC at Day 15; a significant drop from D1 CON-PC was seen even for 22C-PRT PC by Day 5 (8.5% decrease). Activated PLT with P-selectin surface expression (CD62P positive binding) were significantly increased by cold storage for both 4C-CON and 4C-PRT PC by ~two-fold; P-selectin expression on 4C-CON PC was higher than on 4C-PRT PC by d15, but not significantly

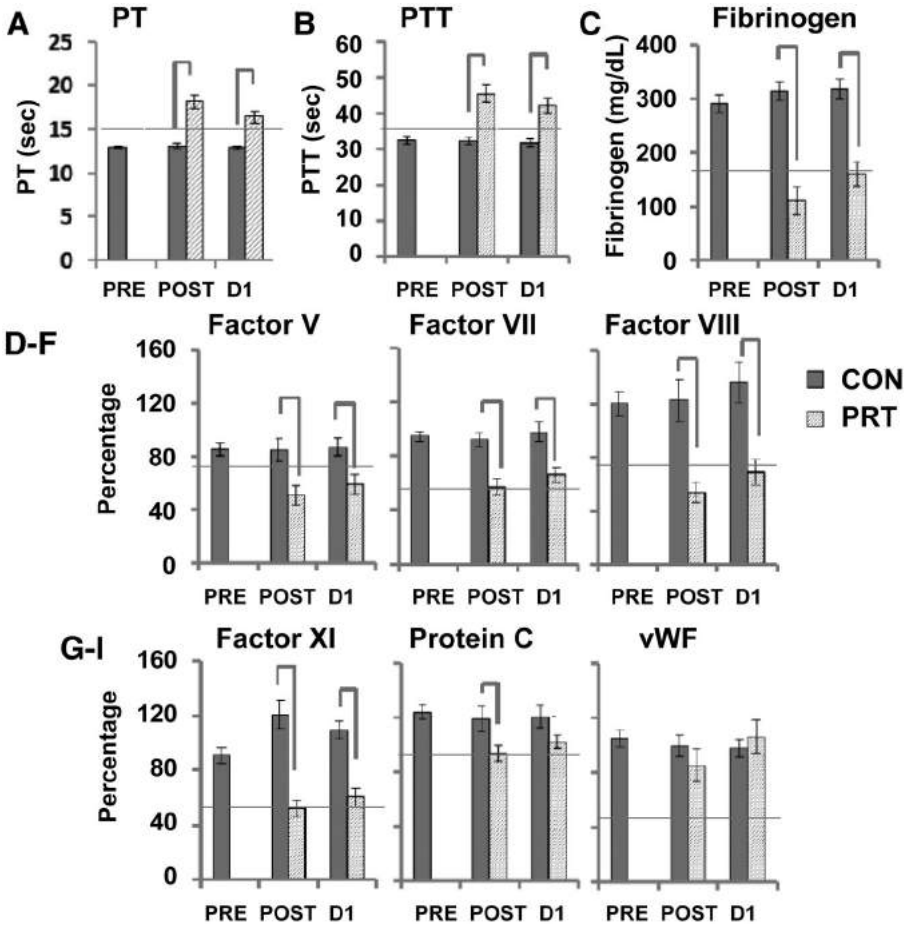

Fig. 3. FFP: In FFP analyses, PT and PTT are prolonged; factors V, VII, VIII, and XI, protein C and fibrinogen are significantly decreased following PRT treatment ($p < 0.05$). This data is represented in Panels A-I and includes the non-significantly changed vWF for comparison. Data are averages ± SEM; significance ($p < 0.05$) between CON and PRT matched samples is shown by a solid bar. The lower limit for each reference range is depicted by the solid gray line.

(p = 0.058, data not shown). Room temperature storage showed no significant changes for 22C-CON PC or 22C-PRT-PC. Storage time of PC, whether 22°C or 4°C, increased phosphatidylserine on the PLT surface (lactadherin binding), but only cold storage or PRT PC demonstrated significant difference with Day 1 CON PC over time. However, apoptotic indicators including caspase 3/7, caspase 8, and caspase 9 were significantly increased by cold storage and by PRT treatment compared to Day 1 CON PC; by Day 15 4C-PRT PC differed significantly compared to Day 15 4C-CON PC (Fig. 2F).

In FFP, assayed within 60 days of Day 1 freezing, levels of several hemostatic proteins were changed by PRT treatment prior to processing. After PRT, the PT averaged 18.1 ± 0.7 sec; the aPTT averaged 45.5 ± 2.5 and 42.2 ± 2.2 for both PRT-PPP and d1 PRT-FFP (Fig. 3A, B). These values exceeded the reference ranges of 9.5-13.5 sec for normal PT values and 30-40 sec for aPTT values. CON values were within normal range. Even after correction for dilution, fibrinogen showed a 43% decrease in PRT samples versus CON samples (Fig. 3C). In fact, fibrinogen, Factor V, VII, VIII, XI, and protein C all show significant decreases for PRT versus CON plasma samples. Fibrinogen, Factor V, VIII, and XI fall below normal reference values while factor VII in PRT FFP lies just within the lower reference value limit (Fig. 3D-G). Protein C, while significantly decreased, was within reference ranges (Fig. 3H). Neither ATIII, protein S (data not shown) nor vWF (Fig. 3I) showed any significant differences compared to CON samples.

