In vitro Quality of Platelets with Low Plasma Carryover Treated with Ultraviolet C Light for Pathogen Inactivation

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Keywords

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Summary

Background: The THERAFLEX UV-Platelets system uses shortwave ultraviolet C light (UVC, 254 nm) to inactivate pathogens in platelet components. Plasma carryover influences pathogen inactivation and platelet quality following treatment. The plasma carryover in the standard platelets produced by our institution are below the intended specification (<30%). **Methods:** A pool and split study was carried out comparing untreated and UVC-treated platelets with <30% plasma carryover (n = 10 pairs). This data was compared to components that met specifications (>30% plasma). The platelets were tested over storage for in vitro quality. **Results:** Platelet metabolism was accelerated following UVC treatment, as demonstrated by increased glucose consumption and lactate production. UVC treatment caused increased externalization of phosphatidylserine on platelets and microparticles, activation of the GPIIb/IIIa receptor (PAC-1 binding), and reduced hypotonic shock response. Platelet function, as measured with thrombelastogram, was not affected by UVC treatment. Components with <30% plasma were similar to those meeting specification with the exception of enhanced glycolytic metabolism. **Conclusion:** This in vitro analysis demonstrates that treatment of platelets with <30% plasma carryover with the THERAFLEX UV-Platelets system affects some aspects of platelet metabolism and activation, although in vitro platelet function was not negatively impacted. This

study also provides evidence that the treatment specifications of plasma carryover could be extended to below 30%.

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Introduction

Pathogen inactivation (PI) methods attempt to prevent the replication of pathogens in blood components, thereby reducing the risk of transfusion-transmitted infection (reviewed in [1]). The INTERCEPT and Mirasol systems use ultraviolet (UV) light in conjunction with a photosensitizing agent, whereas the THERAFLEX UV-Platelets system (referred to herein as UVC treatment) does not utilize a photosensitizer, but relies on short-wave UVC light (254 nm). It is well established that UVC light alone can be microbiocidal and virucidal, inducing DNA damage through the dimerization of adjacent pyrimidine nucleotides [2–4]. However, turbid solutions such as platelet concentrates have low permeability to UVC light [4]. As such, UVC treatment requires the preparation of platelets in platelet-additive solution (PAS) as well as strong agitation to form shallow regions within the platelet component to facilitate sufficient light penetration.

The THERAFLEX UV-Platelets system has been CE-marked, and several investigations have been conducted to determine the effect of UVC treatment on in vitro platelet quality [4–8]. Treatment with the UVC system appears to accelerate the metabolic rate of platelets, which is similar to the effects of other PI methods [9–11]. However, in vivo investigations using the UVC system are showing positive results [5, 12].

To balance PI and minimize platelet damage, the optimal light dose must be achieved. The dose of light effectively applied to a

Table 1. Specifications of platelet concentrates*

Parameter	Specifications	<30% plasma carryover			>30% plasma carryover		
		control	UVC-treated	p value [‡]	control	UVC-treated	p value [‡]
Platelets × 10 ⁹ /ml	0.8–1.4	0.86 ± 0.06	0.86 ± 0.07	0.7655	0.83 ± 0.08	0.84 ± 0.09	0.3576
Volume, ml	325–375	353.1 ± 6.1	353.6 ± 6.0	0.6599	369.8 ± 4.7	369.8 ± 6.3	0.8433
Plasma carryover, %	30–40	27.4 ± 1.7	27.7 ± 1.8	0.6459	31.8 ± 1.0 [†]	32.2 ± 1.3 [†]	0.2327

*Values shown as mean ± SD; n=10 in each group.

[‡]Obtained using paired, two-sided t-test.

[†]p < 0.05 obtained using one-way ANOVA comparing <30% and >30% for each treatment.

platelet concentrate is influenced by the platelet concentration and plasma carryover, and as such, the platelet component must meet certain specifications to be suitable for treatment (table 1). The pooled platelet components routinely produced in Australia do not meet all of these specifications. In particular, the plasma carryover is below the lower limit of the specifications for UVC treatment (30–40%). As such, the aim of this study was to evaluate the in vitro quality of the standard pooled platelet units prepared in Australia following treatment with the UVC system. Further, these data were compared to components which meet the current specifications for UVC treatment. This data is required to provide confidence that these products would be suitable for use in a clinical setting.

