Optimized processing for pathogen inactivation of double-dose buffy-coat platelet concentrates: maintained in vitro quality over 7-day storage

S. Ohlsson, B. Diedrich, M. Uhlin & P. Sandgren
1Department of Clinical Immunology and Transfusion Medicine (KITM), Karolinska University Hospital, Stockholm, Sweden
2Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden
3Department of Clinical Science, Intervention and Technology (CLINTEC), Karolinska Institutet Stockholm, Stockholm, Sweden

Received: 22 March 2018, revised 12 June 2018, accepted 28 June 2018, published online 29 August 2018

Background and objective Efficient pathogen inactivation (PI) offers the possibility of increasing the number of buffy coats per pool without the concurrent increased risk of pathogen transmission. Here, we describe the findings of in vitro analyses of platelets from pools of eight buffy coats treated with amotosalen and UVA light (INTERCEPT Blood System for Platelets) using INTERCEPT disposable processing sets with plastic materials sourced from alternate suppliers and split afterwards to obtain two therapeutic transfusion doses.

Methods Double-dose platelet concentrates were prepared from pools of eight buffy coats in additive solution (SSP+) using either previous 6-lead or new 8-lead pooling sets and PI processing sets in previous or alternate supplier sourced plastics (AS). Platelets were treated with the INTERCEPT Blood System then stored for up to 7 days and tested for in vitro quality.

Results All tested units (n = 30) were in conformity with European guidelines. Using AS sets more effectively maintained glucose reserves (P < 0.01), reduced lactate production (P < 0.01), reduced CD62P expression (P < 0.01) and downregulated levels of surface CD42b (P < 0.01) overtime. AS set maintained JC-positive cells (NS) between day 2 and day 7 and sustained platelet integrin activation (PAC-1) between day 2 and day 7 (NS). Overall sCD40L and PGDF accumulated in an equivalent way (P < 0.01) within series.

Summary/conclusions In summary, our data demonstrate that PI treatment using AS sets, in combination with the new pooling set for double-dose platelet preparation, maintained the platelet in vitro quality over 7 days of storage.

Key words: buffy-coat, pathogen reduction, Platelets.

Introduction

Thrombocytopenia or platelet disorders resulting from disease, therapy or injury can be supported by transfusion of pathogen-inactivated platelets, limiting patient exposure to infectious risks [1, 2] as compared to non-pathogen-inactivated platelets.

The INTERCEPT Blood System for Platelets is a Class III medical device intended for the ex vivo preparation and storage of whole blood-derived platelet-rich plasma (PRP), buffy-coat (BC) and apheresis platelet concentrates (PC). The system has been designed to inactivate a broad spectrum of viruses, bacteria and protozoa, as well as contaminating donor leucocytes. Thus, the process may reduce the risk of transfusion-associated transmission of pathogens and transfusion-associated graft-versus-host disease (TA-GVHD). The device uses amotosalen (a phototoxic active compound) and ultraviolet (UVA) illumination to photochemically treat platelets [3].
An increasing number of blood transfusion establishments adopts the INTERCEPT technology with the aim of increasing the safety of their labile blood components. At the same time, it is crucial that adoption is cost-effective and can easily be incorporated into routine use.

As an approach to allow for the treatment of larger quantities of platelets in one single pathogen inactivation procedure, the pooling of 7–8 BC has been established [4]. Platelets resulting from such a pool can be split afterwards to provide two therapeutic transfusion doses. In our institution, we have applied the concept of BC pooling for the production of BC-derived platelet concentrates since 2015. This extends the traditional approach of preparing platelet concentrates from 4 to 6 BC, generating single-dose products [4].

A limitation has been the unavailability of a platelet pooling and leukoreduction disposable set adapted to this procedure. The IPP buffy-coat platelet pooling set (Kansuk Laboratory, Istanbul, Turkey) was designed for this application, that is, to allow for larger pools of 7–8 BC in platelet additive solution (PAS), and the capability to perform leukoreduction with a filter of sufficient capacity. After this processing step, the platelet concentrate is temporarily stored in a 1-3-l bag before PI treatment.

