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The quality of platelet concentrates produced by COBE Spectra and Trima Accel during storage for 7 days as assessed by *in vitro* methods

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Running head: Quality of platelets stored for 7 days

ABSTRACT

BACKGROUND: The quality of PLT concentrates (PCs) can be evaluated using various *in vitro* methods. A new technique, free oscillation rheometry (FOR), can be used to monitor coagulation properties of PCs and gives information on clotting time and coagulum elasticity. This study compared the quality of apheresis PCs produced by COBE Spectra and Trima Accel during storage for 7 days using *in vitro* tests including FOR.

STUDY DESIGN AND METHODS: Apheresis PCs were collected with the COBE Spectra (n=10) and Trima Accel (n=10) cell separators. Swirling, blood gases and metabolic parameters were analyzed on day 0. Samples taken on day 1, 5 and 7 were also analyzed for hypotonic shock response (HSR), P-selectin and GPIb expression and evaluation of coagulation by FOR

RESULTS: Swirling, HSR and percent GPIb expressing PLTs were well maintained for 7 days whereas glucose decreased and lactate increased significantly during storage for both Spectra and Trima PCs. Percent P-selectin expressing cells increased to the same extent in both types of PCs during storage. pH increased between day 0 and 1 but then decreased. The clotting time remained constant throughout the storage period whereas the development of elasticity was reduced on day 5 and 7 compared to day 1 ($p<0.05$) for both types of PCs.

CONCLUSION: The results indicate that the PLT quality after storage for 7 days is well preserved although activation of PLTs occurs during storage as assessed by *in vitro* tests. No difference in platelet quality was observed between Spectra and Trima produced PCs.

INTRODUCTION

The transfusion of single donor PLT concentrates (PCs) has increased worldwide during the last few years.[1] By apheresis (AP) technique, a large number of platelets can be collected from a single donor during one procedure leading to a reduction of donor exposures to the patients. By the AP technique, the donor's blood is processed in a cell separator with an in-line centrifuge to isolate the PLTs, which are collected in a bag while most of the plasma and the RBCs are returned to the donor. Various AP devices are available for PLT collection and they vary regarding the number of PLTs collected per volume-processed blood, collection time and number of contaminating leukocytes. It has previously been demonstrated that various cell separators induce different degree of PLT activation depending on their collection principle.[2-4]

Until recently, PCs have been stored for no more than 5 days mainly because of the risk for bacterial growth. However, with the introduction of bacterial detection systems, it is possible to extend the standard storage period from 5 to 7 days provided that the PLT function is well maintained.

The aim of this study was to assess and compare PLT function of PCs collected with COBE Spectra and Trima Accel cell separators during storage for 7 days with special emphasis on the prolonged storage time from 5 to 7 days. We used a new *in vitro* technique, free oscillation rheometry (FOR), to evaluate the PLT quality.

By FOR it is possible to monitor the coagulation process and obtain information about clotting time and coagulum elastic properties.[5,6] The elasticity (G') measured by FOR will

give information about the fibrin network of the coagulum and the clot retraction capacity[7], which is an important function of the PLTs during wound healing.

FOR analyses were performed in combination with more conventional *in vitro* tests often used to assess PLT function such as inspection of swirling, analysis of the hypotonic shock response (HSR), and detection of the PLT surface markers P-selectin and GPIb.

MATERIALS AND METHODS

PLT collection, preparation and storage

PLTs were obtained by AP technique from 20 healthy blood donors (14 males and 6 females; mean age 43 ± 11 years, range 24 - 61). PLTs were collected from 10 of the donors with the blood cell separator COBE® Spectra™ v.7.0 LRS Turbo (Gambro BCT, Lakewood, CO) and from the other 10 donors with Trima® Accel™ v.5.0 LRS (Gambro BCT). ACD (Gambro BCT) was used as anticoagulant. Contaminating leukocytes were removed by the leukocyte reduction system (LRS) of the cell separators. The donors were randomly assigned to Spectra or Trima for PLT collection.

The PLTs collected from each donor were divided on the day of donation (day 0) into units (PCs) containing >300 x 10^9 PLTs. One unit from each donation (mean platelet number of 374 ± 38 x 10^9 /unit for Spectra PCs and 358 ± 29 x 10^9 /unit for Trima PCs) was used in this study. The PCs were stored in bags integrated to the collection kit for COBE Spectra and Trima Accel on a PLT agitator (Model LPR-3, Melco Engineering, Glendale, CA or Model Helmer PF S84, Helmer, Noblesville, IN) operating at 60 cycles/min, in a Helmer incubator (Helmer PC2200) with a stable temperature of 22 ± 2 °C. The collection, preparation and storage of the PCs were done according to standard methods at the Department of Clinical Immunology & Transfusion Medicine, University Hospital, Linköping, Sweden.

