表6. SensiMediaによる細菌検査－35℃と室温との比較－

		増殖細菌検出時間(時間)				
細菌濃度(個/ml)		2	100	5000	250000	12500000
細菌種名	孵置温度					
E. Coli	35℃	ND	12	10	9	6
	22℃	47	27	24	24	18
Klebsiella pneumoniae	35℃	13	11	9	7	5
	22℃	24	22	19	15	12
Pseudomonas aerginosa	35℃	25	23	20	17	13
	22℃	52	47	42	35	30
Serratia marcescens	35℃	15	13	10	8	6
	22℃	25	23	20	17	13

＊：生理食塩に浮遊した各濃度細菌浮遊液1mlを検体に使用した
ND　　：検出されず

表7. 採血直後のPCのみ(無菌)での偽陽性の有無を測定

検体量(ml)	陽性表示までの時間
1	13.4
2	6.1
3	51
5	3.1
10	1.9

表8. SensiMedia測定時の偽陽性表示時間と検体量および血小板の採血後時間
（POバッグに保存した血小板濃厚液のデータ）

検体量(ml)	時間		
	測定①	測定②	測定③
	偽陽性表示時間		
0. 5ml	168以上	168以上	168以上
1ml	16.1	168以上	168以上
2ml	7.7	15.1	168以上
3ml	4.6	9.4	168以上
5ml	3.5	7.4	NT
採血から測定開始までの時間			
	5	31	74

孵置温度　35℃
血小板濃厚液の血小板濃度　143万／cmm（POバッグに40ml保存）
NT　測定せず

表9.　SensiMedia測定時の偽陽性表示時間と検体量および血小板の採血後時間
（PO80バッグに保存した血小板濃厚液のデータ）

検体量(ml)	時間		
	測定①	測定②	測定③
	偽陽性表示時間		
0. 5ml	300時間以上	300時間以上	300時間以上
1ml	17.7	24.3	300時間以上
2ml	7	6.7	56.2
3ml	4.5	5.5	9.7
5ml	3.2	4.2	5.2
採血から測定開始までの時間			
	5	30	78

孵置温度　　35℃
血小板濃厚液の血小板濃度　　124万／cmm （PO80バッグに200ml保存）

表10.　SensiMedia測定時の偽陽性表示時間と検体量および血小板の採血後時間
（PO80バッグに保存した血小板濃厚液のデータ）

検体量（ml）	時間		
	測定①	測定②	測定③
	偽陽性表示時間		
0.5ml	120時間以上	120時間以上	120時間以上
1ml	31.9	89.9	120時間以上
1.5ml	7.3	120時間以上	120時間以上
2ml	4.4	70	120時間以上
2.5ml	NT	44	89.9
採血から測定開始までの時間			
	5	5	5
CO2センサー	5N	5N	8N
吸着体	無し	有り	有り

（5Nは感度が高く、8Nは感度を下げたCO2センサー）

孵置温度　　35℃
血小板濃厚液の血小板濃度　　103万／cmm　（PO80バッグに200ml保存）

Ⅲ．研究成果の刊行に関する一覧表

別紙５

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Ⅳ．研究成果の刊行物・別冊

Vox Sanguinis (2008) **94**, 292–298

ORIGINAL PAPER

DOI: 10.1111/j.1423-0410.2008.01042.x

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

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Vox Sanguinis

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, Deerfield, IL, USA).

Materials and Methods *In vitro* characteristics of paired platelet concentrates (PCs; mean $4{\cdot}2 \times 10^{11}$/250 ml plasma/bag) stored in PO-80 or PL2410 were assessed through 9 days of storage. *In vivo* recovery and survival of 7-day-old autologous PCs were assessed according to the Murphy method.

Results Laboratory assessment of platelet quality favoured PO-80 during 9 days of storage with statistically significant differences in glucose consumption (2·75 vs. 4·93 mmol/10^{12}/24 h in the interval 120–168 h), lactate generation (4·37 vs. 8·11 mmol/10^{12}/24 h in the interval 120–168 h), pressure of oxygen (pO_2) (59·3 vs. 38·1 mmHg at day 1), and HCO_3^- (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 7 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 non-inferior to fresh platelets, with upper confidence limits (UCL_{95}) in recovery and survival of stored PCs below the maximum acceptable difference (MAD); 15·3% UCL_{95} < 20·4% MAD and 2·1 days UCL_{95} < 2·1 days MAD.

Conclusions The *in vitro* characteristics of PCs stored in a highly oxygen-permeable container were stable at least 7 days. The *in vivo* study supports the suitability of PO-80 for 7-day platelet storage.