The INTERCEPT Blood System for platelets consists of an integrated disposable plastic processing set with sterile, nonpyrogenic fluid path and INTERCEPT illuminator. Platelets suspended in 35% plasma/65% platelet additive solution (InterSol or SSP+) or platelets suspended in 100% plasma flow through the amotosalen container into the illumination container. The nominal concentration of amotosalen in the platelet mixture prior to illumination is 150 μM. Photoactivation is achieved using an illuminator. This ancillary device is microprocessor controlled and delivers a target ultraviolet A (UVA) light treatment of 3 J/cm². Photocatalyst is added to platelets by incubation with the integrated component adsorption device (CAD) before transfer of the treated platelets to one or two final containers for storage and subsequent release.

Recent changes were made to the INTERCEPT disposable sets using, in particular, plastic materials sourced from an alternate supplier (Renolit, Enkhuizen, The Netherlands) of nonpolyvinyl chloride (non-PVC) and di-(2-ethylhexyl)phthalate-free (DEHP-free) plastic sheeting for the processing and storage containers and by increasing the size of the CAD container used in the dual storage configuration from 1 to 1-3 l capacity. Disposable sets with plastic materials from alternate supplier were proven during the technical validation supporting the European conformity marking (CE Mark) approval to provide equivalent pathogen inactivation capability and postillumination amotosalen and free photoproducts removal by the CAD as sets in current plastic materials. These validations were not repeated in the study.

The IPP buffy-coat platelet pooling set designed for double-dose platelet production was evaluated in comparison with the previously used pooling set and in combination with PI processing sets manufactured with current and alternate supplier plastic materials (defined as alternate plastic set, AS). The effect on platelet quality was evaluated with in vitro storage studies.

The objective of these in vitro studies was to qualify the preparation of pathogen-inactivated double-dose BC platelet concentrates prepared with the IPP pooling set suspended in a platelet additive solution (PAS) and prepared in the AS plastics disposable sets for a maximum duration of 7 days and to compare the findings with platelet components prepared using the current disposable sets.

Materials and methods

General overview

Processing efficiency and in vitro platelet function during storage over 7 days were evaluated. Two pooling sets were used and compared for the preparation of double-dose pooled BC platelets; a routinely available pooling set (Fenwal/Fresenius Kabi, Frankfurt, Germany) used as control (PS-F), and a new pooling set adapted for double-dose (PS-K, IPP pooling set, Kansuk Laboratory, Istanbul, Turkey), both including a Sepacell™ PLX-5 leucocyte depletion filter (Asahi Kasei, Tokyo, Japan). The sets differ essentially for features important for the handling of pools of eight BC. The new PS-K set has [8 + 1] lines available for connecting the BC preparations and PAS container, while the conventional PS-F set has only [6 + 1] lines. The PS-K set has a slightly higher volume pooling bag (~700 mL) made of PVC- tri-(2-ethylhexyl) trimellitate (TEHTM) plastic versus a ~600 mL pooling bag made of PVC-DEHP for the PS-F set. The 1-3-l platelet storage containers are made of different plastics (PVC-TEHTM and ethylene-vinyl acetate (EVA) for, respectively, PS-K and PS-F). The PS-K platelet container has no infusion port as platelets are intended to be transferred after separation into the INTERCEPT dual storage (DS) processing set.

