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P-selectin mRNA is maintained in platelet concentrates stored at 4°C

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Abstract

Background—Platelets (PLTs) contain mRNA and synthesize proteins in response to activation. Most guidelines for PLT concentrates (PCs) recommend ambient temperature for storage but the impact of the storage temperature on PLT mRNA content has not yet been investigated.

Study Design and Methods—Ten leukoreduced apheresis PCs were split and stored at 22 and 4°C. P-selectin mRNA, its expression on PLTs, and its soluble form were quantified. In parallel, cellular (cell count, mean PLT volume), metabolic (pH, pO₂, pCO₂, HCO₃, glucose), and functional markers (swirling, hypotonic shock response, aggregation to collagen) were analyzed. Rotation thrombelastography was used to monitor the hemostatic potential of PLTs. All measurements were performed on Days 1 and 5 of storage.

Results—After 5 days of storage at 4°C, only 31 ± 27 percent of P-selectin mRNA and 29 ± 41 percent of glyceraldehyde-3-phosphate dehydrogenase mRNA were lost, while minute amounts of the mRNAs were detectable at 22°C. In PCs stored at 4°C the percentage of P-selectin-positive PLTs was significantly higher when compared to PCs stored at 22°C. Soluble P-selectin concentrations did not significantly differ between both storage temperatures. Thrombelastography revealed significantly shorter reaction times in PLTs kept at 4°C.

Conclusion—Our data indicate that storage at 4°C is accompanied by maintained mRNA levels. PLTs with intact mRNA levels and short reaction times in thrombelastography might be functionally superior to PLTs that are devoid of mRNA and show less augmented P-selectin surface expression. In therapeutic applications, that is, if PLTs are transfused to control acute bleeding, PLTs kept at 4°C may be advantageous.

Platelet (PLT) transfusions are an indispensable tool in the management of life-threatening bleedings. Nevertheless, transfusion-transmitted diseases are still imposing a serious threat for recipients of PLT concentrates (PCs). Owing to nucleic acid testing the risk of virus transmission has been surpassed by the hazard of bacterial contamination at present. Bacterial growth in PCs is particularly supported by ambient storage temperatures. Therefore, PCs are usually discarded after 5 days. PLT storage at low temperatures could withhold pathogen proliferation and contribute to a safer transfusion policy.

Storage of PCs at low temperatures, however, is associated with rapid clearance of PLTs after transfusion.^{1–3} As a consequence, Murphy and Gardner¹ already concluded in 1969 that “the use of cold temperatures for PC storage should be abandoned.” This statement has become a paradigm; most guidelines for PCs recommend ambient temperatures for PLT storage.^{4,5}

While Becker and colleagues² confirmed the shortened circulation time, they, surprisingly, pointed out that PLTs kept at 4°C are “superior with respect to their capacity to shorten bleeding time and effect hemostasis.” For transfusion purposes these contradictory observations suggest that chilled PLTs could be efficiently used in therapeutic indications but would be less useful in prophylactic applications.

PLTs stored in the cold maintain their metabolic activity, lose swirling and shape change, respond delayed to hypotonic shock, and show differences in their aggregation capacity and in the expression of activation markers, most notably upregulation of P-selectin.^{6–11} These observations, however, have been made in buffy coat or PLT-rich plasma (PRP)-derived PCs.

It has been reported that PLTs actively synthesize proteins upon activation with thrombin (reviewed in Zimmerman and Weyrich¹²) including glycoprotein (GP)Ib, IIb, and IIIa and cytoplasmic proteins.¹³ The presence of mRNA in PLTs as a prerequisite for protein synthesis has been shown in several studies. Analysis of mRNA expression in PLTs has been performed for the description of differentially expressed genes in patients and healthy donors;¹⁴ levels of mRNA for GPIIIa have been determined to further characterize the PLT storage lesion.¹⁵ To the best of our knowledge, the influence of storage temperature on PLT mRNA levels has not yet been described.

Taken together, determination of the mRNA content in PLTs might help clarify their ability of signal-dependent protein synthesis. Hence, it might also provide new understanding of the impact of storage temperature on the functional properties of PLTs.