Sampling

A sample was taken aseptically from each PC using a 30 mL sampling bag (Maco Pharma, Tourcoing, France) on day 0, 1, 5 and 7 for the *in vitro* analyses. pH, pO_2, pCO_2, glucose, lactate, LDH, PLT concentration and mean PLT volume (MPV) were analyzed on day 0, 1, 5 and 7. HSR, flow cytometry and FOR analyses were done on day 1, 5 and 7. Autologous

plasma was obtained by centrifugation of a sample of the PC at 2500 g for 15 min at RT. On day 1, 5 and 7 autologous plasma was frozen at $-70°$ C for later analysis of soluble P-selectin.

The dilution of the sample to various concentrations as needed for HSR, flow cytometry and FOR (see below) was based on the platelet concentration on day 1 throughout the study.

Swirling

On day 0, 1, 5 and 7 the swirling phenomenon of the PLTs in the PCs was examined by visual inspection and graded as 0, +, ++ or +++ (0 = no swirling, +++ = maximum swirling).

PLT concentration, MPV, metabolic parameters and blood gases

PLT concentration and MPV were analyzed with the instrument CELL-DYN 4000 (Abbott Diagnostics Division, Abbot Park, IL). Glucose, lactate and LDH were measured in autologous plasma with the instrument Advia 1650 (Bayer Healthcare LLC Diagnostics Division, Tarrytown, NY).

pH, pO_2, pCO_2 were measured at $37°$ C immediately after sampling using an ABL 725 instrument (Radiometer Medical ApS, Copenhagen, Denmark) or AVL Omni 6 (Roche Diagnostics Scandinavia AB, Bromma, Sweden). All the analyses were performed according to accredited methods at the Department of Clinical Chemistry, University Hospital, Linköping, Sweden.

Hypotonic shock response (HSR)

HSR, which reflects the PLTs ability to regain normal size after exposure to a hypotonic solution, was measured according to Valeri et al.[8]

In short, the PLT concentration was adjusted to 300 x 10^9 PLTs/L by dilution with autologous plasma. The measurements were done with the dual beam spectrophotometer UV-2101 PC (Shimadzu Corporation, Kyoto, Japan) at a wavelength of 610 nm. The beams were zeroed simultaneously with 0.6 mL of autologous plasma mixed with 0.3 mL of NaCl in the reference and test positions. After that 0.6 mL of diluted PC mixed with 0.3 mL of NaCl was placed in the test position and light transmission was recorded for 11 min. Finally, 0.6 mL of diluted PC mixed with 0.3 mL of water was placed in the test position and transmission was once again recorded for 11 min. The results were expressed as percent recovery at 10 min after exposure to the hypotonic solution.

PLT surface markers

PLT surface markers GPIb (CD42b) and P-selectin (CD62P) were analyzed by flow cytometry. The PLT concentration was adjusted to 300 x 10^9 PLTs/L by dilution with autologous plasma. Samples were analyzed as duplicates except for the negative controls.

For measurement of GPIb and spontaneous P-selectin expression, 10 µL of 50 mg/mL anti-CD42b-FITC (monoclonal mouse anti-human CD42b, DakoCytomation, Glostrup, Denmark) or 10 µL of anti-CD62P-FITC (polyclonal chicken anti-human CD62P, Diapensia HB, Linköping, Sweden), respectively, was added to 110 µL of HEPES buffer (137 mmol/L NaCl, 2.7 mmol/L KCl, 1 mmol/L $MgCl_2$, 5.6 mmol/L glucose, 1 g/L bovine serum albumin and 20 mmol/L HEPES, pH 7.40). As negative controls to GPIb and P-selectin 10 µL of 50 mg/mL anti-IgG2a-FITC (monoclonal mouse anti-human IgG2a, DakoCytomation) or 10 µL of anti-insulin-FITC (polyclonal chicken anti-insulin, Diapensia HB), respectively, was added to 110 µL of HEPES buffer. After addition of 10 µL of diluted PC the sample was allowed to

incubate for 20 min before the reaction was stopped by addition of 1.0 mL of HEPES buffer. The sample was then further diluted 1:20 in HEPES buffer.

