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Transfusion. Author manuscript; available in PMC 2015 August 01.

Published in final edited form as:
Transfusion. 2014 August ; 54(8): 1935–1944. doi:10.1111/trf.12602.

Microparticle Profile and Procoagulant Activity of Fresh Frozen Plasma is Affected by Whole Blood-Leukocyte Depletion Rather Than 24-Hour Room Temperature-Hold

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Abstract

BACKGROUND—Microparticles (MPs) are small phospholipid-containing vesicles that have pro-coagulant properties. MPs are thought to contribute to the hemostatic potential of plasma. This study investigated the effects of WB-hold time and leukodepletion (LD) on the MP profile and hemostatic potential of fresh-frozen plasma (FFP).

STUDY DESIGN AND METHODS—WB units (n=12) from healthy donors were divided into two pairs and each pair was held at 20–24°C for 6 or 24 hours. At the designated hold-time, one unit from the pair was leukodepleted while the other unit was not leukodepleted. FFP was prepared by standard procedures, aliquoted and frozen. The MP content was determined by flow cytometry using an absolute count assay and specific labels for red cells (CD235a), platelets (CD41) and phosphatidylserine. The hemostatic potential was determined by thrombelastography (TEG) and coagulation factor assays.

RESULTS—Compared to non-LD FFP, LD-FFP had significantly lower numbers of MPs, particularly $CD41^+$ MPs and phosphatidylserine-positive MPs ($p<0.03$). LD-FFP, compared to non-LD FFP, had a slower clot formation time ($p=0.002$), lower clot strength ($p<0.001$) and lower FVIII, FXII and fibrinogen levels ($p<0.01$). With longer WB hold-time, the TEG profile was unchanged, although FVIII levels were decreased as expected ($p<0.01$). On average FFP units met quality requirements.

CONCLUSION—LD of WB resulted in lower hemostatic potential of FFP in conjunction with depletion of MPs and coagulation factors. Longer WB hold-time did not significantly affect the hemostatic potential of FFP as measured by TEG. Acceptable hemostatic quality was maintained for all FFP processing conditions studied.

Keywords

microparticles; fresh frozen plasma; leukodepletion; storage conditions; coagulation; thrombelastography; whole blood

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Disclosures: No conflict of interest to disclose

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INTRODUCTION

Transfusion of fresh-frozen plasma (FFP) is used routinely to treat patients with a coagulopathy who are bleeding or at risk of bleeding, including critically-ill patients with multiple trauma or hemorrhagic shock.[1] Early administration of FFP has been associated with improved survival rates in these patients.[2,3] The mechanism of action of FFP is not well understood, however it is believed that the function of FFP transfusion in trauma is mainly to replenish coagulation factors and reinstate hemostasis.[4]

More than 1,000 proteins have been identified in plasma.[5] Among these plasma proteins, the coagulation factors associated with hemostatic properties are particularly important. Certain coagulation factors, such as Factor VIII and fibrinogen, are used as indicators of the quality of FFP. The concentration of some coagulation proteins can be affected by the processing steps used for the preparation of FFP,[6–8] which may alter its hemostatic properties.

FFP for transfusion can be prepared from whole blood (WB) or by apheresis collection. In some countries, including Australia and the United States of America (USA), a significant proportion of clinical FFP is derived from WB. Processing variables related to the preparation of WB-derived FFP include variations in the hold-time and temperature of WB prior to processing and pre-storage leukodepletion (LD).

LD is the removal of leukocytes, typically by filtration through proprietary devices constructed of leukocyte-affinity matrices.[9] The primary purpose of LD is to reduce the risk of adverse events in recipients arising from the transfusion of accompanying donor leukocytes present in the blood component.[9] Some of the risks include the transmission of leukocyte-associated viruses (e.g. cytomegalovirus, human T cell lymphotrophic viruses), variant-Creutzfeldt-Jakob disease,[10] alloimmunization[11] and nonhemolytic febrile transfusion reactions.[12] In addition to depletion of leukocytes, WB-LD filtration may deplete platelets and reduce the levels of some coagulation factors in FFP, including factors V, VIII, IX, XI and XII,[6,7] although the effects may be dependent on the type of WB-LD filter. The levels of susceptible coagulation factors in LD-FFP have been reported to be reduced by approximately 15 to 25 percent and still comply with relevant jurisdictional quality criteria. Other investigators have not found a significant effect of WB-LD on the level of coagulation factors in FFP, although an unpaired design was used for most if not all of the studies reported to date.[13–15]

In Europe and other countries that use the buffy-coat depletion method to separate WB, clinical FFP can be prepared from WB held for up to 24 hours after collection providing the WB is rapidly cooled to 20 to 24°C.[16] In Australia, where rapid cooling plates are not used, WB units can be held at room temperature (RT) (i.e. 20 to 24°C) for up to 18-hours before clinical FFP must be frozen.[17] Most of the WB-derived clinical FFP produced in Australia is not LD-filtered. In the USA, clinical FFP can be prepared from WB held for up to 24 hours providing the WB unit has been held refrigerated after the first 8 hours following WB collection.[18] Although the concentration of some coagulation factors, notably labile factors such as FVIII, may be lower in FFP prepared from WB held for 24 hours at RT, quality specifications are typically met.[19,20]