To gain insight into changes of PLT function at different temperatures, P-selectin mRNA, protein expression, and release were investigated in leukoreduced apheresis PCs stored at 22 and at 4°C for 5 days. In parallel, cellular, metabolic, and functional markers were determined. Rotation thrombelastography was used to monitor the haemostatic potential of PLTs.

Materials and Methods

Preparation and storage of apheresis PCs

PCs were collected from 10 healthy donors using the same automated collection system (MCS+, C5, program LDP, Haemonetics Corp., Braintree, MA) following the manufacturer’s instructions. Donors provided informed consent for standard plateletpheresis donations.

PCs were stored at room temperature for 2 hours without agitation and for 10 to 16 hours at $22 \pm 2^\circ\text{C}$ on a flatbed agitator PC incubator (PC42000i, Helmer, Noblesville, IN). Immediately before splitting, the Day 1 samples were aseptically drawn. Thereafter, the PCs

were divided into two equal parts, one stored at $4 \pm 2^{\circ}\text{C}$ and the other one at $22 \pm 2^{\circ}\text{C}$, both on a flatbed PC storage system. The second set of samples was collected on Day 5.

Analysis of cellular and metabolic variables

Blood counts and mean PLT volumes (MPVs) of donors and PCs were analyzed using an automated cell counter (Celldyn 3500, Abbott, Santa Clara, CA). The number of residual white blood cells (WBCs) was determined on a flow cytometer (FACSCalibur, Becton Dickinson Immunocytometry Systems, San Jose, CA) using tubes and reagents (TruCount and LeucoCount, respectively, both Becton Dickinson) following the manufacturer's instructions. The instrument was calibrated with beads (CaliBrite, Becton Dickinson) for fluorescence and light scatter daily before each run.

pH, oxygen tension (pO_2 , mmHg at 37°C), partial pressure of carbon dioxide (pCO_2 , mmHg at 37°C), and concentration of hydrogen carbonate (cHCO_3 , mmol/L) were measured on a blood gas analyzer (ABL 77, Radiometer, Copenhagen, Denmark). Glucose was determined on a modular analyzer (Hitachi 747, Roche, Vienna, Austria).

Functional assays

The assessment of swirling was done by visual inspection (positive/negative). For analysis of the hypotonic shock response (HSR) and aggregation, an optical aggregometer (Chronolog 490 4D, Chrono-Log Corp., Havertown, PA) was used. HSR was measured according to the technique of Holme and colleagues.¹⁶

For the assessment of the aggregation capacity we followed the manufacturer's handbook. In short, an aliquot of the PC was centrifuged at $1500 \times g$ for 15 minutes (without brake) to obtain PLT-poor plasma (PPP). An additional aliquot of the PC was diluted with an according amount of PPP to reach a PLT count of 300×10^9 per L (PRP). Baselines for PPP and PRP light transmission were manually set. Collagen (DiaColgen, DiaMed, Cressier, Switzerland) served as the aggregation agonist. A quantity of $0.77 \mu\text{g}$ of collagen ($7 \mu\text{L}$) was added to the $400\text{-}\mu\text{L}$ PRP with quick and sharp ejection. If existent, an initial PLT shape change was plotted. The aggregation curve was followed for at least 10 minutes. Maximum aggregation, the slope of the curve, and the lag time were recorded.

PLTs' clot formation profiles were assessed with rotation thrombelastography (ROTEM, Pentapharm GmbH, Munich, Germany) following the manufacturer's instructions. Since PLT counts influence the results of thrombelastography, PLT samples were adjusted to a PLT concentration of 300×10^9 per L by adding the according amount of PPP.

P-selectin assays

Aliquots of the PC were diluted 1:10 with phosphate-buffered saline. PLTs were stained either with the antibodies CD62P–fluorescein isothiocyanate (FITC) and CD41–phycoerythrin (PE) or with a monoclonal immunoglobulin G (IgG)1-FITC/IgG1-PE isotype control. All antibodies were purchased from Immunotech (Beckman Coulter, Marseilles, France). After incubation for 15 minutes in the dark, a total of 50,000 PLT events were acquired on a FACSCalibur flow cytometer. Settings were optimized for the acquisition of

PLTs by logarithmic signal amplification in all four detectors. For analysis, a gate was set around an intact PLT population as defined by forward and side scatter characteristics. The percentage of CD62P-positive PLTs above that of background (negative control) was recorded.

