The Mirasol® Pathogen Reduction Technology system and quality of platelets stored in platelet additive solution

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Background. The Mirasol Pathogen Reduction Technology system for platelets and plasma uses riboflavin and UV light to introduce irreparable lesions into nucleic acids thereby inhibiting pathogen and white blood cell replication and reducing the load of infectious pathogens. The aim of the present study was to evaluate low plasma buffy coat platelet concentrates obtained from the OrbiSac System and to examine the effects on the development of platelet storage lesion during storage in platelet additive solution.

Material and Methods. Twenty buffy coat platelet concentrates were generated by pooling five individual units using the OrbiSac System. Riboflavin was added during the final pooling step, and the units were exposed to UV light. The bag was removed after the target energy of 6.24 J/mL had been delivered and 150 mL of platelet additive solution were added prior to storage. Platelet quality was assessed by pH, swirl, CD62P expression, lactate dehydrogenase, lactate production and glucose consumption rates over 7 days of storage.

Results. Buffy coat platelet concentrates generated on the OrbiSac contained an average 3.5 ± 0.6 x 10^{11} platelets at a concentration of 2976± 406 x 10^6/mL. After addition of 150 mL platelet additive solution the storage concentration was 1043 ± 148x 10^6/mL. Values obtained for pH, lactate production and glucose consumption rates were all within the limits of previously established correlations between in vitro cell quality and in vivo performance of Pathogen Reduction Technology-treated platelets in plasma.

Discussion. In vitro studies show that OrbiSac-derived platelets treated with the Mirasol Pathogen Reduction Technology system preserve adequate function, which would indicate acceptable in vitro viability.

Key words: Pathogen inactivation, platelet storage, Mirasol, buffy coat platelet concentrates.

Introduction

New approaches to blood safety have dramatically reduced the risk of infectious contamination of blood components. Currently most of the residual transfusion-transmitted infections occur as the result of the interval between the time the donor is infected and the time at which tests are capable of detecting the agent, the so-called "window period" which has been considerably reduced by the increased sensitivity of nucleic acid testing[1]. However, the safety of blood transfusions is still compromised by contamination of blood products with a variety of pathogens such as viruses, bacteria and parasites, including emerging pathogens[2,3]. Bacterial contamination of platelet concentrates is estimated to occur in between 1 per 900 and 1 per 100,000 units[4]. The most promising

approaches so far to reduce the risk of contamination are different pathogen reduction systems[5]. Two main systems have been developed for platelet concentrates; psoralen light treatment and riboflavin (vitamin B$_2$) light treatment. Among the different psoralen compounds, atmotosalen (S-59) is the most used and has demonstrated promising results for pathogen inactivation while preserving platelet function[6]. Among riboflavin-based systems that target pathogen nucleic acids, the Mirasol Pathogen Reduction Technology (Mirasol PRT; CaridianBCT, Lakewood, CO, USA) system has been well described[7-11]. The Mirasol PRT System for platelets and plasma uses riboflavin and UV light to introduce irreparable lesions into nucleic acids thereby inhibiting pathogen and white blood cell (WBC) replication.

This procedure has been the object of numerous *in vitro* studies to ensure safety margins in terms of toxicity to the cells and to the recipients[11]. Although PRT-treated platelets had significantly poorer post-transfusion recovery and survival than control, untreated platelets, the PRT-treated platelets retained sufficient *in vivo* efficacy for clinical use[12,13].

A clinical evaluation in a multicentre patient trial followed, which established that PRT treatment is safe and platelet functionality is maintained[14]. In order to allow future predictions of *in vivo* platelet performance based on *in vitro* cell quality measurements, analyses were performed to evaluate the relationship between *in vitro* and *in vivo* parameters of platelet function[12,13]. In these studies, pH (at 22°C) and lactate production were found to be most strongly correlated with the *in vivo* recovery and survival of PRT-treated platelets[12,13]. Glucose consumption rate and swirl also showed some correlation with these *in vivo* parameters, though to a lesser extent. pO$_2$, pCO$_2$ and P-selectin expression in PRT-treated platelets were poorly correlated with *in vivo* platelet recovery and survival[12, 13].