Material and Methods

Platelet Production and UVC Treatment

This study had approval from the Australian Red Cross Blood Service Human Research Ethics Committee. Buffy coats were prepared from whole blood donations collected in citrate-phosphate-dextrose (CPD) solution [13]. Platelet components were prepared by pooling four ABO/RhD matched buffy-coats with 300 ml additive solution (SSP+; MacoPharma, Mouvauux, France) within 24 h of collection. The platelets were then separated by slow centrifugation (500 × g; 6 min) followed by in-line leukofiltration (Imuguard III-S PL filter; TerumoBCT, Somerset, NJ, USA).

On day 1 post-collection, platelet components were prepared using a pool and split design (n = 10). Two platelet components were pooled and split, and one unit was UVC-treated whilst the other remained untreated as a control. All platelets were sterile docked onto an illumination set and transferred into a 19 × 38 cm illumination bag composed of UVC-permeable ethyl vinyl acetate (EVA). The untreated units were transferred directly into the associated platelet storage bag, which was composed of n-butyltri n-hexyl citrate (BTHC) plasticized PVC [14]. The UVC units were treated with the THERAFLEX UV-Platelets system, according to the manufacturer's instructions (MacoPharma). Briefly, the component was fixed to the edges of a quartz plate, agitated at 110 rpm and illuminated with a dose of 0.2 J/cm² UVC light (254 nm) from the top and bottom. Following treatment, the components were transferred to the associated platelet storage bag and stored at 20–24 °C with agitation (Helmer Inc., Noblesville, IN, USA).

Platelet samples (10 ml) were removed on day 1 (immediately after treatment), 2, 5, and 7 by sterile docking on a sample pouch.

To compare the low plasma carryover group with components which meet the specifications (>30% plasma) for UVC treatment, an additional study group was prepared (n = 10). To achieve >30% plasma carryover, two platelet components were pooled and supplemented with 45 ml of ABO-matched plasma (freshly thawed plasma) prior to splitting. These pairs (>30%) were treated and tested in the same way as the standard units.

Component Specifications and Quality

The platelet count and MPV were measured using a hematology analyzer (CellDyn Emerald; Abbott Laboratories, Abbott Park, IL, USA). Plasma carryover was measured from the supernatant on day 1 using a bicinchoninic acid (BCA) protein assay kit (Pierce Biotechnology, Rockford, IL, USA). The total protein concentration was calculated from a bovine serum albumin (BSA) standard curve, and plasma carryover in the platelet concentrate was calculated as a percentage of the total protein present in 100% plasma. Supernatants were prepared by double centrifugation at 1,600 × g for 20 min and then 12,000 × g for 5 min at room temperature, and stored at –80 °C until tested in the assays as described below.

Assessment of Platelet Quality and Function

The pH, pO₂, pCO₂, and lactate content of platelet components were measured immediately after sampling using an i-STAT blood gas analyzer and CG4+ cartridges (Abbott Diagnostics, Sydney, Australia). These parameters were measured at 37 °C, and bicarbonate was calculated by the i-STAT analyzer. Extracellular glucose was measured from the platelet supernatant using a colorimetric assay measuring the enzymatic conversion of glucose to hexokinase/glucose-6-phosphate dehydrogenase (Fisher Diagnostics, Middletown, VA, USA). Extracellular lactate dehydrogenase (LDH) was measured from supernatants using an in vitro toxicology assay (Sigma Aldrich, St. Louis, MO, USA), against a standard curve of L-lactic dehydrogenase. Adenosine triphosphate (ATP) concentration was measured using an ATP bioluminescence kit (Roche, Mannheim, Germany), as previously described [9]. Samples for glucose, LDH, and ATP were tested in triplicate against a standard curve.