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Longer WB hold-time prior to component separation is known to lead to changes in cellular properties, including release of soluble mediators such as cytokines, complement activation, leukocyte fragmentation and release of microparticles (MPs).[21–24] MPs are small vesicles that are generated from all types of cells and carry some of the cell surface markers of their parent cell.[25] MPs participate in a wide range of biological activities in normal and disease states, including coagulation, thrombosis and inflammation.[26–29] Increased generation of MPs can occur when cells become activated or stressed, which could happen to a varying extent during blood donation or soon thereafter. The hold-time of the WB units before processing and the status of the cells could alter the amount and type of MPs in the final products.

We have previously reported that longer hold-time of WB units prior to processing is associated with increased numbers of MPs in FFP.[30] The WB units were not LD-filtered in our previous study. Other investigators have reported that pre-storage LD-filtration of WB lowered the amount of platelet-derived MPs in the WB units.[24] Based on these observations, we hypothesized that FFP derived from WB, with or without LD, are different in their MP content, which may lead to differences in the hemostatic potential of FFP.

In the study reported here, we examined the difference in MP content of FFP prepared from paired units of WB held for 6 hours or 24 hours at RT with or without WB-LD prior to processing. The coagulation properties of the FFP were investigated using thrombelastography (TEG) to provide a holistic functional measure,[31] and conventional specific coagulation factor assays. To assess the impact of MP content on the procoagulant potential of FFP, MP-depleted and MP-spiked FFP were compared using TEG.

MATERIALS AND METHODS

Donor selection and sample preparation

Standard WB units (n = 12) were obtained from healthy male donors (mean age 47 ± 13 years) attending the Australian Red Cross Blood Service, Melbourne. Six donors were blood group A and six were group O. The study was approved by the Blood Service's Human Research and Ethics Committee.

WB (470 mL ± 10%) was collected into polyvinyl chloride collection packs containing citrate-phosphate-dextrose anticoagulant (Pall Medical, Portsmouth, UK). Each WB unit was divided into two pairs of pediatric-size packs (Terumo, Tokyo, Japan) using a sterile-docking device. Each pair was held at RT for the nominated holding period (6 hours or 24 hours). At the designated hold-time, one unit from the pair was leukodepleted by filtration (WBF3 filter: Pall Corporation, NY, USA), while the other unit was processed without LD. Subsequently, FFP was prepared by centrifugation of the packs at 5005 × *g* for 10 minutes at 22°C, according to standard Blood Service protocols. FFP samples (500 μL aliquots) were frozen immediately at −30°C until analysis, which was performed within one month of sample collection. Figure 1 shows a schematic of the experimental design.

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Flow cytometric quantitation of MPs

MPs were labelled for flow cytometric analysis as previously described.[30] Briefly, for the determination of the cellular source of MPs, 25 μL of freshly thawed FFP were mixed with 2.5 μL of fluorescein isothiocyanate (FITC)-labelled anti-CD41 (platelet marker) or phycoerythrin (PE)-labelled anti-CD235a (RBC marker) (BD Biosciences, San Jose, CA). Phosphatidylserine (PS) expression on MPs was determined by labelling 12.5 μL of FFP with 5 μL of allophycocyanin (APC) labelled-annexin-V (BD Biosciences) or FITC labelled-lactadherin (Haematologic Technologies, Essex Junction, Vermont). Samples were diluted with 0.2 μm-filtered phosphate buffered saline (PBS), pH 7.2 or annexin-binding buffer to a final reaction volume of 100 μL in an absolute count tube (TruCount tubes, BD BioSciences). After incubation in the dark at RT for 30 minutes, 300 μL of PBS or annexin-binding buffer was added to each sample. Concentration-matched isotype antibodies were used as controls. Analyses were performed on a FACSCantoII (BD Biosciences) with instrument settings and gating optimized for detection of MPs less than 1 μm diameter as previously described.[30] Absolute count of MPs was calculated according to the manufacturer's instructions (BD Biosciences).

Factors VIII (FVIII), XII (FXII) and fibrinogen assays

Factors VIII, XII and fibrinogen levels were measured using one-stage clotting assays with automated coagulation analyzers (Factor VIII and fibrinogen: Sysmex CA 1500, Siemens Healthcare Diagnostics, NY, USA; Factor XII: STA-R, Diagnostica Stago S.A.S., France).