During the last few decades, the development and use of platelet additive solutions have improved both the quality of platelet storage and the results in transfusion medicine[15]. Storing platelets in a platelet additive solution has become an attractive alternative to their storage in plasma because the former strategy increases the amount of plasma available for other purposes and may reduce the incidence of non-haemolytic transfusion reactions and transfusion-related acute lung injury (TRALI)[15].

The aim of this study was to examine the effects of the PRT process on the development of platelet storage lesion during the storage of platelets in a platelet additive solution.

Material and methods

Twenty plasma buffy coat platelet concentrates (BCPC) were generated by pooling five individual units, using the Gambro BCT OrbiSac Buffy Coat System (OrbiSac System, Gambro Bct, Lund, Sweden). The buffy coat method involves a hard-spin of the original whole blood collection, separating the whole blood into three distinct layers: red cells, white cells and platelets (buffy coat) and plasma. Plasma and red cells were removed and used as transfusable components. Buffy coats of approximately 50 mL and a 30-40% haematocrit were generated. The OrbiSac BC system then pooled five buffy coats with suspension medium (30-35 mL Riboflavin), centrifuged the pooled buffy coats and passed the platelet concentrate through an in-process leucoreduction filter. The platelet concentrate was transferred into an Illumination/Storage Bag. The bag was placed in the Illuminator and exposed to UV light with a linear agitation of 120 cpm and a product temperature of = 37°C. After the target energy of 6.24 J/mL had been delivered, the bag was removed and 150 mL of platelet additive solution were added prior to storage.

The *in vitro* cell quality of the platelet products was evaluated over 7 days. This study was intended to verify the compatibility of platelet concentrates obtained using the OrbiSac System with the PRT System. Five ABO-identical whole blood units were collected for each single BCPC product made. The BCPC was treated in a small volume of plasma (approximately 30-45 mL plasma/1×10^{11} cells) and stored in platelet additive solution at a final storage concentration of $980\text{-}1920 \times 10^6$/mL.

All platelet concentrates were stored for 7 days under standard blood bank conditions. A small sample was removed from the storage bag on days 0, 1, 3, 5 and 7 for cell quality analysis of the following parameters; cell counts, pH (days 1, 3, 5 and 7), glucose (days 5 and 7), lactate concentrations (days 5 and 7), swirl (days 0, 1, 5 and 7), P-selectin (CD62)

Table I - The results from different parameters for *in vitro* cell quality of the platelet products evaluated over 7 days

	Day 1	Day 3	Day 5	Day 7
pH	6.89 ± 0.057	7.02 ± 0.043	6.90 ± 0.047	6.90 ± 0.052
Glucose consumption rate; mmol/10^{12} platelets/hour			0.041 ± 0.007	0.035 ±0.006
Lactate; mmol/L)	18.43 ± 1.108	11.35 ± 1.089	15.84 ± 1.455	17.81 ± 1.384
Swirl	3.00 ± 0	2.75 ± 0.380	2.20 ± 0.523	1.30 ± 0.523
P-selectin/CD62; percent positive cells			77.27 ± 4.316	53.57 ± 3.181
LDH (IU/L)		147.85 ± 45.297	102.90 ± 18.767	111.2 ± 23.410

(days 5 and 7) and lactate dehydrogenase (LDH)(days 3, 5 and 7).

Glucose and LDH were measured using a Modular (Hitachi High-Technologies corp., Tokyo, Japan), lactate was measured with an ABL 725 (Radiometer, Copenhagen, Denmark), and cells were counted with an Advia 2120 machine (Bayer HealthCare, Tarrytown, NY, USA). P-selectin was measured by flow cytometry (FACS-Alibur and CellQuest, Becton-Dickinson, Franklin Lakes, NJ, USA). The swirling phenomenon was recorded by visual inspection and graded 0 (no swirl) to 3 (excellent swirl) by trained technicians. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 15.0 (SPSS Inc., Chicago, IL, USA). Data are expressed as mean ± standard deviation (SD).

Results

BCPC generated from the OrbiSac contained an average 3.5 ± 0.6 x 10^{11} platelets at a concentration of 2,976± 406 x 10^{6}/mL. After addition of 150 mL platelet additive solution the storage concentration was, on average, 1,043 ± 148x 10^{6}/mL. The final platelet volume after addition of platelet additive solution was 290.3 ± 13.72 mL.