The pathogen inactivation of double-dose platelets was conducted in processing sets (Cerus Europe BV, Amersfoort, the Netherlands) either made of current plastic materials (DS-C) used as control or plastic materials sourced from alternate suppliers (DS-AS). The current plastic material for processing and storage bags is an EVA blend (PL2410) sourced from Baxter (Lessines, Belgium), compared to EVA blend (Transfufol 8300) sourced from Renolit (Enkhuizen; The Netherlands) for the DS-AS sets. The CAD bag size was increased from 1 to 1-3 l in
the DS-AS configuration. Drawings of the pooling sets and PI processing sets are shown in Fig. 1. Three combinations were tested for double-dose pooled BC platelet preparation and pathogen inactivation treatment: 1) PS-F and DS-C, 2) PS-K and DS-C and 3) PS-K and DS-AS. Combinations 1 and 2 were tested simultaneously with a pool and split design (Experiment 1), while combination 3 was evaluated subsequently (Experiment 2) as schematically described in Fig. 2.

Preparation and storage of platelet units

Whole blood (WB) units with a volume of 450 ± 45 ml were collected from normal blood donors who gave their informed consent in accordance with our institutional guidelines. The blood was stored for at least 2 hours (h) on butanediol cooling plates to reduce the temperature to approximately 20°C before further processing. BC was separated on the day of collection by a hard-spin centrifugation (2700× g for 10 min) on a Macospin centrifuge and a separation step utilizing a Macopress Smart (Macopharma, Mouvaux, France) following the blood bank standard operating procedures.

Experiment 1

Sixteen ABO identical BC’s obtained from routine production were pooled together with two PAS (SSP+) bags in the primary container of a DONOpack pooling system (LMB, Hamburg, Germany) to create a ‘maxi pool’. 

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![Fig. 1](image_url)

Fig. 1  (a) Schematic representation of current (PS-F, top) and new (PS-K, bottom) buffy-coat platelet pooling and leucocyte depletion sets. PS-K is designed for double-dose platelet preparation, ready for PR treatment, with eight tubing leads instead of six for sterile docking to buffy coats, a pooling bag size increased by approximately 15% and no transfusion ports on the final platelet storage bag. (b) Schematic representation of INTERCEPT DS processing sets made of current (DS-C, top) or alternate plastics (DS-AS, bottom), both EVA-based. The CAD bag capacity in the DS-AS configuration is increased by approximately 30%.
Connections were done using a sterile-docking system (TSCD, Terumo BCT, Tokyo, Japan). As there are six tubing leads on the ‘Octopus’ assembly of this set, sterile connections were done two or three times on the same lines. After gentle mixing, half of the ‘maxi pool’ was transferred to each of the secondary containers creating two identical pools (subpools) equivalent to eight BC’s each.

One ‘subpool’ was sterile connected to the pooling bag of the Kansuk IPP pooling and leucodepletion set (PS-K).

Fig. 2 General overview, study design. Pooling set to prepare maxi pools of buffy coats, n = 16. [Colour figure can be viewed at wileyonlinelibrary.com]
The other ‘twin’ subpool was connected to the pooling bag of the Fenwal set (PS-F).

Platelet separation and leucodepletion by filtration were performed for the two subpools using a soft-spin centrifugation (speed 1100 rpm for 9 min) on a Macospin centrifuge and a Macopress separator (Macopharma, France).

This manipulation was repeated to create eight replicates of ‘twin’ Test (PS-K) and Control (PS-F) platelet concentrates (PC) ready for PI. The requirements defined by the supplier for these platelet units to be compatible with the INTERCEPT process were a platelet dose of 2.5–8.0 x 10^11, a plasma ratio of 32%–47% and a red-blood-cell (RBC) contamination <4 x 10^9/ml.

The PC obtained with each type of pooling set were in the next step, immediately sterile-docked onto current INTERCEPT dual storage processing sets DS-C. Each platelet product was exposed to 3 J/cm^2 UVA light (corresponding for the DS set to a delivered dose of 3.9 J/cm^2) after addition of amotosalen. Following illumination, the treated units were transferred by gravity flow into the CAD container in the integrated disposable set and incubated at 22°C in a temperature-controlled incubator at 22 ± 2°C (model PC900i, Helmer, Noblesville, IN, USA). All platelet units were then split into two equal single-dose units and transferred to the PL2410 plastic storage container (Cerus) and stored for 7 days at 22°C with agitation (PC900i, Helmer).