MP-depleted and MP-spiked FFP

To assess the impact of MP content on the procoagulant potential of FFP, MP-depleted and MP-spiked FFP samples were prepared. MPs were isolated from freshly-thawed FFP (500 μL) by sequential centrifugation, firstly at 18,000 × *g* for 30 minutes at 4°C, followed by at approximately 100,000 × *g* for 30 minutes at RT, as previously described.[30] The MP-depleted FFP was kept for TEG analysis. Pelleted material from both centrifugation steps were combined and added to a freshly-thawed aliquot of FFP (500 μL) to create the MP-spiked samples, which contained approximately twice the amount of MPs compared to the native FFP sample. The MP-depleted FFP and MP-spiked FFP were kept at 4°C for TEG analysis, which was performed on the same day as sample preparation.

TEG analysis

A computerized thrombelastography analyzer (TEG 5000, Haemonetics, Braintree, MA) was used to measure clot formation in the native FFP, MP-depleted FFP and MP-spiked FFP. The TEG measurements assessed included the initial clot formation time (R), clot formation rate (α-angle) and maximum clot strength (MA). Decreased R-time and increased α-angle and MA values indicated heightened coagulation potential. The reverse indicated decreased coagulation potential.

Analysis was performed according to the manufacturer's instructions. Briefly, TEG sample cups containing 20 μL of 0.2 M calcium chloride (both from Haemonetics) were pre-warmed to 37°C by the TEG analyzer. The FFP sample (400 μL) was activated by

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incubation for 4 minutes at RT in a kaolin tube (Haemonetics), after which 340 μL of the kaolin-activated FFP were added to the TEG sample cup and analysis was initiated immediately. The reaction was terminated once the MA value was acquired.

Statistical analysis

All data passed the test for normal distribution. Results shown are mean ± standard deviation (SD), unless otherwise indicated. Paired *t*-test was used to determine the effect of LD and the hold-times. A sample size of 12 was calculated to achieve at least a power of 0.8 at an error limit of 0.05. Statistical significance was defined as p less than or equal to 0.05. Calculations were performed by software (Excel 2003, Microsoft, Redmond, WA or Prism v5.04, GraphPad Inc, La Jolla CA).

RESULTS

Flow cytometric analysis of MPs

Previously established flow cytometry settings for MP analysis were used in this study.[30] Representative flow cytometric plots are shown in Figure 2 of non-LD FFP (Fig 2A – E) and LD-FFP (Fig 2F – J) from 6-hr hold WB. The events outside the MP and counting bead gates seen in Fig 2A and Fig 2F were most likely residual platelets in the FFP samples. A reduced number of un-gated and MP-gated events were observed in the LD samples compared to the non-LD samples.

WB-LD depletes MPs from FFP

A significantly lower number of MPs was found in FFP prepared from LD-WB compared to their non-LD FFP counterparts ($p < 0.03$) (Table 1). There was a 31% reduction of total MPs in FFP prepared from WB that had been leukodepleted after a 6-hr WB-hold time and a 43% reduction of MPs in LD-FFP from WB leukodepleted after 24-hr WB-hold time.

Of the marker-specific MPs, platelet-derived $CD41^+$ MPs and PS^+ MPs were significantly reduced in the LD-FFP compared to their non-LD counterparts ($p < 0.01$ for 6-hr and 24-hr WB hold time) (Table 1). The number of $CD41^+$ MPs was reduced by 71% in the 6-hr WB-hold LD-FFP and 58% in the 24-hr WB-hold LD-FFP, respectively compared to their non-LD counterparts ($p < 0.001$). PS^+ MPs, whether detected by annexin-V or lactadherin binding, were reduced by 85% and 64% respectively in the 6-hr WB-hold LD FFP ($p < 0.01$) and 60% and 49% respectively in the 24-hr WB-hold LD FFP ($p < 0.001$), compared to their non-LD counterparts. Lactadherin is reputedly a more sensitive marker for PS detection compared to annexin V,[32] which our findings support.

In contrast, the number of RBC-derived, $CD235a^+$ MPs showed an increased trend in LD-FFP compared to their non-LD counterparts, although in this study the increase reached significance only for the blood group A, 24-hr-hold WB sub-group (425 ± 179 versus 879 ± 627 $CD235a^+$ MPs $\times 10^3$/mL; $p = 0.03$). RBC derived MPs were the predominant type of cell-specific MPs present in LD FFP and accounted for 22 – 25 % of total MPs, compared to only 9 – 12 % in non-LD FFP.

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The number of MPs that were neither platelet- or RBC- derived, referred to as “undefined” MPs, was calculated by subtracting the number of $CD41^+$ and $CD235a^+$ MPs from the total number of MPs. The undefined MPs were the major proportion of MPs and accounted for 56 to 71% of the total MPs. LD of WB after 6-hr hold resulted in a modest 19% reduction of undefined MPs, which was not statistically significant. However, LD after a 24-hr hold of WB resulted in a statistically significant 44% reduction ($p = 0.007$) in the number of undefined MPs compared to the non-LD counterparts.