For measurement of activator induced P-selectin expression, 10 µL of anti-CD62P-FITC was added to 100 µL of HEPES followed by addition of 10 µL of diluted PC. After an incubation period of 10 min, 10 µL of 0.74 mmol/L thrombin-receptor 1-activating peptide (TRAP-6, SFLLRN, Biotechnology Centre of Oslo, Oslo, Norway) was added followed by another 10 min incubation, after which the reaction was stopped and the sample diluted as described above.

Flow cytometry was performed using the instrument Epics XL-MCL (Beckman Coulter Inc., Fullerton, CA) with the computer software program Expo 32 ADC (Beckman Coulter Inc.). The fluorescence intensity was checked daily with fluorescent beads (Flow set, Beckman Coulter Inc.). 5000 events were collected based on their forward and side scatter properties. The percentage of PLTs expressing GPIb or P-selectin, normalized to the value for an irrelevant control antibody (IgG2a and anti-insulin, respectively), was determined. The mean fluorescence intensity (MFI) was expressed in arbitrary units (AU).

Clotting time and coagulum elasticity (G')

Clotting time and coagulum G' was measured by FOR using the instrument ReoRox® 4 (Medirox AB, Nyköping, Sweden). In FOR a sample cup is set into free oscillation and the change in frequency and damping of the oscillation caused by the coagulating sample is registered over time. Measurement of change in G' over time in platelet-rich-plasma samples requires the use of a reaction chamber that consists of a gold-coated sample cup with a gold-coated cylinder (bob) attached to a shaft in the center of the cup.[6,9] When using the reaction

chamber the changes occurring in frequency and damping during the coagulating phase and the clot retraction phase are registered and the change in G' is calculated from the frequency and damping data as previously described.[7] The G' is measured in the SI unit pascal (Pa).

The PLT concentration was first adjusted to 100×10^9 PLTs/L by dilution with plasma from an AB Rh D positive donor. The plasma was kept at -70 ºC until use and filtered through a 0.2 μm sterile filter (Sartorius Minisart®, Vivascience AG, Hannover, Germany) after thawing. 1.0 mL of the diluted PC sample was re-calcified with $CaCl_2$ (final concentration 0.02 mol/L), activated with TRAP-6 (final concentration 0.09 mmol/L) and 1.0 mL of the mixture was added to a gold-coated reaction chamber pre-mounted in the ReoRox® 4 using a disposable 1 mL Plastipak™ syringe (Becton Dickinson, Franklin Lakes, NJ). Samples were analyzed in duplicates at 37 °C.

The clotting time was evaluated using the ReoRox® 4 Viewer v.2.11k (Medirox AB) and was determined when the sum of change in damping and frequency reached a preset value.[9] Maximum elasticity (G'max) and the time between clotting time and G'max (time to G'max) were evaluated using an in-house designed computer program as previously described.[7] The average change in elasticity per minute (i.e. G'max/time to G'max) was calculated. A typical elasticity curve is shown in Figure 1.

Soluble P-selectin (sP-selectin)