Experiment 2

Eight ABO identical BC’s obtained from routine production were pooled with one PAS bag in the pooling bag of the PS-K. The subsequent platelet separation process was identical to the one described for Experiment 1. This manipulation was repeated to obtain 14 PS-K platelet DD concentrates ready for PI. Each PC was sterile-docked to an INTERCEPT dual storage alternate sourced plastic processing set DS-AS. The PI product entry requirements and processing steps were the same as described for Experiment 1. Tests were performed on one of the two PC-units produced from n = 14 PI-treated DD donations.

Platelet storage study and laboratory assays

Platelet samples (10 ml) were removed on days 2 (immediately following transfer from the CAD), 5 and 7 by sterile-docking a sample pouch (Fenwal Inc., Lake Zurich, IL, USA) and used to assess in vitro quality as follows.

Cellular and biochemical in vitro parameters including platelet counts (10^9/l) and 10^9/unit) and mean platelet volume (MPV) were measured using the CA 620 Cellguard (Bole Medical, Stockholm, Sweden). The extracellular lactate dehydrogenase (LDH) activity (% of total), a marker for cell disintegration, was measured with a spectrophotometric method (Sigma-Aldrich kit 063K6003; St Louis, MO, USA; Spectrophotometer Jenway 6500; Staffordshire, UK). The extracellular metabolic environment was studied by use of routine blood gas equipment (ABL 800, Radiometer, Copenhagen, Denmark) including pH (22°C), pCO₂, pO₂ (kPa at 37°C), glucose (mmol/l), lactate (mmol/l) and bicarbonate (mmol/l) and the total adenosine triphosphate (ATP) concentration (µmol/10^11 platelets), a marker of anaerobic as well as aerobic energy generation, was determined with a Luminometer (Orion Microplate Luminometer, Berthold Detection Systems GmbH, Pforzheim, Germany). Swirling was scored by visual assessment, and the white-blood-cell count (WBC) was determined with a Nageotte chamber and a microscope (Zeiss, standard, Chester, VA, USA) all according to previously described methods [5, 6].

Analysis of functional, phenotypic, mitochondrial membrane potential and biologic response modifiers

To determine the background activation level preceding stimulation as well as the cellular response capacity, the expression of a conformational epitope on the GPIIb/IIIa complex of activated platelets was assessed using the FITC-conjugated monoclonal antibody PAC-1, (IgM, Beckton Dickinson, San Jose, CA, USA) as read out. For integrin activation purpose, unfixed platelets (10^7/ml) were incubated with 20 µL ADP (Sigma-Aldrich, Schnelldorf, Germany) at 37°C for 15 min preceding the staining with PAC-1 at 20°C for 20 min in the dark. Control specimens were processed as above with the exception that they are not stimulated with ADP.

For the phenotypic analysis, platelet samples, fixed by adding an equal volume of 1% paraformaldehyde (PFA), PFA-phosphate-buffered saline (pH 7.2–7.4) at 22°C for 10 min, were then stained for 20 min at the same temperature in the dark by incubating with 20 µl of fluorochrome-labelled monoclonal antibodies (mAb) per 1 x 10^8 platelets. The following mAbs were used: fluorescein isothiocyanate (FITC)-conjugated (IgG1 isotype) CD42b (clone SZ2), phycoerythrin (PE)-conjugated (IgG1 isotype) CD62P (P-selectin/GMP-140/platelet activation dependent granule-external membrane protein; clone CLB Thromb/6) purchased from Immunotech (Beckman Coulter, Marseilles, France) and Monoclonal Anti-CD31 platelet endothelial cell adhesin molecule (PECAM-1) purchased from Sigma-Aldrich. Control specimens were processed as above but incubated with a FITC- or PE-conjugated mAb (IgG1 isotype) with irrelevant specificity, purchased from Immunotech (Beckman Coulter). After
incubation with fluorochrome-conjugated antibodies, the samples were washed twice by adding 2-0 ml filtered PBS-ethylenediaminetetraacetic acid (PBS-EDTA) and were centrifuged at 2760 g (Eppendorf 5810R) for 10 min at 10°C.

