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In Vitro Quality Evaluation of 8 Days Storage of Random Donor Platelets Using Platelet Additive Solution

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ABSTRACT

Background: The use of platelet additive solution (PAS) as plasma substitute during platelets (PLTs) storage could be an effective step in reducing plasma volume to decrease adverse events and prolonging PLT concentrate (PC) shelf life beyond the traditional 5 days. This study aimed at evaluating the applicability of using PAS as PLT storage media for extending PLT shelf life up to 8 days. **Methods:** 36 random donors (PCs) were prepared by platelet-rich plasma method and divided equally according to their storage media into: Group (1): 100% autologous plasma; Group (2): 30% autologous plasma and 70% PAS (SSP+); Group (3): 100% SSP+. All units were stored at 22° C on flatbed agitator for 8 days. PLT count and indices, metabolic variables, CD42b and CD62p expression were assessed on days 1, 5 and 8, besides automated BACTEC aerobic blood culture system for bacterial screening on days 1 and 8. **Results:** At 8 day storage, the mean PLT yield, mean PLT volume, PLT distribution width, swirling scores, metabolic parameters (glucose, LDH, PO_2, and PCO_2) levels showed best optimum values among group (2) units, compared to other groups. pH was maintained > 6.8 in all groups. The lowest CD62p expression and the highest CD42b expression were found among group (2), on days 1 and 8. No bacteriological growth was detected in all studied units. **Conclusion:** At 8 days storage, PCs using30% autologous plasma and 70% (SSP+) could provide the highest quality, particularly when assisted by a good and rapid bacterial detection system.

KEYWORDS: platelet storage, non-filtered random PCs, platelet additive solutions, quality assessment of PCs, BACTEC.

INTRODUCTION

Platelet transfusion is often considered a life-saving measure, being essential for the prevention and treatment of bleeding in patients who have quantitative and/or functional PLT disorders. Nowadays, in many western countries, the demand for PCs is obviously growing, almost up to 80% increase, compared to a decline in the use of packed red blood cells [1].

This steady increase in PLT therapy is referred mainly to the increased use of chemo/ radiotherapeutic regimens associated with prolonged periods of bone marrow aplasia, which in turn placed considerable pressure on the logistics of PLT supply [2].

It is well known that when PLTs are removed from circulation and stored under blood-bank conditions, they will be subjected to a variety of mechanical and chemical influences resulting in series of changes, collectively referred to as the PLT storage lesion (PSL), with consequent poor post-transfusion function and survival. In addition during storage time, programmed cell death (apoptosis) begins [3].

Furthermore, room temperature storage and the biological composition of PCs and their media make PCs close-to-ideal culture media for a wide variety of Gram-positive and Gram-negative organisms derived fromasymptomatic bacteremic donors, insufficient skin disinfection or contamination during processing, storage and handling [4].

Collectively, these factors result in limitation in the extension of PCs shelf life beyond 5 days [3] and imply

the need for continuous renewal of stock [5].

Many studies have been focused on extending the storage time of PCs more than 5 days and assessing their acceptable in vivo performance, in order to reduce the bulk of outdated and wasted products. successful achievement of this goal will confer additional flexibility to blood centers and transfusion services [6].

The idea of storing PLTs in plasma-free artificial media emerged in the 1950s, in order to improve storage conditions. Since that, all efforts in developing PASs are directed at producing a storage environment that allows reduction of PSL, in other words, optimization of viability, energy metabolism, and the ability of PLTs to undergo haemostatic activation after transfusion [7].

The aim of this study is to evaluate the applicability of using PAS in different concentrations with autologous plasma as a PLT storage medium for extending PLT shelf life up to 8 days, while monitoring PLTs quality parameters during storage in order to ensure the optimum clinical effectiveness and safety of PLT transfusion. This will be an important step in transfusion services if PLTs can be rendered available in a timely manner while minimizing the wastage of time-expired PLTs and plasma transfusion reactions.