Soluble P-selectin concentrations were measured from PPP with a commercially available enzyme immunoassay (R&D Systems, Oxon, UK). Samples were tested in duplicate. The concentrations of soluble P-selectin were corrected for the volume of each PC and the number of PLTs collected per PC.

mRNA contents

Samples used for mRNA extraction were gel filtered on Sepharose 2B to remove contaminating WBCs. Total RNA was extracted from PLTs by an isolation system (MagnaPure, Roche) followed by reverse transcription to cDNA using random hexamer priming and the Moloney murine leukemia virus reverse transcriptase. Real-time polymerase chain reaction for the expression of P-selectin (*SELP*), glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) and CD45 (*TVI-3*) genes was performed with a light cycler (LightCycler FastStartDNAMaster SYBRGreen1 kit, Roche) following the manufacturer's instructions. Forward and reverse primers used for the *SELP* mRNA were 5'-GTCAACTACCGTGCCAACCTGCCAAG-3' and 5'-CTGTGAGGATTCAAGGGGCATTTCCTCCATC-3'. The reaction was carried out using the following thermal cycling program: one cycle of cDNA/RNA hybrid denaturation at 95°C for 10 minutes, 50 cycles of amplification of cDNA with melting at 95°C for 15 seconds, annealing at 72°C for 12 seconds, and elongation at 85°C for 1 second. Reference curves for *SELP*, *GAPDH*, and *TVI-3* copy numbers were generated with plasmids containing the corresponding sequences in a TopO2.1 vector. Results are presented as copy numbers. CD45 mRNA was determined to detect residual WBCs in each sample. Samples containing between 1 and 10 CD45 mRNA copies corresponding to 100 to 1000 WBCs per 10⁹ PLTs were considered acceptable and were used for further analysis.

Statistical analysis

All data were expressed as the arithmetic mean $\pm$ standard deviation (SD). Statistical comparisons were done with computer software (WinSTAT statistics for Windows, Version 3.0, © 2008 Robert K. Fitch, <http://www.winstat.com/default.htm>) using the Wilcoxon matched pairs. A two-tailed p value of less than 0.05 was considered significant.

Results

In this study, apheresis PCs (n = 10) were split and stored in parallel at 4 and 22°C, respectively. Over a period of 120 hours PLTs showed differences in a variety of cellular, metabolic, and functional markers as well as in their mRNA content.

Cell counts

The mean PC volume was 262 ± 36 mL, and the mean PLT content was $3.84 \times 10^{11} \pm 0.4 \times 10^{11}$. The number of residual WBCs was $0.19 \times 10^6 \pm 0.3 \times 10^6$. After storage for 5 days, PLT counts and MPV differed significantly at 4°C versus 22°C (Table 1).

Metabolic assays

Metabolic markers were in general better maintained at 4°C (Table 1): the pH ($p = 0.026$) and the bicarbonate concentration ($p = 0.003$) declined less, glucose ($p = 0.003$) was less consumed, and the oxygen tension (pO_2) ($p = 0.003$) was higher when compared to storage at ambient temperature.

Assays reflecting PLT function

Table 1 summarizes the results of the function tests of PLTs stored for 5 days at 4°C and at ambient temperature. No swirling was observed in PCs stored at 4°C, while it was maintained throughout the entire 5 days when stored at ambient temperature. After storage at 4°C, the HSR was lower ($p = 0.005$), no initial shape change before aggregation was observed, and the aggregation curve was significantly less steep ($p = 0.011$). The initial shape change was present in PLTs stored at ambient temperature. The maximum aggregation amplitudes in response to collagen did not differ significantly between the two storage conditions ($p = 0.789$). Rotation thrombelastography revealed significantly shorter reaction times in PLTs kept at 4°C ($p = 0.016$) but maximum clot firmness was not significantly different.