Changes in platelet mitochondrial transmembrane potential (Δψ) were determined using the lipophilic cationic fluorochrome JC-1 (Biotium Inc., Hayward, CA, USA). The percentage of platelets displaying a polarized mitochondrial membrane (red fluorescence) was measured by flow cytometry, as previously described [9].

The expression of platelet glycoproteins was measured by flow cytometry as previously described [15]. PAC-1 binding was measured at basal levels, where platelets (3 × 10⁶) were stained with 20 μl PAC-1-FITC (BD Biosciences, Franklin Lakes, NJ, USA) for 20 min at room temperature in the dark, and the median fluorescence intensity (MFI) was assessed by flow cytometry. For p-selectin, platelets (3 × 10⁶) were stained with CD62P-PE (BD Biosciences) for 20 min at room temperature, and percentage of positive cells was assessed compared to an isotype control. For phosphatidylserine exposure, platelets (1 × 10⁶) were stained with 5 μl annexin-V-FITC binding in calcium-containing buffer (BioLegend, San Diego, CA, USA) for 15 min in the dark, and the percentage of positive cells was reported.

The absolute number of platelet microparticles was determined by flow cytometry using TruCount tubes (BD Biosciences), as previously described [13]. Platelet microparticles were stained with CD61-APC and annexin-V-FITC and defined as events ≤1.0 μm. The procoagulant activity of platelet microparticles was assessed using the STA-Procoag-PPL kit (Diagnostica Stago Ltd, Asnieres, France), as previously described [13].

Cytokine release was measured from the supernatants of platelet concentrates. Commercially available ELISA kits for CD40L, RANTES, soluble CD62P, PDGF-AB, PF4 and NAP2 (as a measure of β-thromboglobulin) were used ac-

Fig. 1. UVC treatment alters platelet metabolic parameters. Platelet concentrates with <30% plasma carryover were treated and stored for 7 days. The concentration of **A** glucose and **B** lactate were measured and consumption and production rates were calculated using the platelet count and are given as mmol/10¹² platelets/h during the indicated time periods. **C** HSR was measured using phosphate buffer solution and water. The data represent mean $\pm$ SD (error bars). The p value indicates the interaction between storage and treatment as determined by two-way repeated measures ANOVA; *Indicates $p < 0.05$ between untreated and UVC-treated platelets at the same time point.

Table 2. In vitro metabolic parameters of platelets containing <30% plasma carryover*