The results of the main cellular and biochemical measurements performed in the study are summarised in table I.

pH values, corrected for storage temperature, are shown in figure 1. The prediction for clinically acceptable platelet recovery and survival requires pH values greater than 6.85[12,13]. All pH values measured during the entire storage period were above 7.0 (Figure 1).

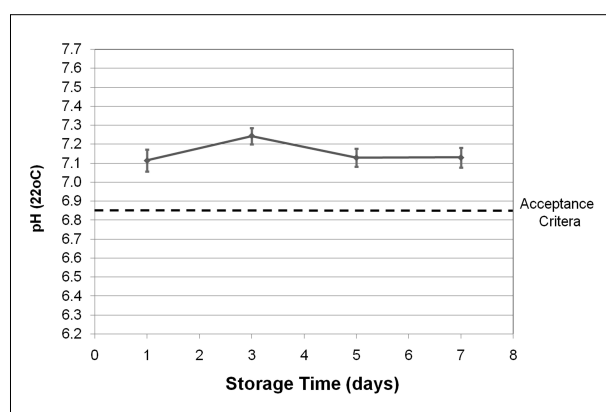The average glucose consumption rate measured on days 5 and 7 was below the previously established

Figure 1 - pH (at 22℃) as a function of time (error bars indicate one standard deviation). The lowest pH value predicting acceptable platelet recovery and survival is represented by the dotted line.
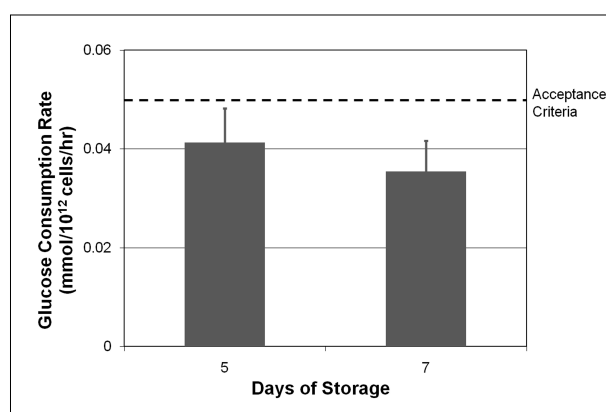

Figure 2 - Glucose consumption rate as a function of time (error bars indicate one standard deviation). The highest glucose consumption rate to predict acceptable platelet recovery and survival is represented by the dotted line.

acceptance criteria of 0.050 mmol/10^{12} platelets/hour (Figure 2).

The results on lactate production rate are shown in figure 3. The average lactate production rate was within the limits of acceptable according to the predefined criterion (<0.110 mmol/10^{12} platelets/hour).

The swirl values decreased during the storage period, but swirl was detected for all units evaluated on days 0, 1, 5 and 7. The results are illustrated in figure 4.

Surface expression of P-selectin (CD62P) was elevated, but within previously reported ranges (Figure 5).

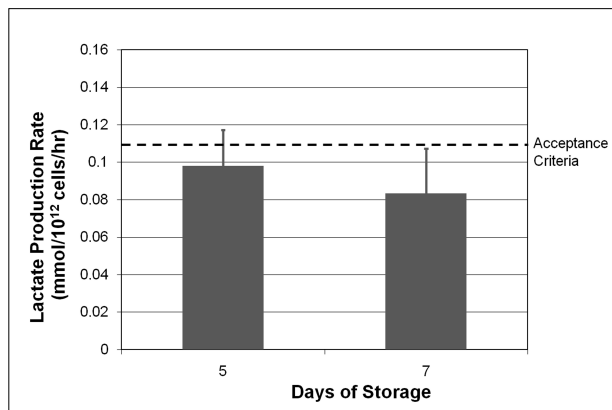Results of the measurement of LDH on days 3, 5 and 7 are presented in figure 6.

Figure 5 - P-selectin (CD62P) expression as a function of time (error bars indicate one standard deviation).

Figure 6 - LDH as a function of time (error bars indicate one standard deviation).