The quality of the RBC components was analyzed by hemolysis products, free hemoglobin, ATP, and 2,3 DPG content. Both CON and PRT RBCs showed an increase in free hemoglobin indicating increased hemolysis that was significant over CON d1 values (Fig. 4A). However, the PRT RBCs were significantly higher than CON RBCs at both d21 and d29. While immediate PRT WB did show a significant decrease in 2,3 DPG compared to non-treated WB and both 2,3 DPG and ATP both showed a decrease with storage in RBCs, no significant differences between CON and PRT RBCs could be demonstrated (Fig. 4B, C).

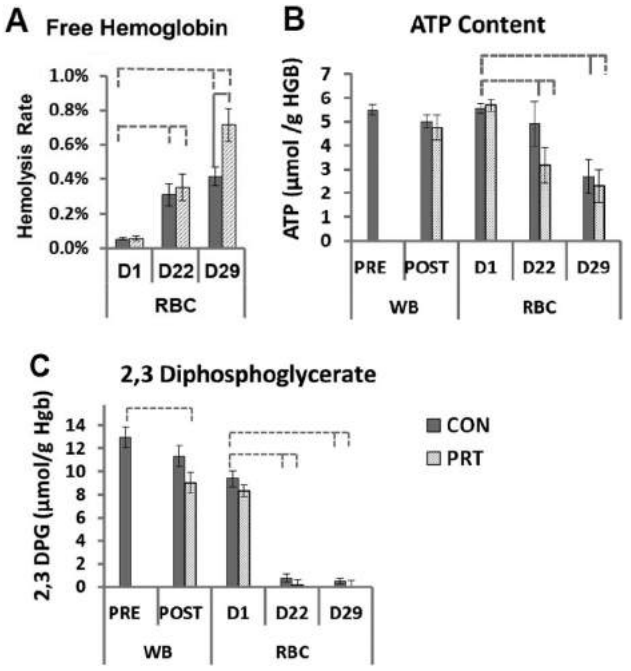

Fig. 4. RBCs: While free hemoglobin was elevated two-fold at D28, all other RBC measures were comparable for CON-RBC and PRT-RBC at all time points. Free hemoglobin is depicted in Panel A, ATP content in Panel B and 2,3 DPG in Panel C. Data are averages ± SEM. Significance ($p < 0.05$) between initial controls and later samples is shown by a dashed bar; significance ($p < 0.05$) between CON and PRT matched samples is shown by a solid bar.

DISCUSSION

The safety of the blood supply continues to be a primary goal of transfusion medicine. An ever-increasing array of bacterial or viral tests is becoming cost-prohibitive.[25,26] The time spent validating new tests leaves the blood supply at risk. A priority for blood technology development continues to be for a treatment that inactivates pathogens while sparing the functionality of the blood or blood products. Ultraviolet irradiation following riboflavin treatment has been shown to reduce many pathogens to non-detectable levels.[27–29] This study was performed at 80 J/mL RBC for WB irradiation, shown to be effective at reducing *Trypanosome cruzi* parasites inoculated in WB.[12]

Previous studies included PRT treatment of PLTs, WB, and of component processing by the buffy coat method.[11,30–34] Component processing of single blood units in the U.S. is performed by the PRP method of sequential centrifugation, which is the process described in this manuscript. Platelets stored at room temperature to preserve recovery and survival after transfusion are susceptible to bacterial contamination, limiting storage times to 5 or 7 days. Therefore, functional testing of PC after PRT and storage was our highest priority. After PRT, PC showed definite decrements in functionality; however all PLTs stored at room temperature show significant decrements in function over time and the differences in PRT PC versus CON PC are not likely to be of clinical significance.

