Cryopreservation of UVC pathogen inactivated platelets

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Abstract

Background:

Extending the platelet shelf life and enhancing product safety may be achieved by combining cryopreservation and pathogen inactivation (PI). Although studied individually, limited investigations into combining these treatments has been performed. The aim of this study was to investigate the effect of PI-treating platelets prior to cryopreservation on *in vitro* platelet quality and function.

Study Design and Methods:

ABO-matched buffy-coat derived platelets in platelet additive solution (SSP+; Macopharma) were pooled and split to form matched pairs (n=8). One unit remained untreated and the other was treated with the THERAFLEX UV-Platelets System (UVC; Macopharma). For cryopreservation, 5-6% dimethylsulfoxide (DMSO) was added to the platelets and they were frozen at -80°C. After thawing, untreated cryopreserved platelets (CPPs) and UVC-treated CPPs (UVC-CPPs) were resuspended in plasma. *In vitro* quality was assessed immediately post-thaw and after 24 hours of room-temperature storage.

Results:

UVC-CPPs had lower *in vitro* recovery compared to CPPs. By flow cytometry, platelets demonstrated a similar abundance of GPIX (CD42a), GPIIb (CD41a), and GPIbα (CD42b-HIP1), while the activation of GPIIb/IIIa (PAC-1) was increased in UVC-CPPs compared to CPPs. UVC-CPPs demonstrated greater phosphatidylserine exposure (annexin-V) and microparticle shedding, but similar P-selectin (CD62P) abundance compared to CPPs. UVC-CPPs displayed similar functionality to CPPs when assessed using aggregometry, thromboelastography (TEG) and thrombin generation.

Conclusion:

This study demonstrates the feasibility of cryopreserving UVC-PI treated platelet products. UVC-PI treatment may increase the susceptibility of platelets to damage caused during cryopreservation, but this is more pronounced during post-thaw storage at room-temperature.

Key words: UVC, pathogen inactivation, platelet, cryopreservation

Introduction

Cryopreservation of platelets at -80 °C with dimethylsulfoxide (DMSO) allows a significant extension of shelf life from five days to at least two years.[1,2] Over the past decade, cryopreserved platelets (CPPs) have become more widely investigated in research and clinical practice.[3] While the extension of shelf life afforded by cryopreservation addresses a major issue associated with conventional platelet storage, improvements to product safety could be made through the use of pathogen inactivation (PI).

Currently, three PI technologies are available for the treatment of platelet products: THERAFLEX UV-Platelets System (UVC-PI; Macopharma, Mouvaux, France), INTERCEPT Blood System (INTERCEPT; Cerus, Concord, CA, USA), and Mirasol Pathogen Reduction Technology System (Mirasol; Terumo BCT, Lakewood, CO, USA).

The UVC-PI system utilizes UVC light in combination with strong agitation to form cyclobutane pyrimidines and pyrimidine pyrimidone dimers.[4,5] This inhibits nucleic acid elongation during transcription, preventing pathogen replication.[4,5] The UVC-PI system effectively inactivates a broad range of bacterial, viral and parasitic species,[6-8] although it has limited efficacy for inactivation of HIV and bacterial spores.[9,10] While not yet approved for clinical use, transfused autologous UVC-PI treated platelets have a similar circulation time to untreated platelets.[11] A stage III clinical trial is currently underway investigating platelets treated with the UVC-PI system (EudraCT 2015-001035-20).

Both cryopreservation and UVC-PI treatment result in phenotypic alterations when compared to conventionally stored platelets. For CPPs, these alterations are characteristic of platelet activation and/or damage, as evidenced by phosphatidylserine externalization, microparticle shedding, and granule release.[12-14] The changes induced by UVC-PI treatment are more in line with an acceleration of the platelet storage lesion, including an increased abundance of P-selectin and phosphatidylserine, and activation of the GPIIb/IIIa receptor.[5,15,16] Glycolytic metabolism also occurs more rapidly during the latter part of storage in UVC-PI treated platelets, leading to a fall in pH,[10,17] although still within component specifications (pH >6.4).[18] Importantly, several key platelet receptors, including GPIbα and GPIX, are similar in abundance to untreated platelets[15,17,19] and platelet function appears to be unchanged, as measured by thromboelastography.[15,20] Therefore, while combining PI treatment and cryopreservation may enhance product safety, it is important that the combination of these processes does not result in further damage.