Parameter	Day 1		Day 2		Day 5		Day 7		p value [†]
	control	UVC-treated	control	UVC-treated	control	UVC-treated	control	UVC-treated	
Platelets $\times 10^9/l$	863 $\pm$ 61	861 $\pm$ 69	861 $\pm$ 63	834 $\pm$ 64	871 $\pm$ 53	792 $\pm$ 50 [‡]	841 $\pm$ 60	756 $\pm$ 52 [‡]	<0.0001
MPV, fl	5.0 $\pm$ 0.2	5.2 $\pm$ 0.2	5.1 $\pm$ 0.1	5.1 $\pm$ 0.1	4.9 $\pm$ 0.1	5.1 $\pm$ 0.2	4.8 $\pm$ 0.1	5.2 $\pm$ 0.1 [‡]	<0.0001
pH ₂₂ °C	7.17 $\pm$ 0.04	7.17 $\pm$ 0.04	7.23 $\pm$ 0.04	7.22 $\pm$ 0.04	7.30 $\pm$ 0.04	7.25 $\pm$ 0.05 [‡]	7.30 $\pm$ 0.05	7.21 $\pm$ 0.05 [‡]	<0.0001
Glucose, mmol/l	6.1 $\pm$ 0.3	6.1 $\pm$ 0.3	6.0 $\pm$ 0.3	5.7 $\pm$ 0.2	4.7 $\pm$ 0.3	3.9 $\pm$ 0.3 [‡]	3.7 $\pm$ 0.3	2.3 $\pm$ 0.3 [‡]	<0.0001
Lactate, mmol/l	4.8 $\pm$ 0.5	4.8 $\pm$ 0.5	5.4 $\pm$ 0.4	5.6 $\pm$ 0.4	7.3 $\pm$ 0.5	8.6 $\pm$ 0.5 [‡]	8.9 $\pm$ 0.5	11.2 $\pm$ 0.7 [‡]	<0.0001
pO ₂ , mm Hg	144 $\pm$ 5	139 $\pm$ 8	136 $\pm$ 7	134 $\pm$ 10	146 $\pm$ 4	145 $\pm$ 7	138 $\pm$ 7	139 $\pm$ 5	0.4869
pCO ₂ , mm Hg	30.5 $\pm$ 1.2	30.1 $\pm$ 1.3	21.9 $\pm$ 0.6	22.6 $\pm$ 1.2	16.7 $\pm$ 0.7	18.3 $\pm$ 0.6 [‡]	16.0 $\pm$ 0.7	17.4 $\pm$ 0.7 [‡]	0.0007
Bicarbonate, mmol/l	8.9 $\pm$ 0.3	8.7 $\pm$ 0.2	8.0 $\pm$ 0.3	7.8 $\pm$ 0.2	7.7 $\pm$ 0.3	6.9 $\pm$ 0.3 [‡]	7.5 $\pm$ 0.4	5.8 $\pm$ 0.4 [‡]	<0.0001
ATP, μ mol/10 ¹¹ platelets	5.4 $\pm$ 0.9	4.5 $\pm$ 0.7	5.5 $\pm$ 0.9	4.4 $\pm$ 1.2	5.4 $\pm$ 0.9	4.2 $\pm$ 1.1	4.9 $\pm$ 1.2	3.9 $\pm$ 1.0	0.8046
Polarised $\Delta\psi$, %	98.0 $\pm$ 1.0	97.8 $\pm$ 1.1	97.7 $\pm$ 1.1	97.1 $\pm$ 1.4	97.4 $\pm$ 1.3	97.4 $\pm$ 0.8	97.0 $\pm$ 1.5	96.0 $\pm$ 2.6	0.4611
Extracellular LDH, U/l	231 $\pm$ 30	242 $\pm$ 29	251 $\pm$ 33	257 $\pm$ 31	274 $\pm$ 33	288 $\pm$ 36 [‡]	279 $\pm$ 34	311 $\pm$ 36 [‡]	0.0003

MPV = Mean platelet volume; ATP = adenine triphosphate; $\Delta\psi$ = mitochondrial membrane potential; LDH = lactate dehydrogenase.

*Values shown as mean $\pm$ SD; n=10 in each group.

[†]Obtained using a two-way ANOVA indicating an overall difference induced by UVC treatment and storage.

[‡]p < 0.05 compared to untreated controls at same time point using post-hoc Bonferroni multiple comparisons.

Fig. 2. UVC treatment alters selected aspects of platelet activation. Platelet concentrates with <30% plasma carryover were treated and stored for 7 days. Platelets were stained with **A.** PAC-1-FITC, **B** CD62P-PE or **C** Annexin-V-FITC and analyzed by flow cytometry. **D** CD61- and Annexin-V-positive microparticles were assessed by flow cytometry **E** Clotting times were measured with a Procoag-PPL assay using platelet supernatant. The data represent mean $\pm$ SD (error bars). The p value indicates the interaction between storage and treatment as determined by two-way repeated measures ANOVA; * Indicates $p < 0.05$ between untreated and UVC-treated platelets at the same time point.

cording to the manufacturer's instructions (R&D systems Inc., Minneapolis, MN, USA). All samples were tested in triplicate against a standard curve.

Hypotonic shock response (HSR) and platelet aggregation were measured with an aggregometer (Helena Laboratories, Beaumont, TX, USA) [11], using 20 μ mol/l ADP (Sigma) or 10 μ g/ml collagen (Helena Laboratories). All samples were tested in duplicate, and the mean of the values was stated.