Key words: kinetics, *in vitro* platelet quality, *in vivo* platelet recovery, platelet storage, platelet survival.

Received: 14 November 2007, revised 27 January 2008, accepted 31 January 2008, published online 6 March 2008

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 5-day storage time has been extended to 7 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,

Conflict of interest statement: Shoji Ezuki is an employee of Kawasumi Laboratories, Inc., Tokyo, Japan. All remaining authors declare that they have no conflict of interest.

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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 carbon dioxide (CO_2) occurs [5]. Since CO_2 is produced both as a product of oxidative metabolism and as a result of disappearance of bicarbonate buffer, too low a level of pCO_2 may diminish the buffer capacity. Therefore, better oxygen and adequate CO_2 gas exchange may slow the platelet storage lesion and improve PC shelf life. However, multilaboratory 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 CO_2 permeability (PO-80; Kawasumi Laboratories, Tokyo, Japan) can preserve *in vitro* characteristics of platelets, including pH, partial pressure of CO_2 (pCO_2), 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, IL, 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, analysed 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. Platelet concentrates 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 $4{\cdot}2 \times 10^{11}$ platelets. The oxygen permeabilities of PO-80 and PL2410 were, by our measurements, 2·660 l/m^2/day/atm and 2·024 l/m^2/day/atm, respectively. The capacity of each bag was 1·0 l.

The characteristics of platelets stored for up to 9 days at 20–24°C with agitation at 50–60 strokes/min on a flat shaker (PC900i with PF48i; Helmer, Noblesville, IN, USA) were evaluated on days 0, 1, 3, 5, 7 and 9 of storage. Each bag was sampled with a syringe six 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, pO_2, pCO_2, and HCO_3^- of the PCs were measured at 37°C using a pH/blood gas analyser (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, MD, USA).

In vivo assay

A minimum sample size of 7 has been required to demonstrate non-inferiority of stored PCs to fresh platelets [8]. In this study, eight healthy donors gave PCs by apheresis: five using the Amicus and three using the COBE Spectra (Gambro, Lakewood, CO, USA). Both cell separators were configured for double-needle access. PCs were collected in PL2410 and extended life platelet (ELP) bags. Within 2 h 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, NY, USA) 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 h after collection, and sample bottles (aerobic and anaerobic) were taken from each aliquot to ensure sterility. The eBDS system was used for sampling 48 h 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 labelled with radioisotopes on day 7.

To prepare fresh platelets [8,10], whole blood was drawn into an acid-citrate-dextrose A (ACD-A) bag. Carefully prepared fresh platelet pellet was gently resuspended in ACD saline before labelling. Fresh (within 6 h of collection) and stored (7 day) platelets were radiolabelled using standard techniques before reinfusion into the original donor [10]. The radiolabel $Na_2{}^{51}CrO_4$ (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 min. Isotope assignments for fresh and stored PCs were alternated randomly. By using a dose calibrator, about 20 μCi of each platelet suspension labelled with ^{111}In or ^{51}Cr was reinfused into the donor. Blood samples were taken from

the contralateral arm 15 min, 1 h and 3 h after infusion, as well as daily for 1 week and again on day 10 (to allow for the correction of activity associated with red blood cells). The radioactivity of the samples was measured using a gamma counter (Autowell Gammasystem, 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 non-inferiority hypothesis test with two-stage analysis was performed [11]. An upper confidence limit (UCL_{95}) was calculated as shown:

$$UCL_{95} = \bar{\delta}_{(control-test)} + t_{\alpha,d.f.}(sd/\sqrt{n})$$

where $\alpha = 0{\cdot}05$; d.f. (degrees of freedom) and $t_{0{\cdot}05}$ (control – test) were obtained from the software package SYSTAT, version 11 (HULINKS Inc., Tokyo, Japan).

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

$$MAD = \bar{X}_{control} - \bar{X}_{control} \times 0{\cdot}667.$$

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 interval (CI) [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 ± SD. The paired *t*-test (two-tailed) or G-test with William's correction was used to compare the values of the components, with $P < 0{\cdot}05$ 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 pO_2 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 ($P < 0{\cdot}01$) on days 1 and 3 (not shown). The pCO_2 of PCs in both bags continuously decreased during storage. The pCO_2 and HCO_3^- in PO-80 decreased more slowly than that in PL2410, achieving statistical significance on day 1 ($P < 0{\cdot}05$).

