



Title	Survival and recovery of apheresis platelets stored in a polyolefin container with high oxygen permeability
Author(s)	Ezaki, Shoji; Kanno, Takahiro; Ohto, Hitoshi; Herschel, Louise; Ito, Takatoshi; Kawabata, Kinuyo; Seino, Osamu; Ikeda, Kazuhiko; Nollet, Kenneth E
Citation	Vox Sanguinis. 94(4): 292-298
Issue Date	2008-05
URL	http://ir.fmu.ac.jp/dspace/handle/123456789/18
Rights	Journal compilation © 2008 Blackwell Publishing Ltd. The definitive version is available at www.blackwell-synergy.com
DOI	10.1111/j.1423-0410.2008.01042.x
Text Version	author

This document is downloaded at: 2023-03-28T11:11:13Z

Survival and recovery of apheresis platelets stored in a polyolefin container with high oxygen permeability

A running short title: High oxygen permeable platelet storage container

Shoji Ezuki^{1,2}, Takahiro Kanno¹, Hitoshi Ohto¹, Louise Herschel³, Takatoshi Ito², Kinuyo Kawabata¹, Osamu Seino⁴, Kazuhiko Ikeda¹, and Kenneth E. Nollet¹

¹Division of Blood Transfusion and Transplantation Immunology, Fukushima Medical University Hospital, Fukushima, Japan,

²Kawasumi Laboratories, Inc., Tokyo, Japan,

³Cell Labeling Laboratory, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire, USA,

⁴Department of Radiology, Fukushima Medical University School of Medicine, Fukushima, Japan.

Address correspondence to: Hitoshi Ohto, MD, PhD, Division of Blood Transfusion and Transplantation Immunology, Fukushima Medical University Hospital, Hikariga-oka, Fukushima City, Fukushima 960-1295, Japan

Telephone: +81-24-547-1536; Fax: +81-24-549-3126; e-mail: hit-ohito@fmu.ac.jp

Conflict of interest statement

Shoji Ezaki is an employee of [Kawasumi Laboratories, Inc.](#), Tokyo, Japan. We declare that we have no conflict of interest.

Abstract

Background and Objectives Oxygen permeability is important in platelet storage media. We compared a new polyolefin container with enhanced oxygen permeability (PO-80, Kawasumi, Tokyo, Japan) to a widely used alternative (PL2410, Baxter Healthcare, Illinois, USA).

Materials and Methods *In vitro* characteristics of paired platelet concentrates (PCs, mean 4.2×10^{11} /250 ml plasma/bag) stored in PO-80 or PL2410 were assessed through nine days of storage. *In vivo* recovery and survival of seven-day-old autologous PCs were assessed according to the Murphy method.

Results Laboratory assessment of platelet quality favored PO-80 during nine days of storage with statistically significant differences in glucose consumption (2.75 vs. 4.93 mmol/ 10^{12} /24hrs in the interval 120-168 hrs), lactate generation (4.37 vs. 8.11 mmol/ 10^{12} /24hrs in the interval 120-168 hrs), pO₂ (59.3 vs. 38.1 mmHg at day 1), and HCO₃⁻ (14.7 vs. 13.4 mmol/L at day 1). Statistically significant differences were not seen in aggregation, hypotonic shock response, or pH. *In vivo* assessment of autologous platelets stored seven days in the PO-80 container revealed that recovery was 82.1% and survival was 81.0% of fresh control. Seven-day-stored PCs in PO-80 were shown *in vivo* to be noninferior to fresh platelets, with upper confidence limits (UCL₉₅) in recovery and survival of stored PCs below the maximum acceptable difference (MAD); 15.3% UCL₉₅ < 20.4% MAD and 2.1 days UCL₉₅ < 2.1 days MAD.

Conclusions The *in vitro* characteristics of PCs stored in a highly oxygen-permeable container were stable at least seven days. The *in vivo* study supports the suitability of PO-80 for seven-day platelet storage.

Ezuki et al

Keywords: platelet storage, *in vitro* platelet quality, *in vivo* platelet recovery, platelet survival, kinetics

Introduction

Modern medical practices have increased the demand for platelet transfusion. Moreover, the ageing population of many developed countries tends to increase the demand for platelets while decreasing the potential supply. In concert with more effective donor recruitment and increased collections, it seems prudent to extend the storage period of platelets, provided that safety and efficacy are not compromised. In some countries, the widely accepted five-day storage time has been extended to seven days with the introduction of bacterial screening systems [1, 2]. The threat of episodic platelet shortages provides a strong motivation to investigate technologies that might safely and efficaciously extend platelet shelf life.