In order to avoid kinetics effects, flow cytometric analyses were immediately performed after staining, regardless of whether the cells had been fixed or not. A total of 10 000 platelet events were acquired using a FC500 cytometer; (Beckman Coulter, Villepinte, France). The flow cytometer settings were optimized for the acquisition of platelets by logarithmic signal amplification in all detectors (forward and side scatter channels and fluorescence channels FL1 and FL2). For all analysis, a gate was set around intact platelet population as defined by forward and side scatter characteristics. The percentage of positive platelets of total platelet expressing PAC-1, CD42b, CD62P and PECAM-1 above that of background (negative control) as well as the mean fluorescence intensity (MFI) was recorded. Changes in the platelet mitochondria transmembrane potential (ΔΨ) as determined by JC-1 staining were performed as recently in detail described [4].

For the BRM analysis, samples from the PCs were collected in citrate theophylline adenosine dipyridamole (CTAD) tubes, posed on the ice bath. The samples from the collected PCs were then centrifuged at 2500 g per relative centrifugal force (Eppendorf 5810R) for 30 min at 10°C, and the supernatants were stored in aliquots at – 80°C pending analysis.

CD40 Ligand/TNFSF5, PDGF-BB and TGF-β1 concentrations were determined with commercial ELISA kits (Quantikine, DCDL40, DBBOO and DB100B) in accordance with the manufacturer’s (R&D Systems Inc., Minneapolis, MN, USA) recommendations. sCD40L was prediluted 1:5 to fit the standards provided by the manufacturer. No additional samples were taken from the supernatant for detection of potential filtration of extracellular vesicles. All measurements were performed on the Spectra Max 190 Micro Plate Reader (Molecular Devices, Sunnyvale, CA, USA) at 450 nm, and the results for the sCD40L, PDGF and TGF-β concentrations are given in pg/ml.

Statistical analyses
The mean values and standard deviations are usually given unless otherwise indicated. A repeated-measures analysis of variance was used to determine whether there were differences within groups over time. Three diverse groups (1) PS-F/DS-C, (2) PS-K/DS-C and (3) PS-K/DS-AS were studied over time (days). The results are presented in Table 1, Figs 3, 4 and 5. The P-value represents the differences within groups between specific time-points (day 2 and day 7) and was considered significant at P-values of less than 0.01. The analyses were carried out using the STATISCA software, version 12, StatSoft, Inc. 1984–2013 (SPSS, Chicago, IL, USA).

Results
All PC produced for the Experiments 1 and 2 were in conformity with EDQM guidelines [9] and Karolinska University Hospital requirements with minimum 2 × 10¹¹ platelets per unit in all of the units, a residual leucocyte content < 1 × 10⁶ per unit in 100% of the units and pH (22°C) >6.4 at the end of the storage period (7 days) in 100% of the units.

For Experiment 1, a pool and split design was used to compare the two combinations PS-F/DS-C with PS-K/DS-C. The two pooling sets use the same leucocyte depletion filter and differ mostly for features relative to ease of handling, PS-K being adapted to the processing of pools of eight BC. Plastics used for the pooling bag and platelet container of the pooling sets are different. The pathogen inactivation set used was the same in these two arms. Platelet characteristics and storage data (Table 1) do not show any statistical difference for these two arms. For Experiment 2, a variation of the source of plastic materials used was introduced for the DS-AS PI processing set and the larger 1.3 l CAD processing container.