Effect of WB-hold time on FFP MP content differs for non-LD and LD

There was a significant increase in the total number of MPs and the number of undefined MPs in FFP prepared from non-LD WB held for 24 hours compared to 6 hours ($p < 0.02$) (Table 1). In particular, non-LD FFP from blood group A donors had the greatest increase in numbers of MPs, which reached significance for $CD41^+$ platelet-derived MPs, undefined MPs and total MPs ($p < 0.03$) (data not shown). These results for blood group A FFP were consistent with our previously published findings, which also used non-LD FFP from group A donors.[30] Non-LD FFP from group O donors had increased numbers of MPs with extended WB hold time, but did not reach significance in this study.

For LD FFP, there were no significant differences in the MP content of FFP prepared from WB that had been LD after a 6-hr hold compared to a 24-hr hold.

LD, but not WB hold-time, affects TEG profile

No significant differences were found in the TEG profiles of FFP from blood group A and O donors (results not shown). Therefore, the results of the two groups were combined.

FFP prepared from WB that was LD after 6-hr hold-time had significantly longer clot formation time (R-time) ($p = 0.002$) and lower clot strength (MA) ($p < 0.001$) compared to their non-LD FFP counterparts (Table 2). The results suggest that FFP produced from LD WB may have lower coagulation potential than FFP produced by non-LD WB. Similar results were found for FFP prepared from the 24-hr WB hold FFP samples, although significant difference between LD and non-LD FFP was only seen for clot strength (MA) ($p = 0.001$). This suggests that LD has a greater impact on the hemostatic properties of FFP when filtration of WB was performed at 6 hours compared to 24 hours.

Extended WB hold-time had no significant effect on any of the TEG parameters for non-LD FFP or LD FFP compared to 6-hr WB hold time (Table 2).

MP-depletion reduced coagulation potential

Depletion of MPs resulted in significantly lower kinetic of clot formation (α-angle) in all FFP samples compared to the corresponding native FFP samples ($p < 0.03$). Increased clot formation time (R time) was found for all MP-depleted FFP samples compared to the corresponding native FFP samples (Table 2), and was statistically significant for non-LD FFP samples (6-hr sample, $p = 0.001$; 24-hr sample $p < 0.001$). Clot strength (MA) was not significantly different between the MP depleted FFP and native FFP.

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MP-spiking enhanced coagulation potential

Shorter R time and increased MA value was seen in the MP-spiked FFP samples, which contained approximately twice the MP content of the native FFP (Table 2). The differences were statistically significant ($p < 0.05$), except for the R-time of the 6-hr hold non-LD FFP ($p = 0.072$) and MA of 24-hr hold non-LD samples. These changes demonstrated increased coagulation potential in MP-spiked FFP. No significant difference was found for the α-angle of MP-spiked FFP compared to native FFP.

WB-LD reduces FVIII, FXII and fibrinogen content of FFP

The levels of FVIII, FXII and fibrinogen were significantly less in FFP prepared from LD-WB compared to their non-LD counterparts ($p < 0.001$) (Table 3). FVIII levels decreased by 0.2 IU/mL, FXII decreased by 0.15 IU/mL and fibrinogen decreased by 0.4 mg/mL in FFP following LD of WB held for 6-hr. FVIII and fibrinogen levels following LD in 24hr FFP WB-hold samples were reduced by 0.1 IU/mL and 0.3 mg/mL, respectively.

No statistically significant differences were found in the levels of FVIII and fibrinogen in the FFP prepared from blood group A and O donors in this study (Table 3), which was consistent with the FFP TEG analysis results.

FVIII levels, but not fibrinogen, are affected by WB-hold time

Longer WB-hold time was associated with significantly lower levels of FVIII for FFP produced from non-LD WB (1.2 IU/mL ± 0.4 vs. 0.9 IU/mL ± 0.5; $p < 0.001$) and LD-WB (1.0 IU/mL ± 0.3 vs. 0.8 IU/mL ± 0.3; $p < 0.001$). The FVIII level of eight of 24 (33%) non-LD and LD FFP samples prepared from 24-hr hold WB were below the Council of Europe acceptance limit of 0.7 IU/mL.[16] Among these samples with low FVIII levels, five were blood group A (3 non-LD and 2 LD) and three were blood group O (2 non-LD and 1 LD). Only one FFP sample (from a blood group O donor) in the 6-hr WB-hold time group fell below 0.7 IU/mL.

Fibrinogen levels were not significantly affected by longer WB-hold time for FFP produced from either non-LD or LD WB.

DISCUSSION

The results of this study showed that the MP content and procoagulant potential of FFP were reduced by LD-filtration of WB. TEG analysis suggested that LD-filtration, rather than longer WB-hold time, had a greater effect on the procoagulant potential of FFP. The results from the MP-depletion and MP-spiking experiments suggested that MPs contribute to the coagulant properties of FFP. We believe the findings of this study add new insights into the effects of WB-processing conditions on the MP content and the hemostatic potential of FFP.