If the pH of platelet concentrates (PCs) at 20-24 °C falls below 6.2, viability *in vivo* significantly decreases [3]. Thus, a European standard is to maintain pH at or above 6.4 [4]. Hypoxic metabolism provokes a fall in pH due to lactic acidosis; lactic acid displaces bicarbonate and an efflux of CO₂ occurs [5]. Since CO₂ is produced both as a product of oxidative metabolism and as a result of disappearance of bicarbonate buffer, too low a level of pCO₂ may diminish the buffer capacity. Therefore, better oxygen and adequate carbon dioxide gas exchange may slow the platelet storage lesion and improve PC shelf life. However, multi-laboratory examinations show no correlation between high pH and *in vivo* recovery [6].

Our laboratory previously demonstrated that a polyolefin container with high oxygen and adequate carbon dioxide permeability (PO-80, Kawasumi Laboratories, Tokyo, Japan) can preserve *in vitro* characteristics of platelets, including pH, pCO₂, and lactate, better than an alternative container (KBO-PO, Kawasumi) during storage for 7 days [7]. Here, we report *in vitro* effects of platelet storage for up to 9 days, comparing

PO-80 with another polyolefin, PL2410 (Baxter Healthcare, Deerfield, Illinois, USA). Next, to assess the clinical utility of PO-80, we recruited healthy volunteers to compare *in vivo* survival and recovery of autologous platelets stored in PO-80 for 7 days with fresh platelets manually separated from whole blood [8] and radiolabelled with either ^{111}In or ^{51}Cr . This is one of only a few platelet studies to date in which the Murphy method [9] has been properly executed, analyzed, and reported.

Materials and methods

Donors

Following a protocol approved by the Institutional Review Board of Fukushima Medical University, healthy donors were enrolled after informed consent was obtained and documented. Donor health histories were unremarkable and none had taken any medication known to affect platelet function within 10 days of donation.

In vitro assay

Apheresis PCs were collected from 12 healthy donors using the Amicus cell separator (Baxter Healthcare) configured for double-needle access. PCs collected from two donors with the same ABO blood type were pooled using a sterile connecting device (TSCD; Terumo, Tokyo, Japan) and divided equally into PO-80 and PL2410 (n=6). Each bag contained 250 mL of plasma and a mean of 4.2×10^{11} platelets. The oxygen permeabilities of PO-80 and PL2410 were, by our measurements, 2.660 L/m²/day/atm and 2.024 L/m²/day/atm, respectively. The capacity of each bag was 1.0L.

The characteristics of platelets stored for up to 9 days at 20-24 °C with agitation at 50–60 strokes/minute on a flat shaker (PC900i with PF48i, Helmer, Noblesville, Indiana, USA) were evaluated on days 0, 1, 3, 5, 7 and 9 of storage. Each bag was sampled with a syringe 6 times (7 mL per sample) during 9 days of storage. Platelet counts and mean platelet volume (MPV) were determined using an electric cell counter (Sysmex K-2000; TOA, Kobe, Japan). Hypotonic shock response (%HSR) and the degree of aggregation were determined as previously written [7]. The pH, pO₂, pCO₂, and HCO₃⁻ of the PCs were measured at 37 °C using a pH/blood gas analyzer (ABL3, Radiometer, Copenhagen, Denmark). The pH measured at room temperature was

automatically calculated as the pH at 37°C. Swirling degree was estimated visually with a light source and graded from 0 (no swirling) to 2+ (optimal swirling).

To confirm sterility, all PCs were cultured on day 9 for bacteria and fungi in two liquid media, namely, BACTEC Plus Aerobic/F and Plus Anaerobic/F (Becton Dickinson, Sparks, Maryland, USA).