PLT activation assays

The glycoprotein P-selectin is stored in the alpha granules of PLTs. Upon PLT activation it is transported to the PLT surface. Simultaneously, soluble P-selectin is released into plasma. In PLTs stored at 4°C the percentage of P-selectin-positive PLTs was significantly higher when compared to PLTs stored at 22°C (Fig. 1A, $p = 0.003$). Soluble P-selectin concentrations increased under both temperature conditions during storage. Although the increase was higher in PLTs stored at 4°C compared to PLTs stored at ambient temperatures the difference did not reach statistical significance (Fig. 1B, $p = 0.213$).

mRNA contents

P-selectin and *GAPDH* mRNA was present in PCs. At 4°C, both mRNAs were kept well over 5 days (Fig. 2). Notably, P-selectin mRNA was slightly more stable than *GAPDH* at 4°C. After 5 days at 4°C the loss was 31 ± 27 percent for *GAPDH* mRNA and 29 ± 41 percent for P-selectin mRNA. Expectedly, during storage at 22°C mRNA degradation was more pronounced, and after 5 days only minute amounts were detectable.

Discussion

Our experiments intended to characterize the impact of different storage temperatures on PLT function clearly indicate a better preservation of PLT mRNA at a storage temperature of

4°C. Maintained mRNA levels in PLTs may contribute to their functionality by replenishing membrane glycoproteins and alpha granule proteins.

Transcription of remnant megakaryocytic mRNA is tightly regulated¹² and a correlation between high transcript abundance and protein expression has been observed.¹⁸ It is now well established that PLTs have the ability for de novo protein synthesis in vivo.¹³ Signal-dependent translation by activated PLTs, defined as “synthesis of protein products with important biologic activities in a rapid and sustained fashion in response to cellular activation,” is increasingly considered relevant for PLT function.¹²

A matter of concern in studies of PLT mRNAs is the contamination of PLT preparations with transcriptional active nucleated blood cells.¹⁴ Therefore, we performed leukoreduction twice before mRNA isolation. To control for WBC contamination the copy number of the WBC-specific CD45 mRNA was quantified.

In addition to quantification of P-selectin mRNA levels, surface expression and released P-selectin was determined. When compared to initial values, the percentage of P-selectin-positive and thus activated PLTs^{19–21} increased almost sixfold at 4°C, whereas at ambient temperature only a twofold increase was observed ($p = 0.003$). This is in accordance with the findings of Straub and colleagues²² and Xavier and colleagues²³ showing enhanced PLT activation in volunteers under conditions of hypothermia indicating that exposure to low temperatures leads to PLT activation. Our data confirm PLT activation upon exposure to cold temperatures in vitro and show that this activation is paralleled by maintained mRNA levels.

Soluble P-selectin is generated by proteolytic cleavage of membrane bound P-selectin and is considered a marker for PLT activation.^{24,25} In our experiments, there was no difference in the amount of soluble P-selectin secreted into the plasma during storage at 4°C (2.1-fold increase) and 22°C (2.7-fold increase; $p = 0.213$). One may hypothesize that low temperatures do not induce full PLT activation but rather lead to a state of increased reactivity that may be an advantage in therapeutic applications of PC.

When PLTs were stored at 4°C, a significant increase of MPV, a loss of shape change, and swirling were observed, while metabolic parameters (e.g., glucose) were better preserved. These observations are in line with other investigators.^{6–11} High MPV has been shown to be an independent risk factor for myocardial infarction²⁶ and thromboembolic events after primary percutaneous coronary intervention.²⁷ Presumably, this enhanced procoagulatory state indicates functional integrity of PLTs and might be beneficial in therapeutic transfusions.

We assessed the hemostatic potential of PLTs by rotation thrombelastography, an assay that analyses the entire process of clot formation, clot stabilization, and retraction.^{28–30} The reaction time, that is, the time needed to generate the first clot, was significantly shortened in PLTs kept at 4°C. Using rotation thrombelastography, Svendsen and coworkers³¹ have shown impaired hemostatic potential of PLTs during storage at ambient temperature. These results are in line with our findings.

A major concern for PLT storage at 4°C is their rapid clearance from the circulation.¹ This phenomenon is entirely separated from the hemostatic function of PLTs as demonstrated by Hoffmeister and colleagues.³ This group elucidated the mechanism of the rapid clearance of chilled PLTs and showed enhanced GPIIb/IIIa expression to be responsible. Therefore, strategies are currently investigated to modify PLTs to overcome their rapid clearance from the circulation.^{9,32–36} These approaches, however, failed to prevent rapid loss of PLTs so far. In addition, chemical alteration of the PCs may be associated with adverse reactions in the recipients.