The experimental design of this study reflected as closely as possible the current standard blood manufacture practice for WB-derived FFP as well as utilized a paired design to minimize inherent inter-donor variability. WB units from male donors were selected consistent with current transfusion policy aimed at reducing the risk of transfusion-related acute lung injury and other adverse transfusion reactions elicited by donor allo-antibodies.[33]

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The WBF3 filter used in this study is a negatively-charged filter and is the WB-filtering system currently used in Australia.[34]

The significantly lower amount of MPs found in FFP prepared from LD-WB suggests that the LD filter traps MPs, as well as intact leukocytes and platelets. In particular, the number of $CD41^+$ MPs and PS^+ MPs were significantly reduced, which suggests specific interaction of the MPs with the filter matrix and/or co-adhesion to blood-derived elements retained within the filter. It was not possible to specifically identify the cell origin of the PS^+ MPs as we used a single fluorochrome-label method for MP characterization. Our results were consistent with the findings by Krailadsiri and colleagues[10] who reported a significant reduction in the number of platelet-derived MPs in LD-FFP prepared using three different WB-LD systems, while the number of RBC-derived MPs increased such that RBC-derived MPs were the predominant type of MPs in LD-FFP. Similarly, Lawrie and colleagues[35] reported that RBC-derived MPs were the predominant type of MPs in their LD-FFP that had been prepared from WB leukodepleted after overnight-hold at 4°C. Thus, WB-LD filtration may induce increased RBC vesiculation. A small proportion of RBCs are inevitably trapped within the WB-LD filter during the filtering process and these RBCs may be subjected to greater shear stress, which could induce vesiculation of the trapped RBCs.

The cell origin of the major proportion of MPs present in FFP, whether non-LD FFP or LD-FFP, could not be identified as the MPs did not bind the anti-CD41 or anti-CD235a antibodies used in this study. These findings are consistent with our previously reported characterization of MPs in non-LD FFP, which found a large proportion of MPs present in FFP were devoid of the markers used to identify RBCs, platelets, leukocytes and endothelial cells.[30] Further investigation will be required to determine the specific characteristics of this “undefined” population of MPs present in non-LD FFP and LD-FFP.

MPs are known to have immunomodulatory and procoagulant properties.[25–29] Therefore differences in the content and cellular profile of MPs in FFP prepared by different procedures may contribute to a wider diversity of clinical responses than solely hemostatic effects. The TEG results reported here suggest that MPs contribute to the procoagulant potential of FFP. Depletion of MPs from non-LD FFP resulted in significantly prolonged R-time, although not for LD-FFP. In contrast, the R-time was significantly shortened when LD-FFP was spiked with a double-dose of MPs, whereas a somewhat lesser effect was observed for non-LD FFP. For the MP-spiked experiments, each FFP sample was spiked with a double-dose of its own MP material, the content of which would have varied between non-LD FFP and LD-FFP samples. The variation in MP content may have contributed to the different effects of MP addition or removal on the TEG profiles seen for non-LD FFP compared to LD-FFP. Platelet- and RBC- derived MPs have been shown to initiate thrombin generation via a FXII-dependent pathway.[36] The TEG results reported here used the FXII-activator, kaolin to initiate the FFP clotting reaction. The use of kaolin in the TEG reaction may have masked differences in the procoagulant potential of MPs in non-LD FFP compared to LD-FFP.

In addition to the depletion of MPs by LD-filtration of WB, the concomitant reduction in FVIII, fibrinogen and FXII levels reported here could contribute to differences in the

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coagulation potential of FFP produced from non-LD WB or LD-WB. FXII binds to negatively-charged surfaces, including negatively-charged lipids such as PS.[37] The entrapment of PS^+ MPs by the LD-filter could have created additional surfaces for FXII to bind and be retained within the filter. Other coagulation factors also bind to PS and/or negatively-charged surfaces, including Factor XI and the γ-carboxyglutamic acid (Gla)-containing, vitamin K-dependent coagulation proteins, such as protein S.[38,39] We did not measure the levels of these other coagulation proteins that bind to negatively-charged phospholipids. Other studies have not found a reduction in these factors, although different LD-WB filters were used to the one used here.[6]

Prolonged duration from phlebotomy to freezing of plasma affects plasma quality, particularly labile coagulation proteins, such as FVIII.[19,20] The effects of WB-hold time on other plasma characteristics, such as MP content and TEG profile, have not been widely reported. The results reported here confirmed and extended our previous findings that longer WB-hold time resulted in increased numbers of MPs in FFP,[30] and in particular increased the numbers of "undefined" MPs, regardless of whether the WB was LD-filtered or not. However, longer WB-hold time did not significantly affect the fibrinogen level and TEG profile of FFP. These results provide further evidence in support of the Council of Europe guidelines[16] that allow FFP to be prepared from WB that has been held at 20 to 24°C for up to 24 hours.[20]

TEG and similar thrombelastography technologies, such as rotational thrombelastometry (ROTEM; Tem International GmbH, Munich, Germany) were designed for the analysis of patient WB samples and are increasingly being used as point-of-care tests for real-time monitoring of the coagulation status.[31] TEG and ROTEM are being used for wider applications, including analysis of FFP and platelet concentrates.[20,35,40–43] Our results are consistent with, and extend, the findings of these other investigators who noted the potential value of the data obtained from holistic coagulation measurements, such as TEG, to gain greater understanding about the functionality of blood components.