sP-selectin was measured in plasma obtained from all PCs using an ELISA kit from R&D Systems (Minneapolis, MN) according to the instructions from the manufacturer.
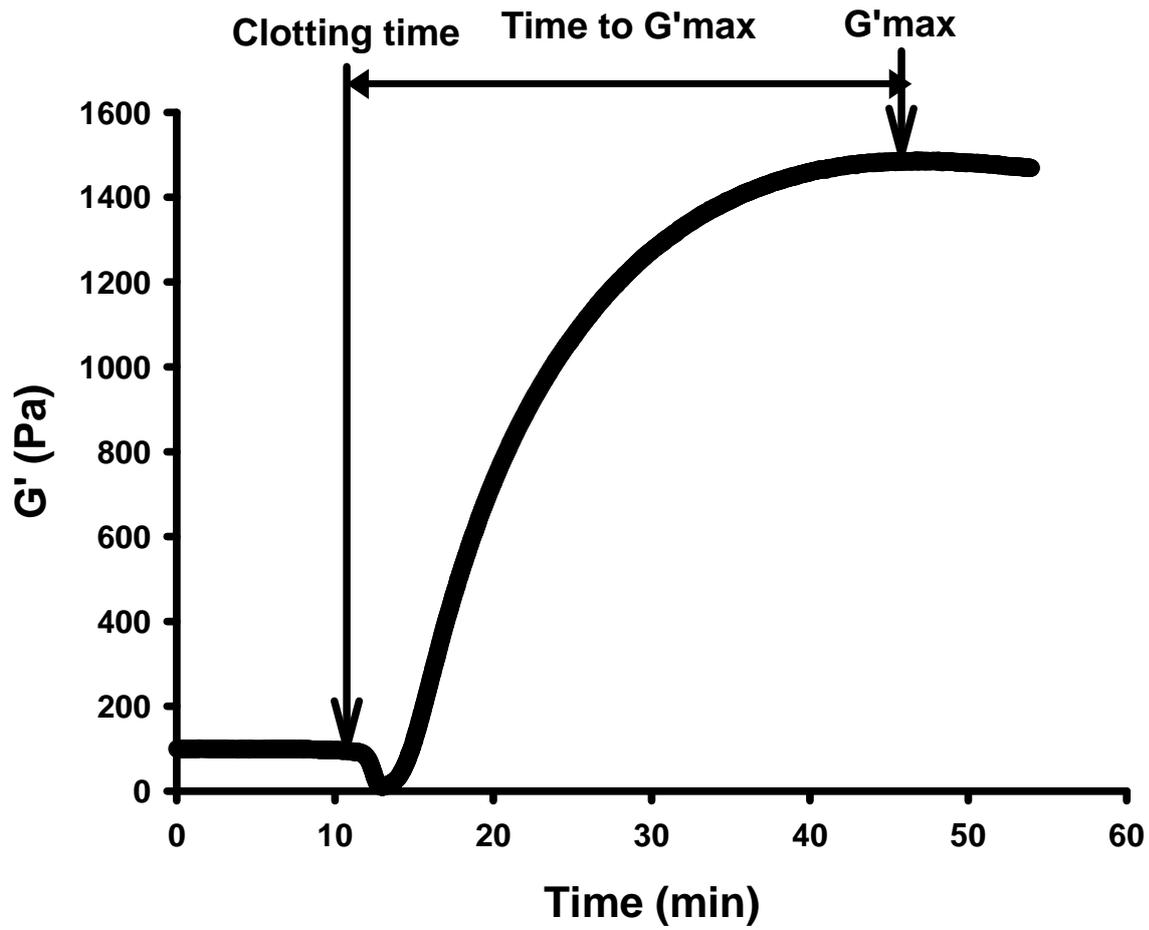
Fig. 1 A typical FOR curve. The change in elasticity (G') over time in a PLT concentrate with the clotting time, maximum elasticity (G'max) and time to G'max indicated.

Statistical analysis

The results are presented as mean ± standard deviation (SD). T-test with paired two-tailed analysis was used within each PC type for comparisons between storage days. T-test with unpaired two-tailed analysis was used for comparisons between the Spectra and Trima PCs. In addition a 3-way ANOVA analysis was performed when appropriate to evaluate changes occurring during storage and for comparison of PCs collected with the two cell separators. p-values apply to the T-test.

RESULTS

Swirling was well maintained (+++) in all PCs during the storage period of 7 days.

PLT concentration, MPV, metabolic parameters and blood gases

The PLT concentration remained constant throughout the storage period in both types of PCs. MPV was reduced from day 0 to day 1 in both types of PCs ($p<0.05$) and then remained constant day 1 to day 7.

Lactate increased and glucose decreased from day 0 to day 5 and from day 5 to day 7 for both Spectra and Trima PCs ($p<0.05$; Table 1). Lactate on day 5 was lower in PCs produced by Spectra compared to Trima PCs ($p<0.05$).

Extracellular LDH, which reflects cytoplasmic leakage, increased during 7 days of storage in PCs collected with Spectra ($p<0.05$; Table 1) whereas no significant changes could be found in PCs collected with Trima. However, there was no significant difference in LDH between Spectra or Trima PCs at any specific day.

The results of the blood gas analyses are shown in Table 1. pH was always within acceptable limits (6.4-7.4) during the storage period. There was no difference between Spectra and Trima PCs at any day.

HSR

HSR was well maintained during storage for 7 days in both types of PCs (Table 2). The HSR for Spectra PCs was slightly but significantly lower on day 7 compared to day 5 ($p<0.05$).