The platelet clotting potential was measured with a thromboelastogram (TEG 5000, Haemoscope Corporation, Niles, IL, USA). Platelets were diluted with platelet-poor plasma to a concentration of $200 \times 10^9/l$, and 1,000 μ l was transferred to a kaolin-containing tube (Haemoscope Corporation) and mixed by inversion. Kaolin-activated platelets (340 μ l) were then added to a plain TEG cup (Haemoscope Corporation) containing 20 μ l of calcium chloride (CaCl_2 ; 0.2 mol/l; Haemoscope Corporation). The following TEG variables were measured at 37 $^\circ\text{C}$ for approximately 60 min: R-time (time to clot initiation; min), maximum amplitude (MA; clot strength; mm), K-time (speed of clot formation; min), and α -angle (clot growth; degrees).

Statistical Analysis

Results are expressed as mean $\pm$ standard deviation (SD). Paired, two-sided t-tests were used to compare the initial specifications of the untreated and

UVC-treated units. A two-way repeated measures analysis of variance (ANOVA) was used to assess the effect of UVC treatment (<30% untreated vs. <30% UVC) or plasma carryover (UVC <30% vs. UVC >30%) and storage. Post-hoc Bonferroni multiple comparisons were performed to determine the differences between samples at each time-point. Pearson's correlation analysis was performed. A p value < 0.05 was considered to be significant.

Results

The average starting parameters of units within each group are shown in table 1, along with the recommended specifications for the treatment of platelets with the UVC system.

The effect of UVC treatment on the metabolic properties of platelets stored in <30% plasma was assessed over the storage time (table 2). UVC treatment reduced the platelet concentration by 10% after 7 days of storage. The MPV was significantly higher in the UVC-treated units compared to untreated platelets by day 7.

Fig. 3. The effect of UVC treatment on platelet aggregation. Platelet concentrates with <30% plasma carryover were treated and stored for 7 days. Platelet aggregation was measured following stimulation with **A** 20 $\mu\text{mol/l}$ ADP or **B** 10 $\mu\text{g/ml}$ collagen. Maximal aggregation (% max) was reported. The data represent mean $\pm$ SD (error bars). The p value indicates the interaction between storage and treatment as determined by two-way repeated measures ANOVA. **C.** Representative aggregation trace of the mean duplicate platelet samples following stimulation with 20 $\mu\text{mol/l}$ ADP on day 5 of storage.

Table 3. Platelet thromboelastography parameters in components containing <30% plasma carryover*

Parameter	Day 1		Day 5		Day 7		p value [†]
	control	UVC-treated	control	UVC-treated	control	UVC-treated	
R-time, min	9.0 $\pm$ 1.2	9.1 $\pm$ 0.5	9.0 $\pm$ 1.3	8.2 $\pm$ 1.2	8.6 $\pm$ 0.7	8.1 $\pm$ 0.7	0.4950
K-time, min	1.2 $\pm$ 0.1	1.5 $\pm$ 0.4	1.3 $\pm$ 0.2	1.1 $\pm$ 0.2	1.1 $\pm$ 0.3	0.9 $\pm$ 0.2	0.0695
α -angle, degrees	64.4 $\pm$ 11.9	57.0 $\pm$ 14.5	67.0 $\pm$ 8.7	68.9 $\pm$ 12.4	75.0 $\pm$ 2.7	69.4 $\pm$ 13.5	0.6620
MA, min	74.2 $\pm$ 5.6	72.0 $\pm$ 2.8	72.7 $\pm$ 5.2	72.6 $\pm$ 6.7	72.4 $\pm$ 2.6	69.5 $\pm$ 7.4	0.7661

*Values shown as mean $\pm$ SD; n = 5.

[†]Obtained using a two-way ANOVA indicating an overall difference induced by UVC treatment and storage.