In summary, our results demonstrated that LD-filtration of WB, rather than WB-hold time, reduced the procoagulant potential of FFP. This effect of LD may be related to depletion of MPs and coagulation factors by WB-LD filtration. The results suggest a qualitative difference in the hemostatic potential of FFP produced from LD-filtered WB compared to non-LD WB. Nevertheless, on average the hemostatic quality met relevant jurisdictional requirements for all FFP processing conditions studied. The clinical significance, if any, of these results is not known.

Acknowledgments

We thank Australian Red Cross Blood Service, Melbourne staff of Donor Services, Processing and Process Control Laboratories for their assistance in the collection, processing of WB units and performing the FVIII and fibrinogen assays, respectively. We thank Ms Lisa Kelly, Haemonectics Corporation, Melbourne for assistance with the TEG assays.

Funding support: Australian governments fully fund the Australian Red Cross Blood Service for the provision of blood products and services to the Australian community. This work was funded in part by NIH Grant 1R01 HL095470-01A1.

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Abbreviations

FFP	fresh frozen plasma
LD	leukodepletion
MP	microparticle
PS	phosphatidylserine
RT	room temperature
TEG	thrombelastography
WB	whole blood

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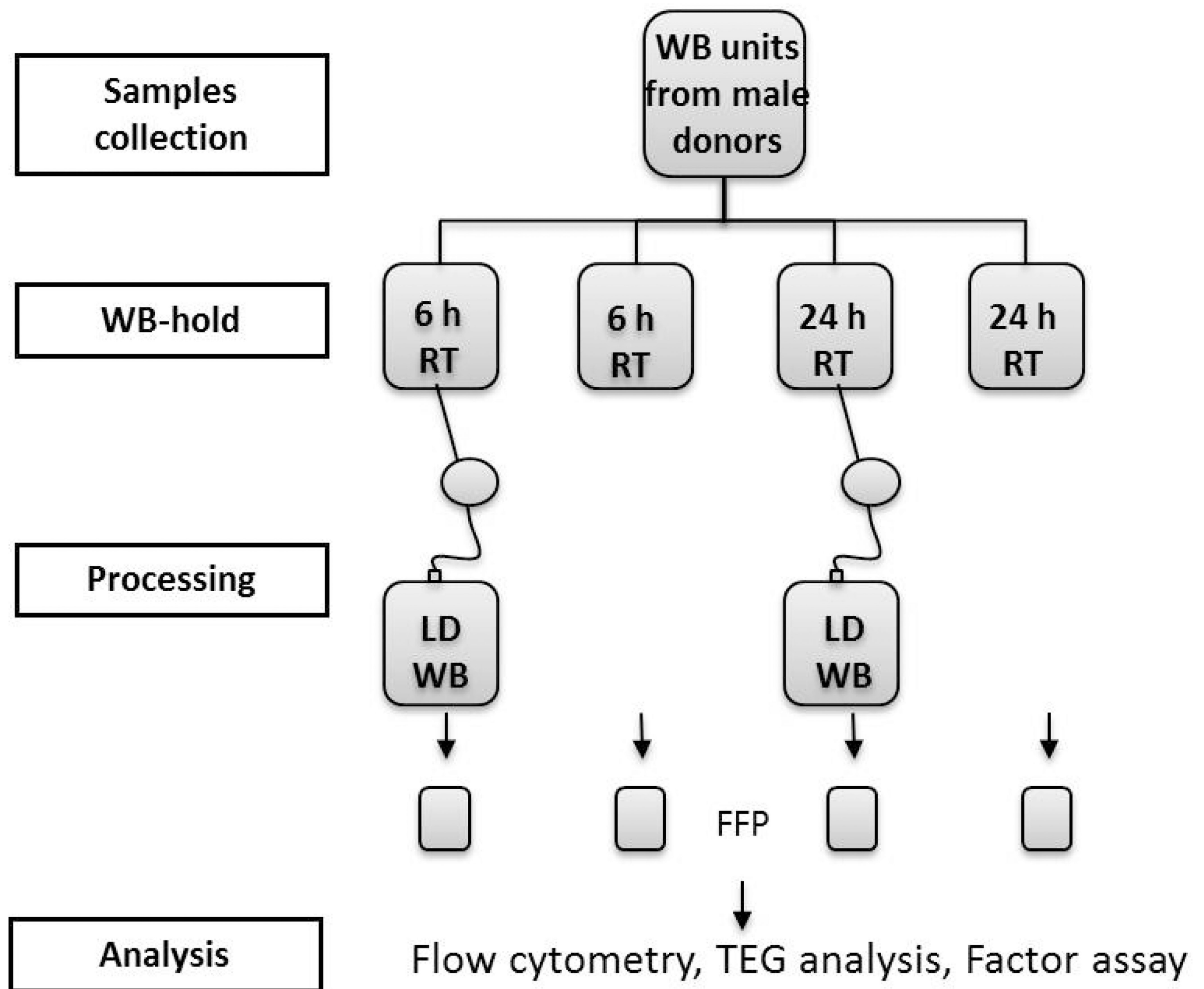