Plasma glucose levels steadily decreased in both bags. The rate of glucose consumption in PO-80 (2·75 mmol/10^{12} platelet/24 h) was, however, slower ($P < 0{\cdot}02$) than in PL2410 (4·34 mmol/10^{12} platelet/24 h) during the interval 120–168 h. The rate of lactate generation in PO-80 (4·37 mmol/10^{12} platelet/24 h) was also slower ($P < 0{\cdot}05$) than in PL2410 (8·11 mmol/10^{12} platelet/24 h) during the interval 120–168 h, but not statistically significant in other intervals (Table 1).

The degree of platelet aggregation induced by double stimuli, namely, adenosine 5′-diphosphate 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-selection 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 favoured PO-80, however, with a statistical difference ($P < 0{\cdot}05$) by G-test on day 7; of six bags in each cohort, none of the PO-80 bags and three of the PL2410 bags had a pH fall below 6·2 up to day 7 (Table 2).

Swirling scores also favoured 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 radiolabelled 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% ($P < 0{\cdot}01$, 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 ($P < 0{\cdot}01$). 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 $UCL_{95,recovery}$ (15·3%) of platelets stored in PO-80 was not more than the $MAD_{recovery}$ (20·4%). Likewise, in the survival time evaluation, the $UCL_{95,survival}$ (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 pO_2 and pCO_2 values, with a smaller decrease in pO_2

Table 1 *In vitro* study of functional and biochemical parameters of highly concentrated platelet concentrates (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 ± 0·4	(3·7–4·7)	4·4 ± 0·4	(3·7–4·7)	
	1	4·4 ± 0·4	(4·0–4·9)	4·3 ± 0·4	(4·0–4·8)	NS
	7	4·2 ± 0·5	(3·5–4·6)	4·2 ± 0·4	(3·7–4·6)	NS
	9	4·1 ± 0·4	(3·4–4·6)	4·0 ± 0·4	(3·3–4·6)	NS
Mean platelet volume (fl) ($n = 6$)	0	7·3 ± 0·4	(6·9–7·4)	7·3 ± 0·4	(6·9–7·4)	
	1	7·0 ± 0·2	(6·7–7·1)	7·1 ± 0·2	(6·8–7·2)	NS
	7	7·1 ± 0·3	(6·5–7·4)	7·4 ± 0·6	(6·5–8·1)	NS
	9	7·2 ± 0·3	(6·7–7·5)	7·8 ± 0·9	(6·9–8·8)	NS
pO_2 (mmHg) ($n = 6$)	0	84·9 ± 15·6	(56·6–97·1)	84·9 ± 15·6	(56·6–97·1)	
	1	59·3 ± 13·4	(44·0–77·1)	38·0 ± 8·9	(27·1–51·7)	0·01
	7	76·9 ± 35·6	(43·0–114·5)	86·7 ± 25·1	(53·7–118·4)	NS
	9	93·3 ± 32·1	(37·2–125·1)	116·4 ± 39·0	(63·0–163·9)	NS
pCO_2 (mmHg) ($n = 6$)	0	75·1 ± 4·0	(69·2–78·9)	75·1 ± 4·0	(69·2–78·9)	
	1	56·3 ± 3·3	(50·7–59·7)	52·3 ± 6·2	(43·4–58·2)	0·04
	7	36·9 ± 9·9	(21·2–46·7)	29·8 ± 4·2	(24·4–34·3)	NS
	9	32·2 ± 8·9	(20·5–44·0)	15·5 ± 11·7	(0–29·7)	NS
HCO_3^- (mmol/l) ($n = 4$)	0	17·1 ± 1·3	(15·8–18·2)	17·1 ± 1·3	(15·8–18·2)	
	1	14·7 ± 1·0	(13·3–15·8)	13·4 ± 1·1	(12·1–14·5)	0·03
	7	5·5 ± 1·3	(3·7–6·9)	2·2 ± 1·9	(0·9–5·0)	NS
	9	3·3 ± 1·5	(1·9–5·0)	0·7 ± 0·8	(0·2–1·9)	NS
Aggregation (%) ($n = 6$)	0	82·8 ± 5·5	(72·0–86·0)	82·8 ± 5·5	(72·0–86·0)	
	1	79·5 ± 4·5	(72·0–83·5)	79·8 ± 4·3	(74·0–83·0)	NS
	7	72·4 ± 4·4	(64·5–76·5)	67·7 ± 11·4	(46·0–76·0)	NS
	9	62·7 ± 17·7	(28·5–78·0)	42·3 ± 28·6	(9·5–75·0)	NS
Hypotonic shock response (%) ($n = 6$)	0	77·0 ± 5·4	(74·1–86·6)	77·0 ± 5·4	(74·1–86·6)	
	1	76·6 ± 6·4	(71·7–89·0)	74·1 ± 3·1	(70·4–78·8)	NS
	7	69·7 ± 3·0	(64·8–73·0)	63·3 ± 11·8	(40·7–74·4)	NS
	9	59·8 ± 8·3	(43·6–65·5)	32·2 ± 31·0	(0–66·9)	NS
pH at 37°C ($n = 6$)	0	7·00 ± 0·04	(6·94–7·03)	7·00 ± 0·04	(6·94–7·03)	
	1	7·05 ± 0·05	(6·98–7·12)	7·05 ± 0·09	(6·94–7·14)	NS
	7	6·71 ± 0·14	(6·52–6·79)	6·45 ± 0·44	(5·95–6·89)	NS
	9	6·44 ± 0·24	(6·01–6·68)	6·20 ± 0·42	(5·71–6·65)	NS
P-selectin expression (%) ($n = 4$)	0	19·68 ± 8·9	(7·09–28·03)	19·68 ± 8·9	(7·09–28·03)	
	1	12·69 ± 6·3	(5·64–20·37)	14·60 ± 7·3	(5·61–22·76)	NS
	7	36·61 ± 7·6	(27·97–46·42)	58·83 ± 21·4	(32·52–83·79)	NS
	9	54·80 ± 17·6	(40·11–80·29)	80·54 ± 21·1	(49·80–94·90)	NS
	Interval					
Glucose consumption (mmol/10^{12}/24 h) ($n = 6$)	0–72 h	3·61 ± 1·7	(1·89–6·57)	3·68 ± 1·0	(2·78–5·60)	NS
	72–120 h	3·08 ± 0·4	(2·27–3·47)	3·49 ± 0·8	(2·90–4·61)	NS
	120–168 h	2·75 ± 1·1	(0·76–3·85)	4·93 ± 2·1	(3·09–7·07)	0·02
	168–216 h	4·21 ± 1·4	(3·03–6·82)	3·41 ± 2·0	(0·95–6·69)	NS
Lactate generation (mmol/10^{12}/24 h) ($n = 6$)	0–72 h	6·07 ± 3·0	(3·21–11·71)	6·85 ± 1·8	(4·67–9·68)	NS
	72–120 h	4·88 ± 0·7	(4·19–5·85)	5·87 ± 1·5	(4·26–7·78)	NS
	120–168 h	4·37 ± 1·4	(2·68–6·64)	8·11 ± 4·1	(4·31–10·69)	0·05
	168–216 h	5·32 ± 4·6	(0·69–13·11)	6·58 ± 3·6	(2·71–12·83)	NS