TABLE 1. Changes in blood gases and metabolic parameters during storage of PCs produced by COBE Spectra and Trima Accel*

Parameter	PC	Day 0	Day 1	Day 5	Day 7
pH	Spectra	$7.12 \pm 0.11\ddagger$	$7.33 \pm 0.03\dagger\ddagger$	$7.30 \pm 0.06\dagger\ddagger$	$7.21 \pm 0.09\dagger$
	Trima	$7.05 \pm 0.08\ddagger$	$7.33 \pm 0.06\dagger\ddagger$	$7.26 \pm 0.06\dagger\ddagger$	$7.14 \pm 0.10\dagger$
pO_2 (kPa)	Spectra	16.9 ± 2.2	16.3 ± 1.4	16.7 ± 1.6	17.3 ± 1.8
	Trima	$15.8 \pm 2.2\ddagger$	$15.9 \pm 2.1\ddagger$	16.8 ± 2.1	$17.5 \pm 0.8\dagger$
pCO_2 (kPa)	Spectra	$7.5 \pm 1.7\ddagger$	$4.3 \pm 0.3\dagger\ddagger$	$3.1 \pm 0.4\dagger\ddagger$	$2.8 \pm 0.3\dagger$
	Trima	$8.6 \pm 1.3\ddagger$	$4.2 \pm 0.3\dagger\ddagger$	$3.1 \pm 0.4\dagger\ddagger$	$2.7 \pm 0.3\dagger$
Glucose (mmol/L)	Spectra	$18.0 \pm 1.7\ddagger$	$17.6 \pm 1.4\ddagger$	$13.8 \pm 1.9\dagger\ddagger$	$11.5 \pm 2.0\dagger$
	Trima	$17.4 \pm 2.6\ddagger$	$16.4 \pm 2.5\dagger\ddagger$	$12.1 \pm 3.1\dagger\ddagger$	$9.6 \pm 3.3\dagger$
Lactate (mmol/L)	Spectra	$2.7 \pm 0.8\ddagger$	$3.8 \pm 1.0\dagger\ddagger$	$9.8 \pm 2.2\dagger\ddagger$	$15.1 \pm 3.2\dagger$
	Trima	$2.9 \pm 1.1\ddagger$	$4.1 \pm 0.9\dagger\ddagger$	$12.2 \pm 1.4\dagger\ddagger$	$16.6 \pm 2.7\dagger$
LDH (μkat/L)	Spectra	$2.6 \pm 0.4\ddagger$	$2.6 \pm 0.3\ddagger$	$3.1 \pm 0.8\ddagger$	$3.4 \pm 1.0\dagger$
	Trima	2.4 ± 0.3	2.3 ± 0.4	3.6 ± 3.1	3.2 ± 1.9

* Data are given as mean $\pm$ standard deviation (SD) for n=10 except pO_2 and pCO_2 on day 0 for Trima PCs where n=9 due to technical difficulties. Statistical differences between storage days are indicated within each PC type.

$\dagger$ = p<0.05 vs day 0

$\ddagger$ = p<0.05 vs day 7

TABLE 2. Changes of in vitro parameters during storage of PCs produced by COBE Spectra and Trima Accel*

Parameter	PC	Day 1	Day 5	Day 7
HSR	Spectra	84 ± 8	85 ± 6‡	81 ± 4
(%)	Trima	86 ± 7	87 ± 7	84 ± 9
sP-selectin	Spectra	55 ± 19‡	117 ± 44†‡	156 ± 56†
(ng/mL)	Trima	53 ± 15‡	118 ± 41†‡	152 ± 61†

* Data are given as mean ± standard deviation (SD) for n=10. NA=not analyzed. Statistical differences between storage days are indicated within each PC type.

† = $p < 0.05$ vs day 1

‡ = $p < 0.05$ vs day 7

Platelet surface markers

GPIb was well maintained during storage for both types of PCs (above 70 % for all PCs at any day).