The evolution of hemorrhage resuscitation driven by military experience in recent conflicts has shifted practice away from heavy use of crystalloids and red cells to a "balanced" blood product-based approach that delivers the functionality of WB through transfusion of either RBCs, plasma, and PLTs in a 1:1:1 ratio or uses WB directly. Indeed, WB is returning to civilian hemorrhage management.[35–37] A previous study of PRT on WB units revealed limited deficiencies of PLT function that were largely

TABLE 2. Comparison of Control versus PRT treatment at each time point. Significant differences ($p < 0.05$) of PRT-treated components from CON at the same time point are shown by bold, solid arrows (↑↓) while trends ($0.05 < p < 0.15$) are depicted by ↓↑. An upward arrow designates an increased measure while a downward arrow indicates a decrease

CONTROL versus PRT				
Platelets	PRP		PC-22°C	
Platelet count	POST	D1	D3	D5
	↓	↓	↓	↓
TEG				
R				
Angle	↓	↓	↓	
MA	↓	↓	↓	
Platelet function - aggregometry				
ADP	↓	↓		
Collagen	↓			
Trap	↓	↓		
ASPI	↓			
Ristocetin	↓	↓		
Platelet function				
Extent of shape change			↓	↓
Hypotonic shock response		↓		
Platelet activation - flow cytometry				
CD62P (P-selectin)	↑			
Lactadherin				
CD41		↓		
CD42				
PAC1		↑	↑	↑
Apoptosis				
Caspase 3/7				↑
Caspase 8				↑
Caspase 9				↑
Plasma	PPP		FFP	
	POST	D1		
Coagulation times				
PT	↑	↑		
PTT	↑	↑		
Coag protein level				
Factor V	↓	↓		
Factor VIII	↓	↓		
Factor VII	↓	↓		
Factor XI	↓	↓		
ATIII	↓	↓		
Fibrinogen	↓	↓		
vWF	↓			
Protein C	↓			
Protein S				
RBC	WB		RBC	
	POST	D1	D22	D29
Lactate			↓	
pH			↓	

(Continues)

TABLE 2. Continued

CONTROL versus PRT				
Glucose		↓	↓	↓
ATP content	↓		↓	↓
Free hemoglobin			↑	↑
2,3 DPG	↓	↓	↓	↓

ameliorated by storage at 4°C.[11] Overall, WB hemostatic assessment by TEG demonstrated preservation of acceptable function in both CON and PRT over 21 days of storage at 4°C.

The current study extends the findings of our previous study and examines the possibility of first preparing PRT WB and then isolating components from the PRT WB to allow component-optimized storage.

All PRT isolated blood components showed significant differences between CON and PRT in at least one of the tests (Table 2). The RBCs were the least susceptible to PRT deleterious effects. There was a significant increase in free hemoglobin, an indicator of RBC hemolysis; but changes in ATP or 2,3 DPG content were not significantly different over the 29 days of the study. This echoes previously observed data in RBCs.[34,38–40]

PRT FFP was significantly different in several key hemostatic proteins. Fibrinogen was the most susceptible with an 37% decrease compared to CON FFP, however, Factors V, VIII, XI, and Protein C were all just at or below normal reference values. Conversely, levels of ATIII, vWF, and Protein S all remained within reference ranges (data not shown). These data are in agreement with PRT FFP processed by the buffy coat method[34] or PRT treatment of isolated fresh plasma.[41]

PRT PC presented the greatest challenges. PRT PC had decrements in function over a wide range of assays. Clot strength was decreased minimally but significantly by PRT, although this could be attenuated by cold storage. Aggregometry of 4°C stored PRT PC was comparable to 22°C stored CON PC but was significantly decreased compared to cold-stored CON PC—long known to have better function than room temperature–stored PLTs.[19,42–47] There were decrements in HSR, ESC, activation, and apoptosis markers—all significantly different and functionally worse in the PRT PC versus CON PC stored at 4°C. Similar decreases in functionality of PRT-PC were noted in work by Devine and her group[34,48] using the buffy coat method of component separation and in studies of irradiated WB.[11]

However, the most basic parameter of product acceptability, PLT recovery, did not meet AABB minimum PLT concentrations for transfusion of 5.5×10^{10}/blood bag after PRT. While 8 of 10 control units were $>5.5 \times 10^{10}$/blood bag, only 2 of 9 PRT units met this criteria. PLT recovery in WB, pre- and post-PRT at 93%, ±16.3%, was not significantly different from CON WB at similar time points (102%, ±15.8%, $p = 0.27$).

However, PRP fractions isolated from WB post-PRT was only 74.5% ± 9.0% of pre-PRT samples compared to 102.7% ± 7.2% for CON-PRP recovery at similar times ($p < 0.001$). No aggregation of PRT-PRP was detected by flow; this appears to be an issue with PRP centrifugation isolation.

Clearly, while PRT of riboflavin-treated WB will reduce pathogens and allow storage of PRT WB at 4°C for later transfusion, the separation of single unit blood components by the PRP method does not result in PC or FFP products that meet current regulatory standards. While units potentially could be pooled, future research to further optimize PRT and component separation technologies is needed.

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CONFLICT OF INTEREST

The authors have disclosed no conflicts of interest.

AUTHOR CONTRIBUTIONS

HFP, CGF, MCH, BSS, RKM, and APC designed the study. MCH, BSS, and RKM performed the experiments and data acquisition. CFG, BSS, HFP, APC, and MCH interpreted and analyzed the data. MCH wrote the manuscript and CGF, BSS, JAB, and APC provided critical revisions.

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