In vivo assay

A minimum sample size of 7 has been required to demonstrate noninferiority of stored PCs to fresh platelets [8]. In this study, 8 healthy donors gave PCs by apheresis: 5 using the Amicus and 3 using the COBE Spectra (Gambro, Lakewood, Colorado, USA). Both cell separators were configured for double-needle access. PCs were collected in PL2410 and ELP bags. Within 2 hours of collection, products were transferred into PO-80 bags and stored for 7 days in the same manner as the *in vitro* study. Two systems (eBDS, Pall Corporation, East Hills, New York; and BacT/ALERT, bioMerieux, Marcy l'Etoile, France) were used to detect the presence of bacteria. The BacT/ALERT system was used for sampling 24 hours after collection, and sample bottles (aerobic and anaerobic) were taken from each aliquot to ensure sterility. The eBDS system was used for sampling 48 hours before the end of storage, and its sample pouches were connected to the tubing of the PC bags with a TSCD. All the samples were double-checked using both bacterial detection systems. The platelets in PO-80 were labeled with radioisotopes on day 7.

To prepare fresh platelets [8, 10], whole blood was drawn into an ACD-A bag. Carefully prepared fresh platelet pellet was gently resuspended in ACD saline before labeling. Fresh (within 6 hours of collection) and stored (7 day) platelets were

radiolabeled using standard techniques before reinfusion into the original donor [10]. The radiolabel $\text{Na}_2^{51}\text{CrO}_4$ (Daiichi Radioisotope Laboratories, Tokyo, Japan) or ^{111}In -oxine (Nihon Medi-physics, Kobe, Japan) was added to the platelet suspension, which was then incubated at room temperature for 20 - 30 minutes. Isotope assignments for fresh and stored PCs were alternated randomly. By using a dose calibrator, about 20 μCi of each platelet suspension labeled with ^{111}In or ^{51}Cr was reinfused into the donor. Blood samples were taken from the contralateral arm 15 minutes, 1 hour, and 3 hours after infusion, as well as daily for 1 week and again on day 10 (to allow for the correction of activity associated with RBCs). The radioactivity of the samples was measured using a gamma counter (Autowell Gammastem, ARC-370M, ALOKA, Tokyo, Japan). Recovery rate and survival duration were determined using the multiple hit model [8].

To evaluate statistically the effectiveness of PO-80 for platelet storage, a noninferiority hypothesis test with two-stage analysis was performed [11]. An upper confidence limit (UCL_{95}) was calculated as shown:

$$\text{UCL}_{95} = \delta_{(\text{control}-\text{test})} + t_{\alpha, \text{d.f.}} (sd/\sqrt{n})$$

where $\alpha=0.05$; d.f. (degrees of freedom) and $t_{0.05, (\text{control} - \text{test})}$ were obtained from the software package SYSTAT, version 11 (HULINKS Inc., Tokyo, Japan).

The maximum acceptable difference (MAD) was determined as follows:

$$\text{MAD} = \bar{X}_{\text{control}} - \bar{X}_{\text{control}} \times 0.667$$

If the UCL_{95} is less than the MAD, investigators may reject the null hypothesis (i.e., test platelets are inferior to control platelets) and may make a strong statement with 95% confidence [11]. Murphy [9] has proposed a criterion that test platelets retain at least

66 % of the control recovery rate and the survival time should be at least 50% of the control.

Statistical analysis

Data analyses were performed with SYSTAT and STATMATE III (Advanced Technology for Medicine & Science, Tokyo, Japan). Data were expressed as mean \pm SD. The paired t-test (two-tailed) or G-test with William's correction was used to compare the values of the components, with $p < 0.05$ considered statistically significant.

Results

In vitro assay

Platelet count and MPV remained nearly constant in both bags stored for 9 days. As shown in Table 1, the pO₂ of PCs decreased in both bags on day 1; however, the amount of decrease in PO-80 was significantly smaller than that in the control bag (p < 0.01) on days 1 and 3 (not shown). The pCO₂ of PCs in both bags continuously decreased during storage. The pCO₂ and HCO₃⁻ in PO-80 decreased more slowly than that in PL2410, achieving statistical significance on day 1 (p < 0.05).

Plasma glucose levels steadily decreased in both bags. The rate of glucose consumption in PO-80 (2.75 mmol/10¹²platelet/24hrs) was, however, slower (p < 0.02) than in PL2410 (4.34 mmol/10¹²platelet/24hrs) during the interval 120 to 168 hrs. The rate of lactate generation in PO-80 (4.37 mmol/10¹²platelet/24hrs) was also slower (p < 0.05) than in PL2410 (8.11 mmol/10¹²platelet/24hrs) during the interval 120 to 168hrs, but not statistically significant in other intervals (Table 1).