PI treatment of platelet products prior to cryopreservation is a relatively new concept. One study has investigated the treatment of platelets with the INTERCEPT system prior to

cryopreservation, revealing a similar *in vitro* phenotype and functionality compared to untreated CPPs following thawing.[21] Thus, combining PI treatment and cryopreservation appears to be feasible. However, it is well established that each PI system has different effects on platelet quality and function,[9,22,23] thus necessitating an investigation of each PI system in this context. Therefore, the aim of this study was to characterize the effects of UVC-PI treatment of platelets in additive solution prior to cryopreservation on post-thaw *in vitro* platelet quality and function.

Methods and Materials

Study Design

This study had ethics approval from the Australian Red Cross Blood Service Ethics Committee. All donations were collected from eligible, voluntary donors, in accordance with Blood Service guidelines.

Pathogen inactivation and cryopreservation

Platelet concentrates were prepared in platelet additive solution (SSP+; Macopharma) from four buffy coats, as previously described.[24] On day 1, two platelet units were pooled and split to form matched pairs (n=8). Within 4 hours of platelet manufacture, UVC-PI treatment was performed. All platelet units were transferred to an illumination kit (Macopharma) via sterile connection, but only the UVC assigned units were treated with the THERAFLEX UV-Platelets System, according to the manufacturer's instructions.[15] The platelets were then transferred to the associated storage bag.

Platelets were frozen immediately after UVC-PI treatment. For platelet cryopreservation, approximately 100 mL of 27 % wt/vol DMSO/0.9 % saline (Sypharma Pty. Ltd, Dandenong, VIC, Australia) was added to the platelet units to achieve a final concentration of 5-6% (v/v). Platelets were transferred to a 450 mL polyvinylchloride platelet storage bag (Macopharma) and were frozen at -80 °C, as previously described.[24] The untreated cryopreserved units are referred to herein as CPPs, and the UVC-PI-treated cryopreserved units are referred to as UVC-CPPs.

Thawed CPPs and UVC-CPPs were reconstituted in paired, freshly thawed whole blood-derived plasma (275 ± 10 mL). Two plasma units were thawed in a 37 °C water bath, before being pooled and split to form a matched pair. Matched platelet units (CPP and UVC-CPP) were then thawed in a 37 °C water bath, until they reached 30 °C (5 minutes), then sterile welded to a unit of thawed plasma. The plasma was added to the platelet unit and allowed to flow in by gravity, during which the platelets were gently mixed to facilitate resuspension. Platelet units were sampled, then rested at room-temperature for one hour following resuspension, before storage on a platelet agitator (Helmer Inc., Noblesville, IN, USA).

Laboratory analysis

Platelet samples (5 mL) were taken from the pooled unit via sterile transfer into an associated bag prior to UVC treatment and before freezing to determine platelet count and plasma carryover. Following thawing, platelet samples (15 mL) were aseptically removed immediately after thawing and reconstitution (post-thaw) and after 24 hours of room-temperature storage

(post-storage). At the time of sample removal, all platelet units were investigated visually for the presence of swirling and aggregates.

Platelet counts were measured using an automated hematology analyzer (CELL-DYN Emerald, Abbott Diagnostics, IL, USA). The platelet count was used to determine platelet recovery following cryopreservation and to perform dilutions allowing for comparisons between CPPs and UVC-CPPs.

Platelet supernatant was prepared by sequential centrifugation at 1,600 x g for 20 minutes followed by 12,000 x g for 5 minutes at room-temperature (20-24°C), as previously described.[14] Supernatants were stored at -80°C until tested.