At present, the paradigm prevails that PCs should be stored at ambient temperatures,^{4,5} the main reason being the rapid clearance of transfused PLTs that have been kept at 4°C. In our opinion, this issue deserves a differentiated view depending on whether PLTs are transfused prophylactically or therapeutically. In therapeutic indications, that is, if PLTs are transfused to control acute bleeding, PLTs kept in the cold may be advantageous. In the early 1970s, Becker and colleagues² have demonstrated the haemostatic superiority of PLTs stored at 4°C in aspirin-treated volunteers and patients suffering from hematologic malignancies. We are not aware of any further preclinical or clinical studies using untreated PCs kept at 4°C to support this finding. Our in vitro data of maintained mRNAs and short reaction times in thrombelastography during storage at low temperature indicate high functional competence of PLTs. A possible benefit of PCs stored at 4°C for the treatment of acute bleeding must be evaluated in clinical studies.

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Abbreviations

GAPDH	glyceraldehyde-3-phosphate dehydrogenase
GP	glycoprotein
HSR	hypotonic shock response
MPV(s)	mean platelet volume(s)
PC(s)	platelet concentrate(s)
PPP	platelet-poor plasma
PRP	platelet-rich plasma
SELP	P-selectin gene

References

1. Murphy S, Gardner FH. Effect of storage temperature on maintenance of platelet viability—deleterious effect of refrigerated storage. *N Engl J Med.* 1969; 280:1094–8. [PubMed: 5778424]
2. Becker GA, Tuccelli M, Kunicki T, Chalos MK, Aster RH. Studies of platelet concentrates stored at 22 C and 4 C. *Transfusion.* 1973; 13:61–8. [PubMed: 4695593]

3. Hoffmeister KM, Felbinger TW, Falet H, Denis CV, Bergmeier W, Mayadas TN, von Andrian UH, Wagner DD, Stossel TP, Hartwig JH. The clearance mechanism of chilled blood platelets. *Cell*. 2003; 112:87–97. [PubMed: 12526796]
4. Guide to the preparation, use and quality assurance of blood components. 13th ed. Strasbourg: Council of Europe; 2007.
5. Standards for blood banks and transfusion services. 25th ed. Bethesda: American Association of Blood Banks; 2008.
6. Winokur R, Hartwig JH. Mechanism of shape change in chilled human platelets. *Blood*. 1995; 85:1796–804. [PubMed: 7703486]
7. Holme S, Sawyer S, Heaton A, Sweeney JD. Studies on platelets exposed to or stored at temperatures below 20 degrees C or above 24 degrees C. *Transfusion*. 1997; 37:5–11. [PubMed: 9024483]
8. Babic AM, Josefsson EC, Bergmeier W, Wagner DD, Kaufman RM, Silberstein LE, Stossel TP, Hartwig JH, Hoffmeister KM. In vitro function and phagocytosis of galactosylated platelet concentrates after long-term refrigeration. *Transfusion*. 2007; 47:442–51. [PubMed: 17319824]
9. Hornsey VS, Drummond O, McMillan L, Morrison A, Morrison L, Macgregor IR, Prowse CV. Cold storage of pooled, buffy-coat-derived, leucoreduced platelets in plasma. *Vox Sang*. 2008 Apr 2.
10. Sandgren P, Hansson M, Gulliksson H, Shanwell A. Storage of buffy-coat-derived platelets in additive solutions at 4 degrees C and 22 degrees C: flow cytometry analysis of platelet glycoprotein expression. *Vox Sang*. 2007; 93:27–36. [PubMed: 17547562]
11. Sandgren P, Shanwell A, Gulliksson H. Storage of buffy coat-derived platelets in additive solutions: in vitro effects of storage at 4 degrees C. *Transfusion*. 2006; 46:828–34. [PubMed: 16686851]
12. Zimmerman GA, Weyrich AS. Signal-dependent protein synthesis by activated platelets: new pathways to altered phenotype and function. *Arterioscler Thromb Vasc Biol*. 2008; 28:s17–24. [PubMed: 18296586]
13. Kieffer N, Guichard J, Farcet JP, Vainchenker W, Breton-Gorius J. Biosynthesis of major platelet proteins in human blood platelets. *Eur J Biochem*. 1987; 164:189–95. [PubMed: 3830180]
14. Hillmann AG, Harmon S, Park SD, O'Brien J, Shields DC, Kenny D. Comparative RNA expression analyses from small-scale, single-donor platelet samples. *J Thromb Haemost*. 2006; 4:349–56. [PubMed: 16420564]
15. Thon JN, Devine DV. Translation of glycoprotein IIIa in stored blood platelets. *Transfusion*. 2007; 47:2260–70. [PubMed: 17714413]
16. Holme S, Moroff G, Murphy S. A multi-laboratory evaluation of in vitro platelet assays: the tests for extent of shape change and response to hypotonic shock. Biomedical Excellence for Safer Transfusion Working Party of the International Society of Blood Transfusion. *Transfusion*. 1998; 38:31–40. [PubMed: 9482391]
17. Whelan JA, Russell NB, Whelan MA. A method for the absolute quantification of cDNA using real-time PCR. *J Immunol Methods*. 2003; 278:261–9. [PubMed: 12957413]
18. Gnatenko DV, Dunn JJ, McCorkle SR, Weissmann D, Perrotta PL, Bahou WF. Transcript profiling of human platelets using microarray and serial analysis of gene expression. *Blood*. 2003; 101:2285–93. [PubMed: 12433680]
19. Snyder EL. Activation during preparation and storage of platelet concentrates. *Transfusion*. 1992; 32:500–2. [PubMed: 1502701]
20. Holme S, Sweeney JD, Sawyer S, Elfath MD. The expression of P-selectin during collection, processing, and storage of platelet concentrates: relationship to loss of in vivo viability. *Transfusion*. 1997; 37:12–7. [PubMed: 9024484]
21. Leytin V, Allen DJ, Gwozdz A, Garvey B, Freedman J. Role of platelet surface glycoprotein Ibalpha and P-selectin in the clearance of transfused platelet concentrates. *Transfusion*. 2004; 44:1487–95. [PubMed: 15383023]
22. Straub A, Breuer M, Wendel HP, Peter K, Dietz K, Ziemer G. Critical temperature ranges of hypothermia-induced platelet activation: possible implications for cooling patients in cardiac surgery. *Thromb Haemost*. 2007; 97:608–16. [PubMed: 17393024]