The pH of UVC-treated components was significantly lower by day 5. The glucose concentration in UVC-treated platelets was lower than that in untreated platelets by day 5 (table 2), as a result of increased consumption (fig. 1A). Similarly, the lactate concentration and consumption rate was significantly higher in UVC-treated platelets (table 2 and fig. 1B). The mitochondrial membrane potential did not change during storage, and there was no difference in UVC-treated platelets. The HSR of UVC-treated platelets was markedly reduced immediately following treatment, but partially recovered during storage (fig. 1C). Extracellular LDH, an indicator of platelet leakage, increased gradually during storage, and UVC-treated platelet supernatant had a significantly higher concentration of LDH.

UVC treatment of platelet components did not significantly affect the proportion of cells expressing the platelet glycoproteins GPIIb (CD41a), GPIX (CD42a), GPIb α (CD42b), GPIIIa (CD61) and CD47 (data not shown). Basal PAC-1 was significantly higher in the UVC-treated groups compared to untreated controls

(fig. 2A), even on day 1 of storage. The extrusion of P-selectin (CD62P) and CD63 from intracellular stores was not increased by UVC treatment (fig. 2B and data not shown, respectively). However, the percentage of annexin V-positive cells was increased in UVC-treated platelets by expiry (fig. 2C). The total number of platelet microparticles (CD61-positive) did not change over storage or in response to UVC treatment (data not shown). However, a higher number of CD61/Annexin-V-double-positive microparticles were formed as a result of UVC treatment and storage (fig. 2D). The effect of microparticle release was examined using a phosphatidylserine-dependent, FXa-based clotting assay. Clotting time was significantly shorter in the UVC-treated platelets at all points during storage (fig. 2E), and this correlated with the number of CD61/annexin-V-positive microparticles ($r = -0.6372$; $p < 0.0001$). A gradual release of cytokines occurred over the storage period, which is typical of the platelet storage lesion. With the exception of CD40L, the release of the investigated cytokines was accelerated by UVC treatment (data not shown).

Table 4. In vitro quality parameters of UVC-treated platelets*

Parameter	Day 1		Day 2		Day 5		Day 7		p-value†
	<30% plasma	>30% plasma	<30% plasma	>30% plasma	<30% plasma	>30% plasma	<30% plasma	>30% plasma	
Platelets × 10 ⁹ /l	861 ± 69	835 ± 90	834 ± 64	801 ± 88	792 ± 50	784 ± 81	756 ± 52	753 ± 82	0.5922
pH _{7.2} °C	7.17 ± 0.04	7.18 ± 0.04	7.22 ± 0.04	7.23 ± 0.04	7.25 ± 0.05	7.28 ± 0.05	7.21 ± 0.05	7.25 ± 0.05	0.2375
MPV, fl	5.2 ± 0.2	5.1 ± 0.2	5.1 ± 0.1	5.2 ± 0.1	5.1 ± 0.2	5.1 ± 0.1	5.2 ± 0.1	5.2 ± 0.1	0.5881
Annexin-V, % positive	0.73 ± 0.23	0.71 ± 0.16	0.78 ± 0.14	0.68 ± 0.17	1.92 ± 0.36	1.83 ± 0.41	3.16 ± 0.60	2.95 ± 0.73	0.4432
Basal PAC-1, MFI	937 ± 290	794 ± 140	461 ± 102	442 ± 133	328 ± 62	299 ± 50	289 ± 55	256 ± 45	0.2037
Collagen aggregation, % max	102 ± 14	100 ± 16	96 ± 15	92 ± 15	65 ± 21	57 ± 13	43 ± 18	37 ± 15	0.4598
HSR, %	34.8 ± 8.7	40.4 ± 6.8	48.0 ± 6.0	51.8 ± 6.3	46.3 ± 6.6	48.5 ± 6.1	38.3 ± 8.6	43.2 ± 4.8	0.1036
Day 1–2									
Day 2–5									
Day 5–7									
Glucose consumption	0.010 ± 0.010	0.005 ± 0.006	0.010 ± 0.010	0.005 ± 0.006	0.027 ± 0.005	0.025 ± 0.005	0.039 ± 0.006	0.033 ± 0.007‡	0.0115
Lactate production	0.048 ± 0.010	0.048 ± 0.012	0.048 ± 0.010	0.048 ± 0.012	0.057 ± 0.007	0.046 ± 0.009	0.085 ± 0.014	0.070 ± 0.014‡	0.0278

*Values shown as mean ± SD; n=10 in each group.