The spontaneous PLT surface expression of P-selectin increased from day 1 to day 7 ($p < 0.05$) as measured by percent P-selectin expressing cells as well as MFI for both types of PCs (Figs. 2A and 2B). The surface expression of P-selectin was higher on day 7 compared to day 5 ($p < 0.05$) on PLTs collected with Trima as measured by percent P-selectin expressing cells and on PLTs collected with Spectra as measured by MFI. There was no significant difference between the two types of PCs at any specific day.
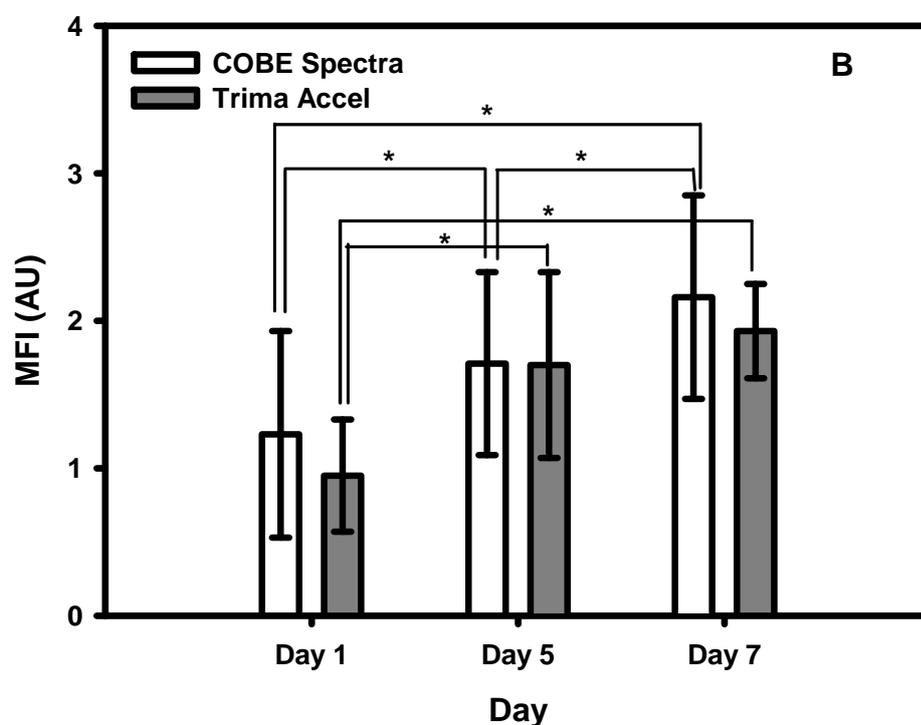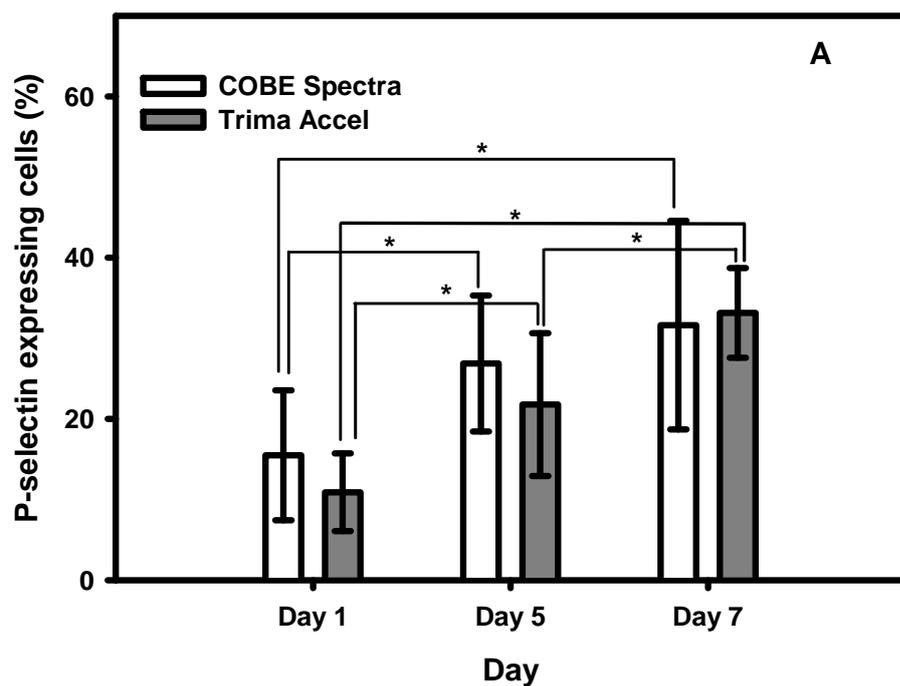
*Fig. 2 P-selectin (CD62P) positive cells as measured by percent (A) and mean fluorescence intensity (MFI; B) in PLT concentrates produced by the COBE Spectra and the Trima Accel. * indicates significant differences (p<0.05) between storage days within each PC type (i.e. day 1 vs. day 5, day 1 vs. day 7 and day 5 vs. day 7) Data are given as mean ± standard deviation for n=10.*

The TRAP-6 induced surface expression of P-selectin decreased for both types of PCs during the 7 days storage period as measured by MFI. For PLTs collected with Spectra MFI decreased from 10.30 ± 2.25 AU on day 1 to 8.43 ± 1.79 AU on day 5 ($p<0.05$) and then continued to decrease to 6.91 ± 1.65 AU on day 7 ($p<0.05$). MFI for PLTs collected with Trima was 8.44 ± 1.99 AU on day 1, 7.61 ± 1.59 AU on day 5 and 6.83 ± 1.31 AU on day 7. The decrease in MFI for Trima PCs was only significant when comparing day 1 to day 7 ($p<0.05$).