The concentration of platelets remained stable in the three series without indication of platelet lysis during storage as shown by LDH levels below 7% on average. Swirling was maintained at the maximum grade 2 throughout storage, and MPV was stable showing no alteration of macroscopic morphologic properties. There was no sign of clumping in the platelet concentrates. The pH value changed on average from 6.98 at day 2 to 7.19 at day 7 in the two series with DS-C PI sets, while a more pronounced rise in pH value from 7.12 to 7.35 was observed for DS-AS sets.

In the DS-C sets, glucose consumption correlated well with lactate production without pH decrease due to the bicarbonate buffering capacity (reserves of about 4 mmol/l at day 7) of the storage medium containing plasma and the permeability of the storage containers with stable pO₂ and decreasing pCO₂. There were sufficient glucose reserves at the end of storage (on average 1.7 and 1.5 mmol/l for the series with PS-F and PS-K both with DS-C).

However, a higher glucose concentration was observed post-PI treatment on day 2 after processing in the DS-AS set with the larger 1.3 l CAD container, averaging 6.9 mmol/l compared with 5.0 mmol/l for platelets prepared with the DS-C set and the same pooling set (PS-K). This difference was maintained throughout storage in DS-
Table 1 Cellular, extracellular, metabolic, mitochondrial depolarization and activation analysis on PS-F/DS-C platelets, n=8; PS-K/DS-C platelets, n=8; PS-K/DS-AS platelets, n=14 stored for 7 days in SS+ platelet additive solution with different pooling bags and INTERCEPT sets

<table>
<thead>
<tr>
<th>Variables</th>
<th>PS-F/DS-C platelets</th>
<th>PS-K/DS-C platelets</th>
<th>PS-K/DS-AS platelets</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DAY 2</td>
<td>DAY 5</td>
<td>DAY 7</td>
</tr>
<tr>
<td>Platelet count ((×10^9)/l)</td>
<td>1378 ± 90</td>
<td>1356 ± 108</td>
<td>1325 ± 102</td>
</tr>
<tr>
<td>Platelet content ((×10^9)/Unit)</td>
<td>282 ± 15</td>
<td>277 ± 11</td>
<td>271 ± 8</td>
</tr>
<tr>
<td>MPV [FL]</td>
<td>9.3 ± 0.4</td>
<td>9.3 ± 0.4</td>
<td>9.5 ± 0.4</td>
</tr>
<tr>
<td>LDH (% of total)</td>
<td>4.7 ± 1.3</td>
<td>4.5 ± 0.3</td>
<td>4.5 ± 1.4</td>
</tr>
<tr>
<td>pH ((22°C))</td>
<td>6.976 ± 0.107</td>
<td>7.174 ± 0.142</td>
<td>7.178 ± 0.113</td>
</tr>
<tr>
<td>Glucose [mmol/l]</td>
<td>5.1 ± 1.3</td>
<td>3.1 ± 1.4</td>
<td>1.7 ± 1.2</td>
</tr>
<tr>
<td>Lactate [mmol/l]</td>
<td>12.6 ± 2.4</td>
<td>15.6 ± 2.8</td>
<td>18.4 ± 2.4</td>
</tr>
<tr>
<td>pCO₂ [kPa at 37°C]</td>
<td>5.67 ± 0.32</td>
<td>2.60 ± 0.15</td>
<td>2.41 ± 0.14</td>
</tr>
<tr>
<td>pO₂ [kPa at 37°C]</td>
<td>19.2 ± 1.2</td>
<td>20.3 ± 0.8</td>
<td>17.2 ± 2.8</td>
</tr>
<tr>
<td>Bicarbonate [mmol/l calculated]</td>
<td>5.9 ± 1.3</td>
<td>4.3 ± 1.3</td>
<td>4.0 ± 1.2</td>
</tr>
<tr>
<td>JC-1 (%)</td>
<td>95.09 ± 2.85</td>
<td>92.70 ± 3.84</td>
<td>90.01 ± 7.65</td>
</tr>
<tr>
<td>CD62P (%)</td>
<td>21.63 ± 5.47</td>
<td>33.21 ± 4.74</td>
<td>38.99 ± 8.51</td>
</tr>
</tbody>
</table>

\(a\) P < 0.01 represents differences within each group between specific time-points (day 2 and day 7).