Figure 1. Schematic diagram of the experimental design

Twelve standard CPD-WB units were collected from male donors (6 blood group A and 6 blood group O). Each WB unit was divided into two pairs of pediatric-size packs and held for 6 or 24 hours at room temperature. A pack from each pair was leukodepleted (LD) (WBF3 filter, Pall) and the other pack from the pair remained unfiltered. All packs were then processed to FFP, frozen and thawed according to standard blood bank protocols. The microparticle content and coagulation potential of the thawed FFP samples were determined by flow cytometric absolute bead count assay, TEG analysis and coagulation factor assays.

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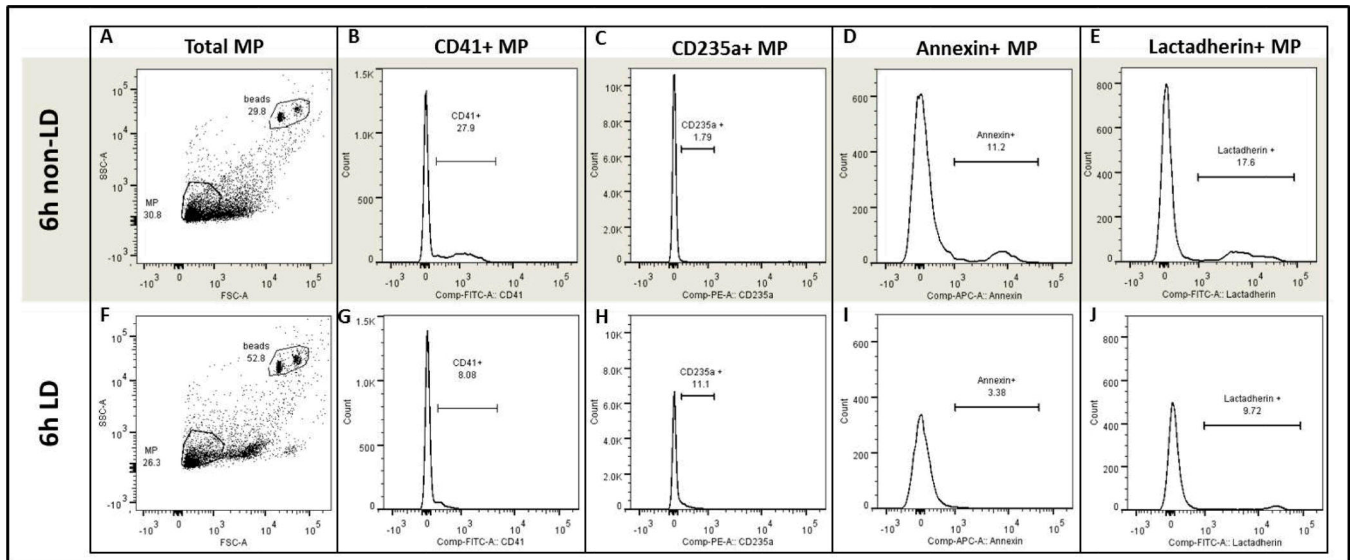

Figure 2. Flow cytometric analysis of MPs in FFP

Representative flow cytometric plots from a 6-hour non-LD (**top panel**) and 6-hour LD (**bottom panel**) FFP samples. The dot plots (**A, F**) demonstrate the positions of the MP gate and the absolute counting bead gate (beads) for the assay. Cell-specific fluorochrome-conjugated antibodies or ligands including, FITC-CD41 (platelet) (**B, G**), PE-CD235a (RBC) (**C, H**), APC-annexin V (PS) (**D, I**) and FITC-lactadherin (PS) (**E, J**), were used to quantitate the cell source and PS expression of MPs. The percentage of events in the MP gate that were positive for the various antibodies and ligands are shown in the histogram.

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Table 1

Microparticle content and cell origin in FFP prepared from whole blood held for 6 hours or 24 hours, with or without leukodepletion.