The degree of platelet aggregation induced by double stimuli, namely, ADP and collagen, decreased gradually and similarly in both bags for up to 3 days. Platelet aggregation in PO-80 was preserved better than that in the control bag on day 5, although the tendency was not significant on days 7 and 9. Storage also reduced %HSR over time, without significant difference between the bags. P-selectin expression increased in both bags during storage. The amount of increase in PO-80 tended to be smaller than that in the control bag, but did not significantly differ thereafter (Table 1).

The average pH of PCs stored over 9 days in PO-80 vs. PL2410 showed no significant differences by paired-T test (Table 1). The values favored PO-80, however, with a statistical difference (p < 0.05) by G-test on day 7; of 6 bags in each cohort, none

of the PO-80 bags and 3 of the PL2410 bags had a pH fall below 6.2 up to day 7 (Table 2).

Swirling scores also favored PO-80, but with no statistical difference, on days 7 and 9 (Table 2).

In vivo assay

The pH of all PCs stored in PO-80 for *in vivo* study was above 6.8 on day 7, and swirling was preserved for 7 days (Table 3). No bacteria were detected through day 7, when the radiolabeled stored and fresh platelets were simultaneously infused into the same donor. The recovery rate of stored platelets for 7 days of storage was 50.3 ± 13.4 %, whereas fresh platelets was 61.2 ± 13.0 % ($p < 0.01$, Table 4). The average recovery of 7-day-stored platelets relative to that of fresh platelets was 82.1 ± 13.2 % [95% CI; 74.0 - 89.4 %]. The average survival of 7-day-stored platelets was 6.3 ± 1.2 days, whereas that of fresh platelets was 7.8 ± 1.1 days ($p < 0.01$). The survival rate of stored platelets relative to that of fresh platelets averaged 81.0 ± 12.8 % [95% CI; 72.2 - 90.0 %].

In the recovery estimation, the $UCL_{95, \text{recovery}}$ (15.3%) of platelets stored in PO-80 was not more than the MAD_{recovery} (20.4%). Likewise, in the survival time evaluation, the $UCL_{95, \text{survival}}$ (2.1 days) was not more than the MAD_{survival} (2.6 days). These values reject the null hypothesis, and indicate that 7-day platelets stored in PO-80 are not inferior to those of the control platelets.

Discussion

In this study, we found that the PO-80 container maintained suitable pO₂ and pCO₂ values, with a smaller decrease in pO₂ than the PL2410 container, suggesting an advantage of the PO-80 container for minimizing platelet storage lesion [12]. Internal pO₂ is affected by the permeability and surface area of a container, as well as by the metabolism of platelets and any contaminant organisms. The present results suggest that the increased oxygen permeability of PO-80 helps maintain a higher internal pO₂, particularly over the first three days. A higher pO₂ promotes aerobic glucose metabolism in platelet mitochondria [5,13,14]. Better aerobic metabolism in PO-80 might be inferred from the smaller decrease in glucose level and smaller increase in lactate level in PO-80 vs. PL2410 (day 1, p <0.01). Alternatively, activation of platelets would likely increase their metabolism, and higher P-selectin values were observed in PL2410 vs. PO-80.

To obtain better gas transfer rates in storage containers for platelets, there are three strategies: 1) enlarge the bag to increase its surface area; 2) decrease the membrane thickness to increase gas permeability; and 3) improve membrane materials. These strategies have been employed to various degrees in commercially available apheresis PC containers, including: PL2410; CLX (PVC plasticized with tri-2-(ethylhexyl)trimellitate; MedSep Corp., Covina, California, USA); and ELP (PVC plasticized with *N*-butyryl tri-*n*-hexyl citrate; Gambro BCT).