Figure 3 - Lactate production rate as a function of time (error bars indicate one standard deviation). The highest lactate production rate predicting acceptable platelet recovery and survival is represented by a dotted line.
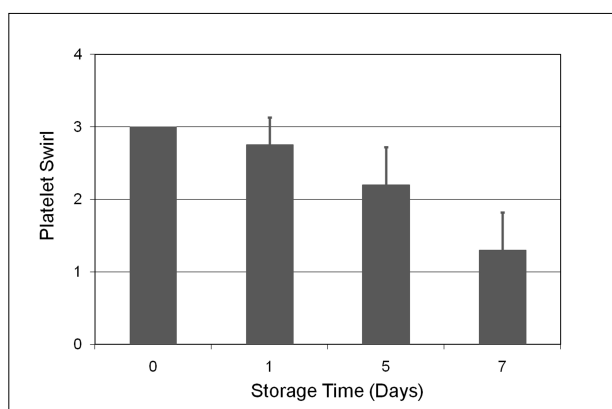

Figure 4 - Swirl as a function of time (error bars indicate one standard deviation).
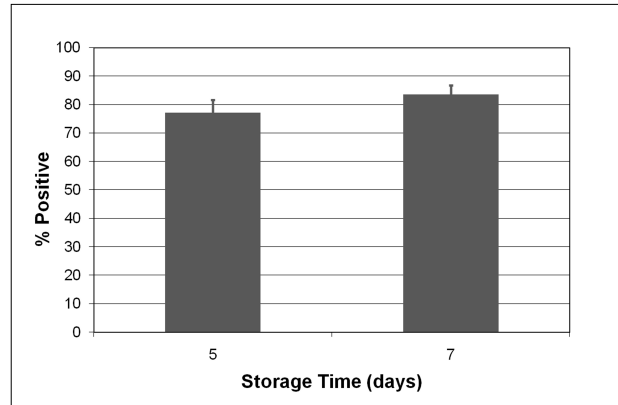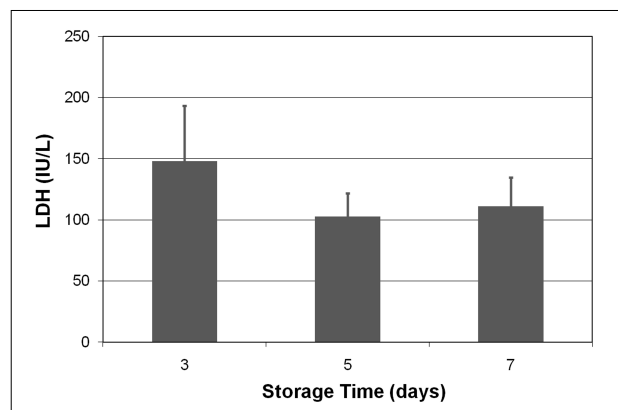
Discussion

Ever since platelet transfusions were shown to reduce mortality from haemorrhage in patients with acute leukaemia in the 1950s, the use of this therapy has grown steadily, such that it has become an essential part of the treatment of cancer, haematological malignancies, marrow failure, and haematopoietic stem cell transplantation[19]. However, platelet transfusions can transmit bacterial infections, which still threaten patients' safety[2,3]. There are several types of platelet components; leucoreduced platelets can be used to reduce platelet alloimmunisation, transmission of infections, and febrile transfusion reactions, while gamma irradiation prevents transfusion-associated graft-versus-host disease. Although, significant progress has been made in reducing the risk of pathogen transmission by transfusions, there remains

a continuing risk of transmission of microbiological agents to recipients. Pathogen inactivation technologies provide additional protection against both known and as-yet-unidentified agents. However, the impact of pathogen inactivation on a product's functional quality and performance in the clinical setting has not yet been fully examined [20]. The Mirasol PRT System for Platelets and Plasma is a new technology utilising riboflavin as a photosensitiser in combination with UV light. Riboflavin is a dietary nutrient with known pharmacokinetic and toxicology profiles [11]. Riboflavin can rapidly traverse lipid membranes where it non-specifically associates with nucleic acids. Upon exposure to light, riboflavin causes modification of the nucleic acid present in pathogens through oxidation of guanine residues and generation of reactive oxygen species. As a result of these photochemical processes, the nucleic acids of pathogens in blood products undergoing this treatment are rendered unable to replicate [21]. The aim of our study was to collect information about the effects of this PRT process on the development of storage lesion in OrbiSac-derived BCPC during storage in platelet additive solution.