The percentage of cells expressing P-selectin following TRAP-6 stimulation remained at a high level during the storage period (>80 %). The PLTs collected with Spectra responded better to TRAP-6 activation than the PLTs collected with Trima on day 1 as measured by percent cells expressing P-selectin ($p<0.05$).

sP-selectin

sP-selectin increased from day 1 to day 7 ($p<0.05$) in both types of PCs (Table 2) with a higher level on day 7 than on day 5 in both types of PCs ($p<0.05$). There was no difference between Spectra and Trima PCs at any day.

Clotting time and coagulum elasticity (G')

The clotting time remained constant during the storage period (Table 3). The G'max and time to G'max increased from day 1 to day 5 ($p<0.05$) and then remained constant to day 7. The average change in G' per minute decreased during storage from day 1 to day 5 ($p<0.05$) and then remained constant. The FOR parameters were similar for Spectra and Trima PCs at any day.

TABLE 3. Changes of FOR parameters during storage of PCs produced by COBE Spectra and Trima Accel*

Parameter	PC	Day 1	Day 5	Day 7
Clotting time	Spectra	13 ± 2	14 ± 3	14 ± 2
(min)	Trima	15 ± 2	15 ± 2	14 ± 2
G'max	Spectra	$1486 \pm 171\ddagger$	$1655 \pm 154\dagger$	$1666 \pm 210\dagger$
(Pa)	Trima	$1468 \pm 186\ddagger$	$1617 \pm 127\dagger$	$1658 \pm 154\dagger$
Time to G'max	Spectra	$36 \pm 4\ddagger$	$43 \pm 4\dagger$	$44 \pm 4\dagger$
(min)	Trima	$34 \pm 4\ddagger$	$43 \pm 3\dagger$	$43 \pm 4\dagger$
Average G' change/min	Spectra	$42 \pm 4\ddagger$	$38 \pm 5\dagger$	$38 \pm 3\dagger$
(Pa/min)	Trima	43 ± 4	$38 \pm 4\dagger$	39 ± 4

* Data are given as mean ± standard deviation SD for n=10. Statistical differences between storage days are indicated within each PC type.

† = p<0.05 vs day 1

‡ = p<0.05 vs day 7

3-way ANOVA analysis

The ANOVA analysis confirmed that MPV, glucose, lactate, blood gases, P-selectin expression (both soluble and surface bound), response to TRAP-6 as measured as P-selectin MFI as well as the elasticity changed during storage.

The ANOVA analysis also showed that there was no difference between Spectra and Trima collected PCs during storage and that the changes that occurred during storage did not differ between Spectra and Trima PCs.

DISCUSSION

Since *in vivo* evaluation of PLT quality is difficult and expensive to perform various *in vitro* methods are often used to predict PLT recovery and viability post-transfusion as previously reviewed.[10-12] Varying *in vitro* results have previously been reported regarding the quality of PLTs collected by different cell separators [13,14] and regarding the quality of PLTs stored for 7 days.[15-18]

This study aimed at comparing the quality of PCs produced by COBE Spectra and Trima Accel cell separators and stored for a period of 7 days. The PLT quality was assessed by *in vitro* tests with special emphasis on clotting properties evaluated by a new method, FOR.

The COBE Spectra has a dual-stage channel for the separation of PLTs from other blood cells. The RBCs and WBCs are removed in the first stage while the PLTs flow into the second stage, where the PLTs are concentrated and transferred to the storage container.[19] The Trima Accel has a single-stage channel where the cells separate at maximum radius and over full channel circumference.[20] Both types of cell separator are equipped with a leukocyte reduction system (LRS).