The pH of platelet components was measured using a pH meter at room temperature (20-24 °C) immediately following sampling (Seven Excellence Multiparameter; Mettler Toledo, OH, USA). The glucose and lactate concentration were measured from the platelet supernatant, as previously described.[25]

The platelet phenotype was characterized by flow cytometry. Platelets (3×10^6 in Tyrode's buffer) were labelled for 20 minutes in the dark with the following antibodies CD41a-PE, CD42a-PE, CD42b-HIP1-PE, PAC-1-FITC, CD62P-PE (all obtained from BD Biosciences, San Jose, CA, USA), CD42b-AN51-PE (Dako, Glostrup, Denmark), or GPVI-eFluor660 (eBioscience Inc., San Diego, CA, USA). The abundance of surface bound fibrinogen was measured by staining platelets (5×10^5 in Tyrode's buffer) with anti-fibrinogen-FITC (Abcam, Cambridge, UK) in the dark for 20 minutes. Phosphatidylserine externalization was analyzed by staining platelets (1×10^6 in annexin-V binding buffer; BioLegend, San Diego, CA, USA) with annexin-V-FITC (Biolegend) in the dark for 15 minutes. Platelets were diluted in 1 mL of Tyrode's buffer or annexin-V binding buffer and were measured by flow cytometry (FACSCanto II, Becton Dickinson, Franklin Lakes, NJ, USA), with a total of 10,000 events collected. The platelet population was established based on FSC and SSC properties and gating for positive fluorescence was determined using relevant isotype controls (IgG1-eFluor660 (eBioscience), IgM-FITC (BD Biosciences), IgG-FITC and IgG1-PE (BioLegend)). The median fluorescence intensity (MFI) or percentage of positive events was reported, as indicated.

The absolute number of microparticles was determined by flow cytometry using TruCount tubes (BD Biosciences), as previously described.[13] Platelet microparticles were defined as events less than 1.0 µm and identified as staining positive for CD61-APC (Dako) and annexin-V-FITC (BioLegend). The distribution of particles in the platelet component was assessed by

dynamic light scattering technology (ThromboLUX system and ThromboSight software, Version 3.11, LightIntegra Technology, Vancouver, BC, Canada).

The concentration of soluble CD62P (sCD62P), RANTES, PDGF-AB, PF4, and NAP2 (as a measure of β-thromboglobulin) were measured from the platelet supernatant using commercially available ELISA kits, according to the manufacturer's instructions (R&D Systems Inc., Minneapolis, MN, USA). Platelet supernatant samples were diluted in reagent diluent (R&D Systems) in a range of 1:50-1:10000, depending on the cytokine being tested. All samples were tested in triplicate against a standard curve and absorbance at 450 nm was measured using a plate reader (VARIOSKAN LUX platform, version 5.0.0.42, Thermo Fisher Scientific).

Aggregation was assessed using light transmission aggregometry (Helena Laboratories, Beaumont, TX, USA). Platelets were diluted to 300 x 10^9/L in freshly thawed plasma and stimulated with 20 µmol/L adenosine diphosphate (ADP; Sigma, St Louis, MO, USA) or 10 µg/mL collagen (Helena Laboratories) for 5 minutes following agonist addition. All samples were analyzed in duplicate and the average maximal aggregation (%) was reported.

The platelet clotting potential was measured using a thromboelastogram (TEG 5000; Haemoscope Corporation, Niles, IL, USA), as previously reported.[26] Platelets were diluted to 200 x 10^9/L in freshly thawed plasma and activated with kaolin and calcium chloride prior to application of sample to a plain cup. The samples were run for approximately 60 minutes at 37 °C and the following variables were recorded: R-time (time to clot initiation; min), K-time (speed of clot formation; min), maximum amplitude (MA; clot strength; mm), and α-angle (clot growth; degrees).