At a pH below 6.2 or above 7.6, the viability of platelets *in vivo* is supposed to decrease [3], suggesting the importance of pH in maintaining the quality of PCs. Recent multilaboratory research, however, shows no relationship between an *in vitro* pH of 6.2 or more, and *in vivo* platelet viability, as determined by recovery and survival of

radiolabeled autologous platelets [8]. There are several reports [15,16,17] on the usefulness of CLX and ELP containers for the long-term storage of PCs, using pH as an index. PCs at high concentrations ($4.0 - 5.0 \times 10^{11}$ platelets/250 mL of plasma) stored in PL2410 showed an average pH of 6.4 on day 7 [18], which fell below 6.2 on day 8 [19]. When the surface area of PL2410 was increased to accommodate a volume of 1.3 L, the PCs maintained a pH > 6.8 for 7 days [20], indicating that a large surface area for gas transfer helps preserve pH. In our *in vitro* study, only one PO-80 bag showed a pH of 6.01 on day 9. With PL2410, three containers showed pHs of less than 6.1 on day 9. Eight PO-80 samples in the *in vivo* study were higher than pH 6.8 on day 7. Therefore, PCs stored in PO-80 almost always satisfy the pH criterion for clinical use for at least 7 days, or possibly 9 days of storage.

We found that platelet function is preserved moderately better in PO-80 than in PL2410, especially in the aggregation test on day 5, although there were large variations in the aggregation test and %HSR. Alternatively, it has been reported that a pH of 6.0 to 6.2 marks the threshold at which expression of P-selectin leads to an irreversible shape change and poor *in vivo* viability [4,21]. Thus, we believe that PO-80 may be capable of storing platelets in plasma for up to 9 days without inducing major damage.

In this study, the recently proposed Murphy method [3,6,8,9,11] was applied to evaluate experimental and control arms with calculation of an upper confidence interval for non-inferiority. Stored PCs were shown to be noninferior to freshly prepared PCs with 95% confidence [11]. As sample size calculations to demonstrate noninferiority suggest a minimum sample size of 7 [11], we investigated the effect of long-term storage on PCs from eight donors, and found that 7-day-stored platelets had recovery and survival rates that compared favorably with freshly separated platelets, meeting the

criterion promulgated by Murphy *et al* [3,9,11]. Although not higher than survival and recovery previously reported by AuBuchon *et al* [8] for an ELP container, neither the volume of the container nor the volume of plasma used for the platelet suspension was explicitly mentioned in that study. Our results for PO-80 were obtained using highly concentrated PCs.

In conclusion, the viability of the PCs stored in the highly oxygen permeable container were stable for a minimum of 7 days storage, suggesting that PO-80 is sufficient for storing PCs for 7 days with good quality.

Acknowledgments

This study was supported by a grant from the Ministry of Health, Labour and Welfare of Japan and funded partly by Kawasumi Laboratories, Inc.

References

1. Dumont L, AuBuchon J, Whitley P, Herschel LHJ, Johnson A, McNeil D, Sawyer S and Roger JC: Seven-day storage of single-donor platelets: recovery and survival in an autologous transfusion study. *Transfusion* 2002; 42: 847-854.
2. Dumont L and VandenBroeke T: Seven-day storage of apheresis platelets: report of an in vitro study. *Transfusion* 2003; 43: 143-150.
3. Murphy S, Rebullia P and Bertolini F: In vitro assessment of the quality of stored platelet concentrates. The BEST (Biomedical Excellence for Safer Transfusion) Task Force of the International Society of Blood Transfusion. *Transfu Med Rev* 1994; 8: 29-36.
4. Kunicki TJ, Tuccelli M, Becker GA and Aster RH: A study of variables affecting the quality of platelet stored at room temperature. *Transfusion* 1975; 15: 414-421.
5. Kilksen H, Holme S, and Murphy S. Platelet metabolism during storage of platelet concentrates at 22 °C. *Blood* 1984;64: 406-414.
6. Dumont LJ, AuBuchon JP, Gulliksson H, Slichter SJ, Elfath MD, Holme S, Murphy JR, Rose LE, Popvsky MA and Murphy S: In vitro pH effects on in vivo recovery and survival of platelets: an analysis by the BEST Collaborative. *Transfusion* 2006; 46: 1300-1305.