Type of MP	MP Marker	FFP process conditions					
		6-hr WB hold			24-hr WB hold		
		non-LD	LD	p	non-LD	LD	p
Total ($\times 10^3$ / mL)	Light scatter	3839 (1391 – 7739)	2631 (888 – 6894)	0.032	5497[†] (1627 – 12095)	3154 (908 – 8011)	0.002
Platelet ($\times 10^3$ / mL)	CD41	898 (597 – 1292)	246 (127 – 371)	0.021	1112 (666 – 1837)	269 (121 – 426)	<0.0001
RBC ($\times 10^3$ / mL)	CD235a	445 (142 – 755)	657 (140 – 1706)	ns	473 (204 – 843)	704 (205 – 1842)	ns
Undefined[*] ($\times 10^3$ / mL)		2269 (291 – 6233)	1728 (152 – 5909)	ns	3911[†] (378 – 9580)	2181 (139 – 6785)	0.007
Phosphatidylserine ($\times 10^3$ / mL)	annexin V	652 (372 – 1066)	277 (120 – 516)	<0.0001	682 (280 – 1094)	271 (115 – 660)	0.0004
Phosphatidylserine ($\times 10^3$ / mL)	Lactadherin	1080 (517 – 1614)	583 (162 – 1300)	0.014	994 (433 – 1614)	508 (200 – 944)	0.001

Results are mean (range); n = 12

[*] Calculated value, Undefined MP = Total MP – (platelet MP + RBC MP)

[†] $p < 0.02$, 6-hr versus 24-hr WB hold-time

ns, not signficant

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Table 2

TEG analysis of FFP, MP-depleted FFP and MP-spiked FFP samples

FFP sample / TEG parameter	FFP process method: 6-hr WB hold			FFP process method: 24-hr WB hold		
	non-LD	**LD**	**p**	**non-LD**	**LD**	**p**
FFP						
R time (min)	7.6 ± 0.8	8.7 ± 1.4	0.002	8.1 ± 0.8	8.6 ± 1.6	ns
α-angle (°)	68.8 ± 5.4	69.1 ± 4.8	ns	68.2 ± 5.5	67.5 ± 5.2	ns
MA (mm)	27.9 ± 4.4	24.4 ± 4.2	<0.0001	28.8 ± 5.5	24.0 ± 4.5	0.001
MP-depleted FFP						
R time (min)	10.7 ± 1.8†	10.4 ± 2.6†	ns	11.6 ± 2.0*†	9.0 ± 1.5	0.006
α-angle (°)	58.7 ± 8.2†	58.2 ± 12.8†	ns	57.3 ± 12.6†	60.3 ± 6.5†	ns
MA (mm)	27.2 ± 5.1	25.9 ± 5.5	ns	27.7 ± 5.1	25.8 ± 5.3	0.01
MP-spiked FFP						
R time (min)	7.0 ± 1.3	6.8 ± 1.0†	ns	7.4 ± 0.7†	6.4 ± 1.5†	0.026
α-angle (°)	64.7 ± 10.3	67.6 ± 6.0	ns	67.0 ± 7.0	68.1 ± 6.5	ns
MA (mm)	30.6 ± 5.7†	26.6 ± 4.9†	0.003	30.4 ± 7.6	26.5 ± 5.2†	0.017

Results are mean ± SD, n = 12

* $p < 0.03$, 6-hr versus paired 24-hr WB hold-time

† $p < 0.05$, FFP versus paired MP-depleted FFP or FFP versus paired MP-spiked FFP

ns, not significant

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Table 3

Factor VIII and fibrinogen in FFP

Factor assays	Blood Group	FFP process method					
		6-hr WB hold			24-hr WB hold		
		non-LD	LD	p	non-LD	LD	p
Factor VIII assay (IU/mL)*							
	A	1.2 ± 0.5	1.0 ± 0.4	0.032	1.0 ± 0.4‡	0.8 ± 0.4‡	0.006
	O	1.2 ± 0.2	1.0 ± 0.2	0.005	0.9 ± 0.2‡	0.8 ± 0.2‡	0.002
	A + O	1.2 ± 0.4	1.0 ± 0.3	0.0002	0.9 ± 0.5‡	0.8 ± 0.3‡	<0.0001
Fibrinogen assay (mg/mL)*							
	A	2.4 ± 0.4	2.1 ± 0.4	0.001	2.4 ± 0.4	2.1 ± 0.4	<0.0001
	O	2.7 ± 0.3	2.3 ± 0.3	0.002	2.7 ± 0.3	2.3 ± 0.3	0.001
	A + O	2.6 ± 0.4	2.2 ± 0.4	<0.0001	2.5 ± 0.5	2.2 ± 0.4	<0.0001
Factor XII (IU/mL)†							
	A + O	0.96 ± 0.27	0.81 ± 0.2	0.014	nd	nd	

*Results are mean ± SD, n = 12 (6 blood group A and 6 blood group O)

†Results are mean ± SD, n = 6 (3 blood group A and 3 blood group O)

‡p < 0.01, 6-hr versus paired 24-hr WB hold-time

nd, not determined