Thrombin generation was measured using a Calibrated Automated Thrombogram (CAT; Thrombinscope BV, Maastricht, The Netherlands). Platelets were diluted to 300 x 10^9/L in freshly thawed plasma and thrombin generation was initiated using PRP reagent (1 pM tissue factor; Thrombinscope BV), as previously published.[27] All samples were analyzed in triplicate and the following variables were recorded: lag time (min), endogenous thrombin potential (ETP; nM/min), peak (nM), and time to peak (min).

Statistical Analysis

The data were analyzed using GraphPad Prism 7.03 (GraphPad Software Inc.; La Jolla, CA, USA) and results are expressed as mean ± standard deviation (SD). Repeated measures two way ANOVA with post-hoc Bonferroni multiple comparisons test was used to assess differences between CPPs and UVC-CPPs after thawing and storage. Linear regression with

Pearson’s correlation (r value) was performed to assess relationships between parameters. A p value < 0.01 was considered to be significant.

Results

UVC-CPPs display reduced platelet recovery

All platelet units used in this study met targeted specifications for UVC-PI treatment (Table 1).[15] Following cryopreservation, the recovery of all CPP units was above the minimum acceptable recovery (>40%) specified by the Council of Europe[18] (Table 2). However, UVC-CPP units had a lower recovery than untreated CPPs (Table 2). The mean platelet volume (MPV) was similar between groups (Table 2). Notably, all UVC-CPP units developed significant aggregates within 1 hour post-thaw that were persistent during storage, compared with only one CPP unit, which developed minor aggregates that dissipated before the 24 hour sampling point. Platelet swirl was absent immediately after thawing, however, it was present at the 24 hour post thaw sampling time point in both CPPs and UVC-CPPs.

UVC-PI treatment did not increase glycolysis during post-thaw storage, with all units having similar glucose and lactate concentrations (Table 2). Further, the rate of glucose consumption and lactate production over the storage period was similar to that of previously published results (data not shown).[12] Although the pH decreased during storage (Table 2), it did not differ between groups and remained within an acceptable range (pH>6.4).[18]

UVC-CPPs have an altered surface phenotype

The abundance of GPIX (CD42a) on platelets was similar between groups immediately after thawing and following storage (Figure 1A). GPVI expression was low on CPPs and UVC-CPPs post-thaw but was re-expressed during subsequent storage, although to a lesser extent on UVC-CPPs (Figure 1B). A similar abundance of GPIbα (HIP1) was present on CPPs and UVC-CPPs following thawing, which increased in both groups following storage (Figure 1C). Interestingly, the conformation of GPIbα was altered on CPPs and UVC-CPPs, as determined by CD42b-AN51 binding, where a reduction in AN51 binding indicates GPIbα clustering and activation.[28] AN51 binding was low but similar between CPPs and UVC-CPPs following thawing (Figure 1D), and increased during post-thaw storage. The abundance of resting GPIIb/IIIa (CD41a) was not altered between CPPs and UVC-CPPs (Figure 1E). However, greater GPIIb/IIIa activation was detected on UVC-CPPs, by PAC-1 binding, which increased approximately 3-fold during storage (Figure 1F). PAC-1 binding correlated with an increase in fibrinogen bound to the platelets after thawing and storage (Figure 1G), where the greatest amount of fibrinogen binding was observed on UVC-CPPs following storage.

The proportion of platelets expressing P-selectin increased during storage to a similar extent in both groups (CD62P; Figure 2A). In contrast, the proportion of UVC-CPPs binding annexin V was higher when compared to CPPs post-thaw and post-storage (Figure 2B). UVC-CPP

units contained a higher concentration of microparticles than CPP units (Figure 2C). However, the concentration of microparticles declined during storage resulting in minimal differences between the groups (Figure 2C). The post-thaw particle content of CPPs and UVC-CPPs was also measured using dynamic light scattering (Figure 2D and 2E). Consistent with the flow cytometry data, UVC-CPP units contained a higher proportion of gated microparticles (CPP: 24.3 ± 7.8 %; UVC-CPP: 35.6 ± 6.1 %; p=0.0020) than untreated CPPs.