†Obtained using a two-way ANOVA indicating an overall difference as a result of plasma carryover.

‡p < 0.05 compared to <30% plasma units at same time point using post-hoc Bonferroni multiple comparisons.

An overall decrease in the maximum aggregation response induced by both ADP and collagen was observed over storage (fig. 3A, B), and, although UVC treatment tended to result in greater aggregation responses, this effect was not statistically significant. However, the kinetics of the aggregation response did differ between the untreated and UVC-treated platelets (fig. 3C) but this effect did not translate into an alteration in platelet function, as the TEG responses were similar between the groups (table 3).

To determine the specific effect of the lower plasma content on quality parameters following treatment, additional units were prepared by supplementation with exogenous plasma to meet the specifications. When comparing the in vitro quality parameters of the standard (<30% plasma) UVC-treated units to those that meet specifications (>30% plasma), very few differences were observed (table 4). Of the parameters tested, the only differences found were a higher glycolytic metabolic rate in the UVC-treated platelets containing less than 30% plasma. These results demonstrate that reducing the plasma content to below the manufacturer's specifications does not cause overt damage to the platelets.

Discussion

This study investigated the impact of UVC treatment on the in vitro functional characteristics of standard whole-blood-derived platelets stored in SSP+ for 7 days. Overall, UVC treatment and subsequent storage increased platelet metabolism and some aspects of activation. However, these observed differences did not negatively impact the in vitro indicators of platelet function, including aggregation and clot formation. Further, the reduction in plasma carryover did not exacerbate these effects.

The standard platelet components used did not meet the manufacturer's specifications for treatment with the THERAFLEX-UV Platelets system in terms of plasma carryover. Further, the components were also at the lower end of the specified limits for the platelet concentration, and all previous studies published to date have reported higher platelet concentrations and plasma carryover than used in this study [4–6, 8, 16, 17]. As both of these parameters influence the amount of UVC penetration, and thus potential platelet damage, it is imperative to establish the effect of UVC on these components. The changes seen in regard to platelet activation and function are no more severe than those previously reported using components that met the specifications for treatment [4–6, 16, 17]. Further, our comparison between units with low plasma carryover and those that meet specification demonstrate that only the metabolic parameters were affected by the reduction in plasma carryover. As such, this study provides evidence to suggest that the specifications for UVC treatment could be extended to encompass products with <30% plasma, thereby increasing the flexibility of the system.

As reported with other PI systems, UVC treatment increased the rate of anaerobic metabolism in the platelets, as evidenced by increased glucose and bicarbonate consumption, lactate production, and the accompanying decline in pH. Although the pH de-

clined following UVC treatment, it was maintained at approximately 7.2–7.3 in all units at day 5 and day 7 of storage, which is well above the Council of Europe recommendation of $\text{pH} \geq 6.4$ [18]. As expected, glycolytic metabolism in the platelets was accelerated towards the end of storage, but the parameters remained within acceptable limits, with the glucose concentration above 1 mmol/l and lactate less than 20 mmol/l [19, 20]. The effect of UVC treatment on oxidative phosphorylation was assessed by examining mitochondrial membrane integrity. A previous report has demonstrated that UVC treatment does not increase mitochondrial membrane depolarization during storage [5] and our results confirm these findings. Thus, UVC treatment of platelets did not adversely affect oxidative metabolism, despite enhanced glycolysis.