Values are reported as mean ± standard deviation [58].
AS sets, maintaining a higher residual glucose level (3.3 versus 1.5 mmol/l) at day 7, while lactate production was also lower (15.0 versus 18.5 mmol/l when considering the series obtained with the same pooling sets. The same observation applies to bicarbonate concentration with reserves of 5.4 mmol/l at day 7. An increased gas permeability in DS-AS sets was not evident in pO2 and pCO2 values.

The maintenance of a balanced aerobic and anaerobic glycolysis was correlated with a preserved mitochondrial membrane potential as assessed by measurements of the JC-1-positive cells throughout storage (≥90% on average for DS-C and ≥95% for DS-AS). No decline in ATP values was detected in any of the series. The two PI processing sets, that is DS-C, DS-AS, did not differ in this respect. The activation level of the platelets in the different preparations was measured by assessment of the CD62P levels and showed an increase from an average of about 22% at day 2 to 39% or PS-F/DS-C and 38% for PS-K/DS-C at day 7. This is consistent with the typical effect of storage in platelet containers and was not found to be affected by the different sets. The use of PS-K/DS AS resulted in a lower activation (from 13% postincubation in the larger CAD to 26% at the end of storage). During storage, platelets retained their ability to respond to stimulation by ADP as assessed by the surface expression of PAC-1 in DS-AS, while a significant reduction was observed for the two series with DS-C on day 7 (Fig. 3a). Base values (unstimulated) were not different (Fig. 3b).

The relative expression of phenotypic markers CD42b and PECAM-1 remains stable at 98-100% with a moderate decrease when expressed as MFI of CD42b (Fig. 4a) by about 16% and increase in PECAM-1 by about 11% (Fig. 4b). A progressive accumulation of cytokine-like biological response modifiers, namely sCD40L (Fig. 5a) and PGDF (Fig. 5b), is observed between day 2 and day 7. For TGF-β, the same pattern was found but this tends to stabilize between day 5 and day 7 for the two series with DS-C, while it appears to show an increase over this period (though not significantly different) for the series in DS-AS (Fig. 5c).

Discussion

Pathogen inactivation of platelet concentrates has the potential to reduce transfusion-related infectious transmission risks. The INTERCEPT Blood System has been in particular shown to inactivate a broad range of bacteria. Therefore, INTERCEPT-treated platelet concentrates are being used in routine in several countries including...
Sweden stored for a maximum of 7 days (official CE mark label claim). In order to have a successful implementation of a pathogen inactivation technology in routine blood bank, it is necessary to manufacture platelet components in an efficient and economical way, while maintaining adequate platelet quality postprocessing and throughout storage.

Platelet production from pooled BC combines efficiency with a reasonable cost. The INTERCEPT technology can be applied to BC platelets to mitigate the risk associated with the multidonor exposure. Traditional methods of preparation are based on pooling 4–6 BC to obtain a single-dose platelet concentrate [10]. More recently, methods were developed based on the pooling of 7–8 BC to obtain a double dose of platelets which can be PI treated with only one disposable set containing two platelet storage containers to allow for a split into two doses for transfusion [4].

Centres adopting this method initially used pooling sets designed for 5–6 BC and stored platelets to 7 days without PI. The limited number of tubing leads required the sequential sterile docking on the same inlets. The pooling bag was limited in size, while the final storage container for pooled BC platelets included transfusion ports despite the product had to be further PI treated before clinical use. To improve this situation, we evaluated a new set designed for the manufacturing of double-dose platelets for PI. Enough tubing leads are available to connect the 7–8 BC and PAS simultaneously, and the size of the pooling bag is increased (up to 700 ml). Additionally, the platelet container has been designed without an access port. The set also features a leucodepletion filter with demonstrated white-blood-cell removal capacity adequate for a double-dose product as recently demonstrated [11].