Supernatant cytokine concentration (ng/mL) after thawing was similar in the two groups (data not shown). However, given the lower platelet count in the UVC-CPP units, the results were presented as the concentration of cytokine per platelet (Table 3). While there was a trend for increased release of cytokines in the UVC-CPPs, the difference was not statistically significant.

UVC-CPPs retain similar functionality to CPPs

Platelet aggregometry was performed to assess the functionality of CPPs. Interestingly, although low, UVC-CPPs were able to aggregate in the absence of agonist stimulation (UVC-CPP: 2.58 ± 1.32%), which was not detectable in untreated CPPs (0.55 ± 0.46%; p=0.0006). The basal aggregation level was moderately associated with the abundance of activated GPIIb/IIIa (PAC-1) (r=0.5889; p=0.0025). UVC-CPPs had higher ADP-induced aggregation at both time points (Figure 3A); while collagen-induced aggregation was similar in both groups immediately after thawing (Figure 3B). Interestingly, CPPs demonstrated a loss of ADP- and collagen-induced aggregation during storage, whilst the UVC-CPPs maintained equivalent aggregation responses to those measured immediately after thawing (Figure 3A and 3B).

The hemostatic potential of UVC-CPPs was assessed by TEG and thrombin generation. Using TEG, the time to clot formation (R time; Figure 3C) and clot strength (maximum amplitude, MA; Figure 3D) was not affected by UVC-treatment. Notably, increasing clot strength was associated with a higher abundance of GPIbα (CD42b HIP1; Figure 3E) and GPVI (Figure 3F). The kinetics of clot development (α-angle and K-time) were not affected by UVC-PI treatment and were similar to CPPs (data not shown). The ability of platelets to generate thrombin was similar between groups, with UVC-CPPs generating a similar amount of thrombin (Figure 3G) at the same rate (Figure 3H) as untreated CPPs.

Discussion

This study examined the effect of combining UVC-PI and cryopreservation on platelet *in vitro* quality and function. While cryopreservation and UVC-PI individually address challenges associated with conventional platelet storage, combining such techniques has the potential to improve the safety profile of CPPs. Although the results confirm that CPPs are activated, the combination of UVC-PI and cryopreservation imparts small additional alterations to the CPP phenotype. However, these, and other effects, became more pronounced during post-thaw storage. Importantly, their *in vitro* functionality remains similar.

The recovery of UVC-CPPs was significantly lower than CPPs after cryopreservation. This reduced recovery in UVC-CPPs was not a result of product loss during transfer through the UVC-PI illumination kit, as both untreated and UVC-PI treated platelets underwent this process. The reduction in platelet count may have arisen due to aggregate formation in UVC-CPP units after thawing.[29] The formation of aggregates during storage is undesirable as it reduces the platelet concentration, may reduce *in vitro* platelet quality, and accelerate the platelet storage lesion.[30,31] It would be of interest to determine whether modifying the thawing process could reduce or prevent aggregate formation.

Activation of GPIIb/IIIa on conventionally stored UVC-PI platelets occurs due to a reduction in disulphide bonds,[29] leading to a conformational change and increased PAC-1 binding, whereas a similar level of resting GPIIb/IIIa is maintained compared to untreated platelets.[15] The data presented here also reflects this. GPIIb/IIIa is responsible for mediating platelet aggregation through fibrinogen crosslinking.[32] Activation of GPIIb/IIIa may also be responsible for the increased *in vitro* aggregation responses seen with UVC-CPPs. Interestingly, the increased PAC-1 staining observed with UVC-CPPs correlated with the increased basal aggregation level and fibrinogen binding. Similar results have previously been described in liquid stored THERAFLEX and Mirasol-treated platelets.[33,34] Therefore, aggregate formation may be facilitated by the increased abundance of activated GPIIb/IIIa, and subsequent binding of fibrinogen from the plasma used for reconstitution.[35,36] Substituting plasma, either partially or completely, with an alternative reconstitution media, such as a platelet additive solution or saline, may reduce or eliminate aggregate formation[36] while still preserving platelet quality.[37,38]