23. Xavier RG, White AE, Fox SC, Wilcox RG, Heptinstall S. Enhanced platelet aggregation and activation under conditions of hypothermia. *Thromb Haemost.* 2007; 98:1266–75. [PubMed: 18064324]
24. Michelson AD, Barnard MR, Hechtman HB, MacGregor H, Connolly RJ, Loscalzo J, Valeri CR. In vivo tracking of platelets: circulating degranulated platelets rapidly lose surface P-selectin but continue to circulate and function. *Proc Natl Acad Sci U S A.* 1996; 93:11877–82. [PubMed: 8876231]
25. Berger G, Hartwell DW, Wagner DD. P-Selectin and platelet clearance. *Blood.* 1998; 92:4446–52. [PubMed: 9834252]
26. Endler G, Klimesch A, Sunder-Plassmann H, Schillinger M, Exner M, Mannhalter C, Jordanova N, Christ G, Thalhammer R, Huber K, Sunder-Plassmann R. Mean platelet volume is an independent risk factor for myocardial infarction but not for coronary artery disease. *Br J Haematol.* 2002; 117:399–404. [PubMed: 11972524]
27. Sezer M, Okcular I, Goren T, Oflaz H, Nisanci Y, Umman B, Mercanoglu F, Bilge AK, Meric M, Umman S. Association of haematological indices with the degree of microvascular injury in patients with acute anterior wall myocardial infarction treated with primary percutaneous coronary intervention. *Heart.* 2007; 93:313–8. [PubMed: 16940390]
28. Carr ME Jr. In vitro assessment of platelet function. *Transfus Med Rev.* 1997; 11:106–15. [PubMed: 9140170]
29. Salooja N, Perry DJ. Thrombelastography. *Blood Coagul Fibrinolysis.* 2001; 12:327–37. [PubMed: 11505075]
30. Luddington RJ. Thrombelastography/thromboelastometry. *Clin Lab Haematol.* 2005; 27:81–90. [PubMed: 15784122]
31. Svendsen MS, Rojkaer R, Kristensen AT, Salado-Jimena JA, Kjalke M, Johansson PI. Impairment of the hemostatic potential of platelets during storage as evaluated by flow cytometry, thrombin generation, and thrombelastography under conditions promoting formation of coated platelets. *Transfusion.* 2007; 47:2057–65. [PubMed: 17958535]
32. Hoffmeister KM, Josefsson EC, Isaac NA, Clausen H, Hartwig JH, Stossel TP. Glycosylation restores survival of chilled blood platelets. *Science.* 2003; 301:1531–4. [PubMed: 12970565]
33. Wandall HH, Hoffmeister KM, Sorensen AL, Rumjantseva V, Clausen H, Hartwig JH, Slichter SJ. Galactosylation does not prevent the rapid clearance of long-term, 4 degrees C-stored platelets. *Blood.* 2008; 111:3249–56. [PubMed: 18096766]
34. Connor J, Currie LM, Allan H, Livesey SA. Recovery of in vitro functional activity of platelet concentrates stored at 4 degrees C and treated with second-messenger effectors. *Transfusion.* 1996; 36:691–8. [PubMed: 8780663]
35. Lozano M, Escolar G, Mazzara R, Connor J, White JG, DeLecea C, Ordinas A. Effects of the addition of second-messenger effectors to platelet concentrates separated from whole-blood donations and stored at 4 degrees C or -80 degrees C. *Transfusion.* 2000; 40:527–34. [PubMed: 10827254]
36. Valeri CR, Ragno G, Marks PW, Kuter DJ, Rosenberg RD, Stossel TP. Effect of thrombopoietin alone and a combination of cytochalasin B and ethylene glycol bis(beta-aminoethyl ether) N,N'-tetraacetic acid-AM on the survival and function of autologous baboon platelets stored at 4 degrees C for as long as 5 days. *Transfusion.* 2004; 44:865–70. [PubMed: 15157253]