In our hands, the adaptation of these features of the pooling set to the double-dose BC process improved its ease of use. In addition, the adoption of a double-dose method overall reduces the number of disposable sets used for both platelet preparation and PI by about half with subsequent reduction in operator interventions and potential environmental impacts of plastics while strongly reducing the overall cost of a treated unit by 21% (Karolinska calculations presented at ISBT, Toronto, Canada 2018). In a side-by-side comparison of the new set adapted to the double-dose method with the conventional set used in the past, we did not notice any detrimental effect on the in vitro storage parameters of platelets after PI.

In this study, we had the opportunity to test a double-dose PI processing set made of plastic materials essentially equivalent but with gas permeability properties of the DEHP-free containers slightly improved. The supplier had validated the PI efficacy and other functionalities of the system like amotosalen and unbound photoproducts removal by the CAD (data on file at Cerus Corporation). This study evaluated potential effects of the INTERCEPT
treatment on in vitro functional, phenotypic and mitochondrial properties of DD-BC platelets during storage in SSP+ for 7 days in INTERCEPT containers with alternate sourced plastic materials and with a larger CAD container. Platelet quality was assessed using a wide range of assays designed to measure platelet metabolism, function and activation.

Despite the fact that the partial pressure of oxygen and carbon dioxide measured in the platelet concentrates were not showing significant differences, glucose consumption and lactate production were reduced in the experiments with the DS-AS and less bicarbonate was consumed, all indicative of a better-balance between the aerobic and anaerobic arms of glucose metabolism [12, 13]. Therefore, using alternate plastic set seems to counteract the slight metabolic shift towards increased anaerobic combustion of glucose/lactate production initially observed in the (PS-K) and (PS-F) units and the efficient use of glucose may provide an advantage, especially at high platelet (PS-K) and (PS-F) units and the efficient use of glucose may provide an advantage, especially at high platelet concentrations [14, 15]. All units in the study, however, met the Council of Europe recommendations (pH >6.4), with higher pH values observed for DS-AS.

The response to ADP stimulation was adequate in both bag systems but improved with DS-AS, indicating maintained cellular responsiveness relative to their activation state. Subsequently, the cell viability as assessed by JC-1-positive cells was significantly improved with the DS-AS set, which may indicate the importance of a well-balanced metabolism for platelet survival during long-term storage [10]. From the large variety of factors released from platelets [7, 8, 16, 17], the measured platelet derived factors sCD40L, and PGDF accumulated in an equivalent way in current and DS-AS set, while TGF-β concentration was significantly higher at day 7 with the DS-AS set. This observation is somewhat surprising and contradicts other parameters that unilaterally reinforce cellular benefits of using the DS-AS set. The significance of the small difference in the TGF-β concentration is difficult to interpret, but the spread is twice as high as generally detected, and only a portion of the cell population originating from Experiment 2 appears to contribute to the increased release of TGF-β, specifically at day 7. Due to very low concentration of TGF-β, this is most likely not clinically relevant.

In summary, our data demonstrate that photochemical PI of DD-BC platelet concentrates with INTERCEPT Blood System using alternate sourced plastic processing have maintained the platelet in vitro quality parameters tested in this study. The use of a platelet pooling and leucodepletion set designed for the double-dose platelet preparation method are also associated with ‘ease of use’.

Acknowledgements

The authors gratefully acknowledge support from Cerus which provided the Kansuk pooling bags and the INTERCEPT disposable processing sets with plastic materials sourced from alternate suppliers used in this study free of charge.

Conflict of interest

PS, MU, BD and SA have no conflict of interest. The study was funded within the framework of the Karolinska University Hospital’s budget for internal evaluation of material changes in current contracted systems.

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