Shown as mean ± SDs and (minimum, maximum). *P*-value by paired *t*-test (two-tailed).
NS, not significant.

than the PL2410 container, suggesting an advantage of the PO-80 container for minimizing platelet storage lesion [12]. Internal pO_2 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 pO_2, particularly over the first 3 days. A higher pO_2 promotes aerobic glucose metabolism in platelet

Table 2 *In vitro* characteristics of highly concentrated platelet concentrates (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			NS		

(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	NS			NS		

P-value from G-test with William's correction on PO-80 and control.
NS, not significant.

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, $P < 0{\cdot}01$). Alternatively, activation of platelets would likely increase their metabolism, and higher P-selectin values were observed in PL2410 vs. PO-80.

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/)	19·2 ± 2·4	10·5 ± 3·3
Lactate (mmol/)	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

Shown are means ± SDs, $n = 8$.

To obtain better gas transfer rates in storage containers for platelets, there are three strategies: (i) enlarge the bag to increase its surface area; (ii) decrease the membrane thickness to increase gas permeability; and (iii) 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

Table 4 Recovery and survival of fresh vs. stored radiolabelled autologous platelets

	Recovery (%)					Survival (days)		
	Control		Test			Control	Test	
Donor No.	(Fresh)	Radiolabel	(7-day)	Radiolabel	Difference	(Fresh)	(7-day)	Difference
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		
UCl_{95}			15·3				2·1	

MAD, maximum acceptable difference; UCL_{95}, upper 95% confidence limit.
Because recovery 15·3% UCL_{95} < 20·4% $MAD_{recovery}$ and survival 2·1 days UCL_{95} < 2·6 days $MAD_{survival}$, we reject the null hypothesis and accept that the test is not inferior to control.

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 radiolabelled autologous platelets [8]. There are several reports [15–17] 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 × 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 pH 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 CI for non-inferiority. Stored PCs were shown to be non-inferior to freshly prepared PCs with 95% CI [11]. As sample size calculations to demonstrate non-inferiority 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 favourably 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 was stable for a minimum of 7 days storage, suggesting that PO-80 is sufficient for storing PCs for 7 days with good quality.

Acknowledgements

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

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