7. Yuasa T, Ohto H, Yasunaga R, Kai T, Shirahama N and Ogata T: Improved extension of platelet storage in a polyolefin container with higher oxygen permeability. *Br J Haematol* 2004; 126: 153-159.
8. AuBuchon JP, Herschel L, Roger J: Further evaluation of a new standard of efficacy for stored platelets. *Transfusion* 2005; 45: 1143-1150.
9. Murphy S. The case for a new approach for documenting platelet viability. *Transfusion* 2006; 46(Suppl):49S-52S.
10. The Biomedical Excellence for Safer Transfusion (BEST) Collaborative. Platelet radiolabeling procedure. *Transfusion*, 2006;46(Suppl), 59S-66S.
11. Dumont, L.J. Analysis and reporting of platelet kinetics studies. *Transfusion*, 2006;46(Suppl), 67S-73S.
12. Seghatchian J and Krailadsiri P: The platelet storage lesion. *Transfu Med Rev* 1997; 11: 130-144.
13. Cardigan R, Turner C and Harrison P: Current methods of assessing platelet function: relevance to transfusion medicine. *Vox Sang* 2005; 88: 153-163.
14. Suzuki H, Yamazaki H and Tanoue K: Immunocytochemical aspects of platelet membrane glycoproteins and adhesive proteins during activation. *Progress in Histochemistry and Cytochemistry* 1996; 30: 1-106.

15. Krailadsiri P, Seghatchian J and Williamson LM: Platelet storage lesion of WBC-reduced, pooled, buffy coat-derived platelet concentrates prepared in three in-process filter/storage bag combinations. *Transfusion* 2001; 41: 243-250.
16. Shanwell A, Diedrich B, Falker C, Jamsson B, Sandgren P, Sundkvist L, Svensson L, Vesterinen M and Gulliksson H: Paired in vitro and in vivo comparison of apheresis platelet concentrates stored in platelet additive solution for 1 versus 7 days. *Transfusion* 2006, 46, 973-979.
17. Meer P, Pietersz R and Reesink H. Leucoreduced platelet concentrates in additive solution: an evaluation of filters and storage containers. *Vox Sang* 2001; 81: 102-107.
18. Wildt-Eggen Jd, Schrijver JG, Bouter-Valk HJ, Fijnheer R, Bins M and Van Prooijen HC: Improvement of platelet storage conditions by using new polyolefin containers. *Transfusion* 1997; 37: 476-481.
19. Vassallo RR, Murphy S, Einarson M, Nixon J and Ziegler: Evaluation of platelets stored for 8 days in PL2410 containers. *Transfusion* 2004; 44(suppl): 28A.
20. Wildt-Eggen Jd, Schrijver JG, Smid WM, Joie M, Bollinne V and Bins M: Platelets stored in a new generation container. *Vox Sang* 1998; 81: 102-107.

21. Holme S, Sweeney JD, Sawyar S and 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: 12-17.

Legends for tables

Table 1

In vitro study of functional and biochemical parameters of highly concentrated PCs stored for 9 days.

Shown as mean \pm SDs and (minimum, maximum). P-value by paired t-test (two-tailed).

N.S.: not significant.

Table 2

In vitro characteristics of highly concentrated PCs during storage in PO-80 and PL2410.

(a) Number of PCs per pH range. (b) Number of PCs per swirling score.

P-value from G-test with William's correction on PO-80 and control.

N.S.: not significant.

Table 3

Functional and biochemical parameters, days 0 and 7, of PCs stored for the *in vivo* study of PO-80.

Shown are means \pm SDs, n=8.

Table 4

Recovery and survival of fresh versus stored radiolabeled autologous platelets.

MAD represents maximum acceptable difference.

UCL₉₅ represents upper 95 percent confidence limit. Because recovery 15.3% UCL₉₅ <

20.4% MAD_{recovery} and survival 2.1 days UCL₉₅ < 2.6 days MAD_{survival}, we reject the

null hypothesis and accept that the test is not inferior to control.

Table 1. In vitro study of functional and biochemical parameters of highly concentrated PCs stored for 9 days.