UVC-PI treatment prior to cryopreservation did not adversely alter basal expression of platelet glycoproteins immediately after thawing. However, small changes became evident after post-thaw storage. The increased abundance of platelet receptors in both CPPs and UVC-CPPs following 24 hours of post-thaw storage suggests that certain receptors may be internalized as a result of the cryopreservation process and are later re-expressed during post-thaw

storage. Alternatively, *de novo* protein synthesis of these platelet receptors may be occurring.[39] UVC-PI treatment may damage the platelet mRNA, as has been shown with other PI systems,[40] or the ability of CPPs to synthesize proteins, resulting in altered expression of platelet receptors following 24 hours of post-thaw storage. While receptor expression improved on thawed platelets over storage, UVC-CPPs did not recover to the same extent as untreated CPPs. CPPs and UVC-CPPs demonstrated recovery of GPIbα during storage, and a reduction in the 'clustered' conformation of this receptor, which may be due to GPIbα re-expression of the receptor in a resting state following shedding. This reduction is interesting given that clustering of GPIbα is linked to enhanced clearance upon transfusion.[41] Similarly, GPVI, a primary receptor for collagen-induced platelet activation, was also re-expressed during platelet storage. This difference appeared to have little impact on collagen-induced platelet aggregation, suggesting some redundancy of GPVI signalling or that alternative collagen-binding receptors may be retained and functional.[42] Notably, the increased abundance of GPIbα (HIP1) and GPVI was positively associated with an increase in the maximum amplitude as measured by TEG. This highlights the importance of these receptors in platelet adhesion and thrombus formation.[43]

Platelet activation occurs during storage (liquid and post-thaw) resulting in the release of platelet granules with concomitant accumulation of cytokines within the platelet product.[44,45] Further, for CPPs, the plasma used to resuspend platelets may contribute to the total amount of cytokines present within each platelet unit. However, the paired CPPs and UVC-CPPs were resuspended in pooled units of plasma. Therefore, any variations in the cytokine profile are a consequence of UVC-PI treatment, the cryopreservation process, or the combination of these two processes.

The externalization of phosphatidylserine on platelets and microparticles is believed to play an important role in the overall functionality of CPPs.[13,26,46] Phosphatidylserine provides a catalytic site for assembly of coagulation factors, ultimately leading to thrombin generation.[47] Although UVC-CPPs contained a larger proportion of platelets with externalized phosphatidylserine and more microparticles, this did not translate to increased thrombin generation. It may be that such a significant proportion of CPPs express phosphatidylserine that maximal thrombin generation had been reached. Therefore, the increased phosphatidylserine exposure observed in UVC-CPPs was not able to potentiate this further. Overall, the functionality of CPPs and UVC-CPPs was similar as measured by numerous assays.

The cryopreservation protocol used in our institute is classified as an 'open' system, which limits the post-thaw shelf life of CPPs to 6 hours.[3,48] However, DMSO products are now

available in sterile bags, which would allow for aseptic addition of the cryoprotectant. The addition of DMSO to the platelet product using a closed system in combination with PI-treatment of platelets prior to cryopreservation would ideally translate to an extension of the post-thaw shelf life beyond 6 hours. Data presented here demonstrate that functional aspects of UVC-CPPs are maintained after 24 hours of post-thaw storage. However, the phenotypical alterations observed following post-thaw storage of UVC-CPPs may limit the shelf-life. A greater understanding of the impact of UVC-PI is required before an appropriate post-thaw shelf life of UVC-CPPs could be established.