Depletion of glucose and lactate levels above 28 mmol/L are associated with low ATP levels and a decrease in pH, which can result in loss of viability.[21-23] In our PCs glucose decreased and lactate levels increased during storage with significantly lower glucose and higher lactate on day 7 of storage compared to day 5 ($p < 0.05$). However, glucose and lactate levels were at acceptable level during the whole storage period (i.e. glucose was never depleted and lactate was always <28 mmol/L). The pH levels were well maintained at a range (pH = 6.4-7.4) which has recently been reported not to influence PLT recovery and survival.[24]

P-selectin, a marker of PLT activation, is exposed on the PLT membrane or released in a soluble form from alpha granules upon activation. Measurement of surface bound P-selectin and soluble P-selectin indicated that the PLTs were activated during the 7 days storage period. P-selectin (both surface bound and soluble) was significantly higher on day 7 of storage compared to day 5. An increase in P-selectin during storage has also previously been reported.[15,17,25,26] We could not detect any difference between PLTs collected with Spectra and Trima cell separators regarding spontaneous activation as measured by P-selectin expression. Differences in PLT P-selectin expression comparing various cell separators have been reported by others.[4,27,28] Previous studies have reported on a negative correlation between P-selectin expression and *in vivo* viability[2,15,29] but in a more recent study by Slichter et al. this correlation was not found.[25]

Hypotonic shock response (HSR), a parameter that has been shown to correlate with *in vivo* survival[30], indicated that the PLT quality was well maintained for 7 days of storage. Rock et al.[31] showed that HSR remained stable for 5 days in PCs produced by the Trima v.4.0 but at a slightly lower level (60-70%) compared to our study (80 %). Dumont et al.[26] showed reduction in HSR by 15 % during 7 days of storage to approximately 50 % in PCs produced by Spectra, which is in contrast to our results. The difference might be due to the use of different instruments for this analysis. Our result is in accordance with the findings reported by others, who also showed a well maintained HSR at a level of 80 % in PCs produced by other cell separators and stored for 5 days[13,14], and in PCs produced by the platelet-rich plasma method and stored for 7 days.[32]

We could not detect any difference between Spectra and Trima PCs regarding any of the FOR parameters. The coagulation (clotting time) and maximum clot elasticity (G'max; reflecting the clot retraction) of PCs stored for 7 days were well maintained but the longer the storage time, the longer the time to obtain maximum clot retraction (prolonged time to G'max, reduced average change in G'/min). The prolonged time to maximum clot retraction could be attributed to a reduction in the ability of the PLTs to respond to activation, as was seen in the flow cytometry analyses in which the response to the PLT activator TRAP-6 was reduced during storage. Gutensohn et al.[33] also showed an increase in elasticity of AP PCs stored for 3-5 days compared to fresh (1-2 days) PCs as measured by a technique with similarities to FOR, i.e. thromboelastography using the roTEG® instrument. This is in contrast to Rock et al.[34] who did not find any change in elasticity of PCs produced with the platelet-rich plasma method, as measured by another tromboelastograph (TEG®), during 5 days of storage. In a previous study[35] it was reported that PLTs contributed only by 55 % to the elasticity measured by thromboelastography. This is much lower than the 90 % we reported for FOR[7] and in our experience tromboelastography has a more limited measuring range than the FOR instrument ReoRox® 4.[36] Thromboelastography might therefore not be able to detect the small differences in elasticity we found between storage days. To our knowledge there are no previous reports on elasticity during storage for 7 days of AP PCs or when comparing PCs collected with various cell separators. No significant correlation could be detected between the FOR parameters and P-selectin (soluble or surface expression) or HSR. The lack of correlation is expected since quite different aspects of hemostasis and blood cells are measured by these methods.

Many of the methods used to evaluate PLT function such as flow cytometry, aggregation and adhesion (e.g. PFA-100) analyses are labor intensive, require a well-equipped laboratory and

only measure a specific PLT function.[12] FOR, in contrast, is easy to use and gives general information on the PLT ability to support clot formation and induce clot retraction. FOR might therefore be a more useful tool in predicting *in vivo* viability but this need to be further elucidated.

In conclusion we found that PCs produced by AP technique and stored for 7 days have a good quality as measured by several *in vitro* tests (e.g. well maintained HSR, swirling, pH, acceptable levels of lactate and glucose) despite PLT activation during storage. The FOR analyses clotting time and clot elasticity also showed that PLTs stored for 5 or 7 days seem to have equal clotting properties. Dumont et al.[15] found a reduction in recovery and survival after autologous transfusion of PLTs stored for 7 days compared with PLTs stored for 5 days but concluded that the magnitude of the changes would not result in diminution of transfusion efficacy in a clinical situation. These findings support a prolongation of the PLT storage time from 5 to 7 days from a functional point of view. Despite differences in collection principle, we found no major difference in PC quality comparing PCs produced by the two cell separators COBE Spectra and Trima Accel.

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