MATERIALS AND METHODS

Sample Collection:

Thirty-six PC units were freshly collected from healthy male and female blood donors who visited the blood bank of Theodor Bilharz Research Institute using triple blood bags, CPDA-1 anticoagulant *(JMS Singapore Pte Ltd),* with proper sterilization of the vein puncture area using blood collection monitor HemoMatic $^{(TM)}$. All units were prepared by the platelet-rich plasma (PRP) preparation method using cooling floor-standing centrifuge (Hettich ROTIXA 50 RS, Germany).

PC preparation method and storage:

Donated whole blood was subjected to two stage centrifugation procedure with different speed and duration of each step. The first is soft spin at 1750 rpm for 11 minutes to produce packed RBCs and PRP. The latter was subjected to the second step of centrifugation (hard spin) at 3940 rpm for 5 minutes to produce platelet poor plasma (PPP) stored as fresh frozen plasma (FFP) and PC.

PCs were classified equally into 3 groups according to the storage medium used: group (1), included 12 PC units where PLTs suspended in 100% autologous plasma, group (2), as well, include 12 PC units containing a mixture of 30% autologous plasma and 70% PAS (SSP+, Macro- Pharma International, Langen, Germany) used as storage medium; and group (3), include 12 units in which PLTs suspended in 100% PAS (SSP+).

Following preparation, PCs were left for 1 hour without agitation at room temperature for resting highly activated platelets during preparation. Subsequently, all the 36 units were kept on a flatbed platelet agitator (Helmer, Inc, USA) with continuous gentle agitation to prevent PLT clumping and facilitate gas exchange at 22-24^{o}c for a total of 8 days.

The study was approved by the local ethics board (Institutional Board Review) and an informed consent was obtained from all subjects.

Assessment of PLT characteristics and metabolic variables during storage:

Samples were drawn aseptically under a laminar flow hood from all units at days 1, 5 and 8 of storage. For each sample, PLT count, and indices (MPV, PDW) were estimated using the automated cell counter (Beckman Coulter Act Diff III).

Swirling phenomenon was evaluated by examining the gently rotated PC units against the light. The normal discoid platelets refract light and produce swirling pattern, which can be identified and scored (0-3) by visual inspection of trained personnel in blood bank [8].

pH, partial pressures of oxygen and carbon dioxide (PO_2 & PCO_2) and glucose levels of all samples were assessed within 10 minutes after sampling at a temperature of 37^{o}C using blood gas analyzer *(Gem Premier 3500).*

Lactate dehydrogenase (LDH) enzyme as an indicator of platelet metabolism was determined using thawed PPP supernatant samples stored frozen at -70^{o}C by using the semi-automated, single-beam filter photometer according to the standard methods (RIEDLE 5010).

In order to detect the studied PLT surface expression markers, PRP was prepared and freshly tested for CD42b (used as a PLT identifying marker) and CD62p (PLT activation marker) expression using fluorescein isothiocyanate conjugated (FITC)and R-phycoerythrin conjugated (PE) monoclonal antibodies (moAbs),Mouse Anti-Human CD62p and CD42b respectively. A nonspecific Isotype Control was used with each sample. All antibodies were of the IgG1κ Isotype and Flowcytometer Epics®Elite “Coulter” system was used for the

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analysis. The Results were expressed as specific CD62p and CD42b percentage of positive platelets, calculated by subtracting the nonspecific fluorescence of the isotype control from the specific fluorescence of the moAbs. For subtraction, the manufacturer's software was used [9].

Bacteriological screening of PC units:

The study had a standardized testing protocol that used aerobic culture bottles (BACTEC Plus Aerobic/F bottles) inoculated with 6ml of PLT samples, and BACTEC 9050 System (BD Microbiology, Cockeysville, MD). These cultures were carried out for each PC unit on days 1 and 8. Continuous monitoring blood culture system in the incubator (37^{o}C) for 8 days after inoculation was performed for the detection of bacterial contaminants in PLT preparations. Even though, an automated system was used, the cultures were also controlled visually for signs of growth, cloudiness, color change, gas bubbles and clumps of bacteria, in the broth.