This study focused on the post-thaw analysis of UVC-CPPs compared to CPPs, meaning some limitations in regards to the study design were observed. A direct comparison of liquid preserved platelets with or without UVC-PI treatment prior to freezing was not conducted due to the well-established differences between liquid-stored platelets and CPPs.[12-14,49] Further, given the minimal time between treatment and freezing, and the fact that alterations induced by UVC-PI treatment appear following storage rather than immediately following treatment,[15,16,19] no immediate UVC-PI induced changes were expected. Platelet components undergoing UVC-PI treatment are required to meet certain component specifications prior to treatment, to minimize damage. The specifications targeted in this study were different to those validated in Europe,[50] and the starting parameters of the platelet units were at the extremes of the targeted treatment specifications, suggesting that the results likely represent the worst case scenario. However, previous data demonstrate that platelet components meeting the specifications used in this study are suitable for UVC-PI treatment followed by conventional storage at 20-24 °C.[15] The results are also in line with recent reports of INTERCEPT treated CPPs, which demonstrate minimal alterations to the CPP phenotype.[21] Additionally, if the rationale for PI-treating platelets is to improve product safety, then PI-treatment of the plasma used as the resuspension medium should also be considered.

To summarize, we have investigated the impact of UVC-PI on CPP products on *in vitro* quality and function. Several differences were identified between CPPs and UVC-CPPs. The combination of UVC-PI treatment followed by cryopreservation, thawing, and resuspension in plasma resulted in the formation of persistent aggregates. However, most other *in vitro* quality and functional parameters were not immediately impacted, although more significant changes developed during post-thaw storage. Clinical studies will be required to determine whether the observed differences translate to clinically relevant effects in the overall context of conventional versus CPP products.

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Author contributions

LJ, LW and DCM conceived and designed the study; LJ and LW performed the experiments, analyzed the data and drafted the manuscript; all authors contributed to writing the paper, critically reviewed and approved the manuscript for publication.

Table 1. Platelet component specifications prior to UVC pathogen inactivation treatment

Parameter	Targeted Specifications	Platelet Components (Pre-treatment)*
Volume (mL)	325-375	364.2 ± 10.0
Platelet concentration ($x10^9$/L)	700-1400	819.6 ± 63.3
Plasma carryover (%)	25-35	27.9 ± 2.6

*Values shown as mean ± SD, n=8

Table 2. Platelet recovery and metabolic parameters of cryopreserved platelets

	Post-Thaw		Post-Storage		
	CPP	UVC-CPP	CPP	UVC-CPP	p value
Recovery (%)	69.66 ± 2.44	61.45 ± 3.00*	47.39 ± 3.37	44.08 ± 2.55	<0.0001
Platelets (x 10^9/L)	704.38 ± 74.64	620.25 ± 57.51	494.38 ± 54.23	459.25 ± 46.29	0.0428
MPV (fL)	5.16 ± 0.14	5.15 ± 0.12	5.64 ± 0.14	5.89 ± 0.23	0.0572
Glucose (mmol/L)	19.05 ± 2.83	17.27 ± 2.10	18.37 ± 2.26	17.03 ± 2.15	0.6874
Lactate (mmol/L)	5.61 ± 1.07	5.70 ± 1.11	8.42 ± 1.18	8.56 ± 1.34	0.8432
pH **(20-24 °C)**	7.24 ± 0.04	7.29 ± 0.07	7.18 ± 0.06	7.21 ± 0.08	0.1680

Post-thaw = immediately following thawing; Post-storage = 24 hours following thawing; CPP= cryopreserved platelet; UVC-CPP = UVC pathogen inactivated cryopreserved platelet; MPV = mean platelet volume

Values shown as mean ± SD, n=8 in each group

p values were obtained using a repeated measures ANOVA to compare treatment at each time point. * indicates p<0.01 compared to CPP at the same time point