Fig. 1.

(A) Increased percentage of P-selectin–positive PLTs stored at 4°C. Box plots indicate the percentage of P-selectin–positive PLTs in PCs on Days 1 and 5 after storage at 4 and 22°C. (B) Storage temperature does not influence soluble P-selectin levels. Box plots show soluble P-selectin levels as measured on Days 1 and 5 after storage at 4 and at 22°C.

Fig. 2. mRNA levels depend on storage temperature. Copy numbers of P-selectin (A) and *GAPDH* mRNA (B) on Days 1 and 5 after storage at 4 and at 22°C are shown.

Table 1
PLTs' in vitro characteristics dependent on storage temperature (n = 10)*

Characteristic	Day 1, 22°C	Day 5, 4°C	Day 5, 22°C
PLT concentration (G/L)	1476 ± 106	1338 ± 147 [†]	1474 ± 106
Mean PLT volume (fL)	7.03 ± 1.0	7.28 ± 0.9 [†]	7.62 ± 0.9
pH (22°C)	7.42 ± 0.14	7.36 ± 0.05	7.21 ± 0.17
pO ₂ 37°C (mmHg)	120 ± 19	169 ± 15 [†]	141 ± 14
pCO ₂ 37°C (mmHg)	35 ± 7	22 ± 2 [†]	16 ± 2
cHCO ₃ (mmol/L)	14.3 ± 1.0	7.6 ± 1.2 [†]	4.4 ± 1.7
Glucose (mg/dL)	373 ± 34	325 ± 34 [†]	266 ± 28
Swirling (yes/no)	Yes	No	Yes
HSR (%)	92 ± 13	45 ± 16 [†]	62 ± 16
Shape change (yes/no)	Yes	No	Yes
Aggregation collagen			
Maximum amplitude (%)	89 ± 11	74 ± 12	77 ± 11
Collagen (slope)	134 ± 25	64 ± 27 [†]	97 ± 28
Thrombelastography			
Maximum clot firmness (mm)	70 ± 8	67 ± 4	68 ± 4
Reaction time (sec)	710 ± 129	650 ± 83 [†]	752 ± 149
Clot formation time (sec)	219 ± 38	207 ± 40	205 ± 67
Alpha angle (°)	57 ± 4	57 ± 5	58 ± 8

* Results are expressed as mean ± SD.

[†] p < 0.05 for PLTs stored at 4°C versus 22°C on Day 5.