Statistical methods:

Results were expressed as mean ± standard error of mean (SEM) or number (%). Comparison between the mean values at different dates within the same group was performed using paired t-test. Comparison between the mean values of different parameters between the different groups were performed using one way analysis of variance (ANOVA) of the mean percent change of each parameter with post hoc using the least significant difference. Correlation between parameters was performed using Spearman's rank correlation coefficient. SPSS computer program (version 18 windows) was used for data analysis. *P* value ≤ 0.05 was considered significant and *p*-value <0.01 was considered highly significant. The percent change of each parameter was calculated by subtracting the baseline (day 1) result from the final result (day 8), then dividing the result of this subtraction by the baseline result, and finally multiplying by 100.

RESULTS

The mean volume of PC units on day 1 for Group I, Group II and Group III was (72.68±1.13, 71.94±13.98 and 78.63±.45 ml respectively). The volume of all units decreased gradually during storage due to sampling (10 ml) each time, on days 1, 5 and 8, in order to monitor the studied parameters during storage period. Results of the studied parameters are shown in tables 1, 2, 3 and 4.

Our results revealed highly significant (p< 0. 01) decrease in mean PLT count/unit in group I and III on day 8 relation to day 1and day 5 meanwhile, it showed no significant difference between all groups. As regards the PLT indices, our results revealed highly significant (*p*< 0.01) increase at the end of storage in all groups in relation to donor regarding PDW and in relation to donor and day 1 regarding MPV with the lowest mean percent change observed in group 2 (table 2).

The metabolic characteristics of the studied PCs during storage revealed that, the pH level was maintained above > 6.8, with no significant difference between all studied days in all groups However, glucose, LDH, PO_2 and PCO_2 levels showed a highly significant difference (*p*< 0.01) at the end of storage in relation to day 1in all groups, with the lowest mean percent change was observed in group 2.

The studied PLT surface expression markers (CD42b and CD62P) showed significant difference on day 8 in relation to day 1 with the lowest mean percent change observed in group 1 regarding CD 42b and in group 2 regarding CD62P.

Correlation studies between pH versus different studied parameters in different platelet products on day 8 of storage were illustrated in table (3), while between CD62P versus different studied parameters in different platelet products on day 8 of storage were shown in table (4).

No bacteriological growth was observed in all units within the studied groups neither in cultures performed on day 1 nor on day 8 which is actually considered as confirmatory culture to day 1.

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Table 1: storage changes as regards: PLT count, indices, metabolic parameters, swirling and PLT surface expression markers.