We demonstrated that BCPC contained an average of $3.5 \pm 0.6 \times 10^{11}$ platelets at a concentration of $2976 \pm 406 \times 10^6$/mL. After addition of 150 mL platelet addition solution the storage concentration was, on average, $1043 \pm 148 \times 10^6$/mL. These conditions are similar to those described by other authors [22]. During normal storage, platelets show activation of caspase 3 and consequent cleavage of gelsolin, which lead to apoptosis [23]. Therefore, independently of platelet activation, a loss of total platelets counts during storage is acceptable. Evaluation of the mechanism of apoptosis in platelets might be the basis for further development of novel strategies to enhance platelet viability during storage [23]. In our study platelet function was assessed by pH, swirl, CD62P expression, lactate production and glucose consumption rates over 7 days of storage. We observed elevated lactate production and glucose consumption and reduced pH rates after PRT treatment. These findings confirm previously reported observations, and are a result of cellular activation and increased metabolism [10,13 22]. Consistent with previously established correlations between *in vitro* cell quality and *in vivo* performance of PRT-treated platelets in plasma, these *in vitro* values predict

acceptable platelet recovery and survival after storage in platelet additive solution [12,13].

Platelet quality was assessed by CD62P expression on the platelet surface, which the level of expression is known to be a predictor of platelet viability [24]. CD62P expression was elevated in our study and increased as the period of storage became longer, reflecting increased platelet activation after PRT treatment. This has also been demonstrated for another pathogen inactivation method that utilises amotosalen and UVA light [25,26]. Measurements of CD62P expression are highly variable and values on day 5 of storage for PRT-treated platelets can range from 18% to 70% [10,16]. Similar variability can be observed in untreated control platelets, in which CD62P expression ranges from 8% to 48% [17,18]. However, the measured CD62P levels after PRT treatment remain within ranges that often are seen at the time of transfusion, and P-selectin positive platelets have been shown to continue to circulate and function *in vivo* [27]. It should also be emphasised that previous studies involving PRT-treated platelets failed to demonstrate a statistically significant correlation between P-selectin expression and *in vivo* platelet recovery and survival of platelets [16].

The visual inspection of swirling is performed before the issue of a platelet concentrate and a swirling score can be recorded. The swirling effect can be seen when a fresh platelet unit is held up to a light source and slightly agitated. When normal discoid platelets are gently rocked, they scatter the incident light in different directions and a moving opalescence is caused by the changing orientation of platelets relative to the incident light. When platelets have undergone shape change (a disk-to-sphere transformation), they lose the ability to change their orientation. As a consequence, all activated (spherical) platelets scatter light in the same direction, resulting in the sample having a dull, unchanging appearance. The discoid shape of platelets can also be determined photometrically by the extent of shape change and the hypotonic shock response (HSR). The extent of shape change and the hypotonic shock response have been demonstrated to correlate well with *in vivo* viability [28], and extent of shape change correlates strongly with morphology scores. In our study swirling remained clearly visible in all PRT-treated units, predicting acceptable *in vivo* viability. Platelet

membrane integrity can also be assessed by measuring the amount the of the LDH enzyme that leaks from the cell. LDH levels in the supernatant of the stored products remained low and stable over time, indicating little or no lysis of platelets after treatment and during storage.

This study lack a potential control group, e.g. untreated platelets or platelets stored in other solutions such as plasma. However, we believe it is important to characterise the changes of platelet function related to activation during the PRT process, and our results suggest that despite the increases in different parameters of platelet activation, the quality of the platelet product is still in line with the criteria for acceptability.

Conclusion

To conclude, the quality of OrbiSac-derived BCPC treated with the Mirasol PRT system and stored in platelet additive solution for 7 days remains with the defined criteria for acceptability of stored platelets.

However, the PRT-treated platelets show signs of increased cellular activation and increased metabolism. The platelets had elevated CD62P expression, lactate production and glucose consumption rates after the PRT treatment. Swirling was detected in all units, reflecting viable platelets. These observations suggest that despite the general activation, platelets treated by this PRT method are suitable for storage.

Acknowledgements

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