The HSR was one of the few parameters tested in this study that was immediately modified by UVC treatment. There appears to be some contradictory evidence regarding the effect of UVC treatment on HSR, with some studies finding a reduction [5, 8] and others not [4, 6, 17]. Similarly, the HSR has been shown to be negatively affected by PI treatment with other systems [10, 11]. This effect is of particular interest given that the HSR is one of the few *in vitro* parameters thought to be associated with post-transfusion efficacy [21]. The increased externalization of phosphatidylserine expression and formation of microparticles is suggestive of membrane asymmetry, which may contribute to the reduced membrane responsiveness to osmotic stress. Further, the MPV was seen to increase to a greater degree in the UVC-treated platelets during storage. This phenomenon has been reported previously with UVC-treated platelets [8, 17] and may be associated with a change in the platelet from the resting discoid shape to a more spherical morphology.

Platelet activation induces a conformational change in GPIIb/IIIa, exposing a ligand binding site. In UVC-treated platelets, basal PAC-1 binding was approximately twofold higher at each point, which has been attributed to disruption of the disulfide bonds [22]. Interestingly, the proportion of cells binding CD41 (GPIIb) and CD61 (GPIIIa) were not affected by UVC treatment and remained stable over storage. These results suggest that, while receptor expression *per se* was not markedly affected by UVC treatment, there was a conformational change associated with platelet activation allowing increased PAC-1 binding. It has been suggested that the activation of the $\alpha\text{IIb}/\beta 3$ integrin may potentiate aggregation, and a previous study has shown that UVC treatment augments ADP-induced aggregation [23]. Our data suggest that, although the kinetics of the aggregation response may be affected by UVC treatment, the maximal response was not higher than in untreated samples, which is consistent with other studies [6, 24]. However, the contribution of platelets to clot formation, as measured by TEG, was not affected by UVC treatment.

Whilst this study and a previous report have shown that UVC treatment does not affect the total number of CD61+ microparticles [5], we have shown that UVC treatment resulted in a significant increase in the number of phosphatidylserine-expressing platelet microparticles. These microparticles were also able to participate in clotting, as measured with a phosphatidylserine-depend-

ent clotting assay. This is of interest as microparticles in blood components have been shown to have hemostatic function *in vitro* [13, 25] and influence clinical outcomes following transfusion [26].

The goal of implementing a PI system is to provide proactive protection against pathogens, thus reducing the need to introduce additional testing and potentially replace processing steps such as gamma-irradiation [27], with minimal impact on the quality of the treated blood components. UVC treatment inactivates a wide spectrum of bacteria, parasites, viruses, and contaminating leukocytes [4, 6, 27–30]. Although HIV, and possibly other retroviruses, appear less sensitive to inactivation by UVC light, this is somewhat compensated by the fact that the residual risk of HIV transmission in Australia is exceptionally small (modelled to be less than 1 in 7 million) [31]. In terms of the impact on *in vitro* platelet quality, the majority of the changes induced by UVC treatment were not seen until at least day 5, which is the current shelf-life of platelets in Australia and several European countries. Further, the average age the platelets issued nationally in Australia during 2013–2014 was 2.9 days. As such, the negative effect of UVC treatment on platelets may be acceptable in order to improve transfusion safety. While less clinical data is available for the UVC system, compared to the other PI systems, UVC-treated platelets have been shown to be well-tolerated in healthy volunteers [12], and the next phase of clinical studies are being designed.

This *in vitro* analysis demonstrates that treatment of platelets with a plasma carryover below 30% with the THERAFLEX UV-Platelets system affected some, but not all, aspects of platelet metabolism and activation. Further, reducing the plasma carryover to below 30% did not significantly affect the overall quality of platelets following UVC treatment. *In vivo* studies will determine whether the changes in platelet quality induced by UVC treatment translate into a measurable clinical effect.

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LJ, FT, CS, AS, and DCM designed the study. LJ, RAH, and ST carried out the laboratory research. LJ and DCM performed the data analysis and wrote the manuscript. All authors critically reviewed the manuscript and approved the final manuscript.

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RAH and ST: none. DCM and LJ professional interest: received corporate sponsorship from MacoPharma for this research project. FT and CS professional interest: employees of MacoPharma. AS professional interest: co-operates with MacoPharma in development of the THERAFLEX UV-Platelets system.

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