	Day	PO-80 (minimum, maximum)	PL2410 (minimum, maximum)	P value
Platelet count ($\times 10^{11}$ /bag) (n=6)	0	4.4 \pm 0.4 (3.7-4.7)	4.4 \pm 0.4 (3.7-4.7)	
	1	4.4 \pm 0.4 (4.0-4.9)	4.3 \pm 0.4 (4.0-4.8)	N.S.
	7	4.2 \pm 0.5 (3.5-4.6)	4.2 \pm 0.4 (3.7-4.6)	N.S.
	9	4.1 \pm 0.4 (3.4-4.6)	4.0 \pm 0.4 (3.3-4.6)	N.S.
Mean platelet volume (fl) (n=6)	0	7.3 \pm 0.4 (6.9-7.4)	7.3 \pm 0.4 (6.9-7.4)	
	1	7.0 \pm 0.2 (6.7-7.1)	7.1 \pm 0.2 (6.8-7.2)	N.S.
	7	7.1 \pm 0.3 (6.5-7.4)	7.4 \pm 0.6 (6.5-8.1)	N.S.
	9	7.2 \pm 0.3 (6.7-7.5)	7.8 \pm 0.9 (6.9-8.8)	N.S.
pO ₂ (mmHg) (n=6)	0	84.9 \pm 15.6 (56.6-97.1)	84.9 \pm 15.6 (56.6-97.1)	
	1	59.3 \pm 13.4 (44.0-77.1)	38.0 \pm 8.9 (27.1-51.7)	0.01
	7	76.9 \pm 35.6 (43.0-114.5)	86.7 \pm 25.1 (53.7-118.4)	N.S.
	9	93.3 \pm 32.1 (37.2-125.1)	116.4 \pm 39.0 (63.0-163.9)	N.S.
pCO ₂ (mmHg) (n=6)	0	75.1 \pm 4.0 (69.2-78.9)	75.1 \pm 4.0 (69.2-78.9)	
	1	56.3 \pm 3.3 (50.7-59.7)	52.3 \pm 6.2 (43.4-58.2)	0.04
	7	36.9 \pm 9.9 (21.2-46.7)	29.8 \pm 4.2 (24.4-34.3)	N.S.
	9	32.2 \pm 8.9 (20.5-44.0)	15.5 \pm 11.7 (0-29.7)	N.S.
HCO ₃ ⁻ (mmol/l) (n=4)	0	17.1 \pm 1.3 (15.8-18.2)	17.1 \pm 1.3 (15.8-18.2)	
	1	14.7 \pm 1.0 (13.3-15.8)	13.4 \pm 1.1 (12.1-14.5)	0.03
	7	5.5 \pm 1.3 (3.7-6.9)	2.2 \pm 1.9 (0.9-5.0)	N.S.
	9	3.3 \pm 1.5 (1.9-5.0)	0.7 \pm 0.8 (0.2-1.9)	N.S.
Aggregation (%) (n=6)	0	82.8 \pm 5.5 (72.0-86.0)	82.8 \pm 5.5 (72.0-86.0)	
	1	79.5 \pm 4.5 (72.0-83.5)	79.8 \pm 4.3 (74.0-83.0)	N.S.
	7	72.4 \pm 4.4 (64.5-76.5)	67.7 \pm 11.4 (46.0-76.0)	N.S.
	9	62.7 \pm 17.7 (28.5-78.0)	42.3 \pm 28.6 (9.5-75.0)	N.S.
Hypotonic shock response (%) (n=6)	0	77.0 \pm 5.4 (74.1-86.6)	77.0 \pm 5.4 (74.1-86.6)	
	1	76.6 \pm 6.4 (71.7-89.0)	74.1 \pm 3.1 (70.4-78.8)	N.S.
	7	69.7 \pm 3.0 (64.8-73.0)	63.3 \pm 11.8 (40.7-74.4)	N.S.
	9	59.8 \pm 8.3 (43.6-65.5)	32.2 \pm 31.0 (0-66.9)	N.S.
pH at 37C (n=6)	0	7.00 \pm 0.04 (6.94-7.03)	7.00 \pm 0.04 (6.94-7.03)	
	1	7.05 \pm 0.05 (6.98-7.12)	7.05 \pm 0.09 (6.94-7.14)	N.S.
	7	6.71 \pm 0.14 (6.52-6.79)	6.45 \pm 0.44 (5.95-6.89)	N.S.
	9	6.44 \pm 0.24 (6.01-6.68)	6.20 \pm 0.42 (5.71-6.65)	N.S.
P-selectin expression (%) (n=4)	0	19.68 \pm 8.9 (7.09-28.03)	19.68 \pm 8.9 (7.09-28.03)	
	1	12.69 \pm 6.3 (5.64-20.37)	14.60 \pm 7.3 (5.61-22.76)	N.S.
	7	36.61 \pm 7.6 (27.97-46.42)	58.83 \pm 21.4 (32.52-83.79)	N.S.
	9	54.80 \pm 17.6 (40.11-80.29)	80.54 \pm 21.1 (49.80-94.90)	N.S.
Interval				
Glucose consumption (mmol/10 ¹² /24hr) (n=6)	0 -72hr	3.61 \pm 1.7 (1.89-6.57)	3.68 \pm 1.0 (2.78-5.60)	N.S.
	72-120hr	3.08 \pm 0.4 (2.27-3.47)	3.49 \pm 0.8 (2.90-4.61)	N.S.
	120-168hr	2.75 \pm 1.1 (0.76-3.85)	4.93 \pm 2.1 (3.09-7.07)	0.02
	168-216hr	4.21 \pm 1.4 (3.03-6.82)	3.41 \pm 2.0 (0.95-6.69)	N.S.
Lactate generation (mmol/10 ¹² /24hr) (n=6)	0 -72hr	6.07 \pm 3.0 (3.21-11.71)	6.85 \pm 1.8 (4.67-9.68)	N.S.
	72-120hr	4.88 \pm 0.7 (4.19-5.85)	5.87 \pm 1.5 (4.26-7.78)	N.S.
	120-168hr	4.37 \pm 1.4 (2.68-6.64)	8.11 \pm 4.1 (4.31-10.69)	0.05
	168-216hr	5.32 \pm 4.6 (0.69 -13.11)	6.58 \pm 3.6 (2.71-12.83)	N.S.