Table 3. Cytokine release from cryopreserved platelets

Cytokine	Post-Thaw		Post-Storage		
	CPP	UVC-CPP	CPP	UVC-CPP	p value
sCD62P (μg/10^{11} platelets)	9.61 ± 1.15	10.12 ± 1.38	25.43 ± 3.08	27.26 ± 3.40	0.2576
RANTES (μg/10^{11} platelets)	9.08 ± 1.61	10.04 ± 1.85	23.43 ± 3.82	24.92 ± 3.13	0.2914
PDGF-AB (μg/10^{11} platelets)	2.14 ± 0.58	2.27 ± 0.39	3.57 ± 0.68	3.79 ± 0.80	0.5623
PF4 (μg/10^{11} platelets)	897.31 ± 113.41	968.85 ± 91.74	1667.41 ± 178.28	1833.41 ± 356.29	0.7433
NAP2 (μg/10^{11} platelets)	804.35 ± 112.78	896.32 ± 92.47	1436.37 ± 249.74	1737.21 ± 207.87	0.0176

Post-thaw = immediately following thawing; Post-storage = 24 hours following thawing; CPP= cryopreserved platelet; UVC-CPP = UVC pathogen inactivated cryopreserved platelet

Values shown as mean ± SD, n=8 in each group

p values were obtained using a repeated measures ANOVA to compare treatment at each time point.

Figure legends

Figure 1. Pathogen inactivation alters glycoprotein expression of platelets following cryopreservation.

Platelets were either untreated or pathogen inactivated (UVC-PI) prior to cryopreservation. Cryopreserved platelets (CPP) and pathogen inactivated CPPs (UVC-CPP) were sampled immediately following thawing (post-thaw) and 24 hours following thawing (post-storage). Platelets were stained with **(A)** CD42a-PE, **(B)** GPVI-eFluor660, **(C)** CD42b-HIP1-PE, **(D)** CD42b-AN51-PE, **(E)** CD41a-PE, or **(F)** PAC-1-FITC and the median fluorescence intensity (MFI) was measured by flow cytometry. **(G)** Scatter plot depicting the correlation between bound fibrinogen and activated GPIIb/IIIa (PAC-1). Data shown as mean ± SD (error bars) or as individual data points (scatter plots), with n=8 in each group. * indicates $p<0.01$ compared to CPPs at that time point.

Figure 2. Pathogen inactivation increases the activation status of platelets following cryopreservation.

Platelets were either untreated or pathogen inactivated (UVC-PI) prior to cryopreservation. Cryopreserved platelets (CPP) and pathogen inactivated CPPs (UVC-CPP) were sampled immediately following thawing (post-thaw) and 24 hours following thawing (post-storage). Platelets were stained with **(A)** CD62P-PE or **(B)** Annexin V-FITC and the percentage positive events was measured by flow cytometry. **(C)** The absolute number of CD61+/annexin-V+ microparticles was enumerated by flow cytometry. Data shown as mean ± SD (error bars), with n=8 in each group. * indicates $p<0.01$ compared to CPPs at that time point. Dynamic light scattering was used to assess the particle size of the platelet components, with representative traces shown for **(D)** CPPs and **(E)** UVC-CPPs immediately post-thaw. The mean size (nm) of the populations and the proportion of particles (%) within that population is presented. The peaks represent exosomes, microparticles and platelets (from left to right) within the sample.

Figure 3. Pathogen inactivation has minimal impact on platelet function following cryopreservation.

Platelets were either untreated or pathogen inactivated (UVC-PI) prior to cryopreservation. Cryopreserved platelets (CPP) and pathogen inactivated CPPs (UVC-CPP) were sampled immediately following thawing (post-thaw) and 24 hours following thawing (post-storage). Platelet aggregation was measured in response to **(A)** 20 µmol/L ADP and **(B)** 10 µg/mL collagen. **(C)** R-time (reaction time; time until clot formation) and **(D)** the maximum amplitude (maximum clot strength) were measured using thromboelastography. Scatter plots depicting the correlation between **(E)** the maximum amplitude and GPIbα (CD42b-HIP1) expression and **(F)** the maximum amplitude and GPVI expression. **(G)** Peak thrombin and **(H)** the time to peak thrombin formation were measured using a calibrated automated thrombogram. Data shown as mean ± SD (error bars) or as individual data points (scatter plot), with n=8 in each group. * indicates $p<0.01$ compared to CPPs at that time point.

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