Table 2 In vitro characteristics of highly concentrated PCs during storage in PO-80 and PL2410

(a) Number of PCs per pH range

	pH on day 7			pH on day 9		
	<6.2	6.2-6.8	>6.8	<6.2	6.2-6.8	>6.8
PO-80	0	5	1	1	5	0
PL2410	3	1	2	3	3	0
P value	<0.05			N.S.		

(b) Number of PCs per swirling score

	Score on day 7			Score on day 9		
	0	1+	2+	0	1+	2+
PO-80	0	0	6	0	3	3
PL2410	0	3	3	3	2	1
P value	N.S.			N.S.		

Table 3. Functional and biochemical parameters, days 0 and 7, of PCs stored for the *in vivo* study of PO-80.

Measure	Day 0	Day 7
Platelet count ($\times 10^{11}$ /unit)	4.3 ± 5.4	4.3 ± 1.1
pH	7.01 ± 0.1	6.89 ± 0.1
pO ₂ (mmHg)	93.6 ± 16.9	103.3 ± 29.6
pCO ₂ (mmHg)	72.9 ± 12.4	34.1 ± 2.8
HCO ₃ ⁻ (mmol/l)	18.1 ± 1.6	3.7 ± 2.3
Glucose (mmol/l)	19.2 ± 2.4	10.5 ± 3.3
Lactate (mmol/l)	1.4 ± 0.4	8.6 ± 1.0
Aggregation (%)	83.0 ± 4.4	76.8 ± 4.1
HSR (%)	75.3 ± 6.6	71.9 ± 6.1
P-selectin (%)	23.5 ± 16.7	31.6 ± 10.0

Table 4. Recovery and survival of fresh versus stored radiolabeled autologous platelets

Donor No	Recovery (%)					Survival (days)		
	Control		Test		Difference	Control	Test	Difference
	(fresh)	Radiolabel	(7-day)	Radiolabel		(fresh)	(7-day)	
1	54.1	⁵¹ Cr	42.7	¹¹¹ In	11.4	7.7	5.9	1.8
2	65.0	⁵¹ Cr	56.1	¹¹¹ In	8.9	7.9	6.7	1.2
3	56.2	⁵¹ Cr	44.9	¹¹¹ In	11.3	7.1	6.3	0.8
4	35.0	⁵¹ Cr	27.9	¹¹¹ In	7.1	9.2	7.3	1.9
5	71.6	¹¹¹ In	47.8	⁵¹ Cr	23.8	6.1	4.0	2.1
6	77.5	¹¹¹ In	73.3	⁵¹ Cr	4.2	6.7	6.7	0
7	65.6	¹¹¹ In	49.2	⁵¹ Cr	16.4	8.9	8.0	0.9
8	64.8	¹¹¹ In	60.1	⁵¹ Cr	4.7	8.5	5.2	3.3
Mean	61.2		50.3		11	7.8	6.3	1.5
SD	13.0		13.4		6.5	1.1	1.2	1
MAD	20.4					2.6		
UCI ₉₅			15.3				2.1	