	Day 1	Day 5	Day 8
PLT Count (x10^{10}/unit)			
Group 1	7.56 ± 0.40	6.68 ± 0.28	5.63 ± 0.42^{dd}
Group 2	6.26 ± 0.36	6.12 ± 0.37	5.46 ± 0.33
Group 3	6.64 ± 0.37	5.98 ± 0.34	4.57 ± 0.22^{dd}
MPV (fl)			
Group 1	6.53±0.14	7.01±0.23^{cc}	7.69±0.22 ^{aa,b,cc}
Group 2	6.89±0.16	7.12±0.16	7.64±0.25 ^{aa,cc}
Group 3	6.89±0.16	7.54±0.21^{cc,a}	8.96±0.44 $^{dd, cc}$
PDW (fl)			
Group 1	11.45±0.55	11.23±0.31^{c}	12.06±0.32^{cc}
Group 2	10.84±0.21	11.23±0.22^{c}	11.72±0.21^{cc}
Group 3	10.84±0.21	12.20±0.14^{cc}	15.7±0.72$^{cc, dd}$
pH			
Group 1	7.12±0.01	7.04±0.03^{a}	6.89±0.03^{aa}
Group 2	7.08±0.016	7.0±0.016^{aa}	6.89±0.02^{aa}
Group 3	7.14±.01	6.93±0.03^{aa}	6.85±0.02^{aa}
Glucose (mg/dl)			
Group 1	544.92±7.86	452.67±13.97	382.25±16.1^{dd}
Group 2	128.58±10.38	97.83±11.79	54.0±6.12^{dd}
Group 3	29.50±1.25	2.75±0.62	0.56±0.11^{dd}
LDH (U/L)			
Group 1	450.0±4.37	672.08±23.04	844.08±8.60^{aa}
Group 2	240.17±9.21	296.50±12.06	359.33±14.06^{aa}
Group 3	143.50±14.46	298.0±29.33	389.17±23.76^{aa}
PO2			
Group 1	106.0±6.63	126.33±5.80	125.83±7.18^{aa}
Group 2	144.67±4.48	160.67±4.29	170.92±3.84^{aa}
Group 3	166.58±4.2	188.25±6.09	157.17±8.53^{aa}
PCO2			
Group 1	45.42±2.73	30.75±1.82	25.75±2.06^{aa}
Group 2	24.08±2.01	20.33±1.33	17.33±1.02^{aa}
Group 3	9.92±0.77	14.92±1.65	20.25±1.48^{aa}
CD42b (%)			
Group 1	95.35±0.63	93.08±0.78	91.30±0.96^{aa}
Group 2	96.65±0.62	94.82±0.89	90.58±1.17^{aa}
Group 3	95.18±.49	90.27±1.04	55.12±11.77^{aa}
CD62P (%)			
Group 1	8.36±0.72	15.47±0.96	25.08±1.54^{aa}
Group 2	5.87±0.18	8.99±0.56	15.45±0.92^{aa}
Group 3	8.12±.44	18.45±1.05	54.866±3.63^{aa}
Swirling score / unit			
Group 1	+++ (12)	+++ (10), ++ (2)	+++ (9),++(2),+(1)
Group 2	+++ (12)	+++ (9), ++ (3)	+++ (7),++(4),+(1)
Group 3	+++ (12)	+++ (7),++(5)	+++ (3),++(5),-(4)

MPV: mean PLT volume. PDW: PLT distribution width. LDH: Lactate dehydrogenase. PO2 & PCO2: partial pressures of oxygen and carbon dioxide.

Data are expressed as mean ± SEM (standard error of mean).

^{a}p >0.05; ^{aa}p >0.01 (relative to day 1). ^{b}p >0.05; ^{bb}p >0.01 (relative to day 5).

^{c}p >0.05; ^{cc}p >0.01 (relative to donor). ^{d}p >0.05;^{dd}p >0.01 (relative to day 1& 5).

^{a}, ^{b}, ^{c,d} = significant difference. ^{aa}, ^{bb}, ^{cc}, ^{dd} = highly significant difference.

Table 2: Mean percent change of evaluated parameters between day 1 and 8 in different studied groups

	Group I PCs suspended in **%100** autologous plasma (n=12)	Group II PC suspended in 30% autologous plasma &70% PAS (n=12)	Group III PCs suspended in 100% PAS (n=12)	F value	*P*
Platelet count(↓)	26.11±1.92	26.10±10	30.932±0.69	1.64	>0.05
MPV (↑)	-17.72±2.61**	-10.93±2.83**	-30.69±6.91	5.089	≤0.01
PDW (↑)	-15.09±2.52**	-7.72±4.26**	-45.19±6.84	17.99	<0.001
pH (↓)	3.33±0.33	2.78±0.33	3.96±0.37	2.9	>0.05
PO2 (↑ or ↓)	-20.28±5.42**	-18.92±3.28**	5.11±5.47	8.72	<0.001
PCO2 (↓or ↑)	41.92±5.23**	25.05±4.40**	-112.30±19.14	51.94	<0.001
LDH (↑)	-87.61±1.24**	-49.78±2.36**	-194.37±25.10	26.47	<0.001
Glucose (↓)	30.08±2.25*	20.73±38.28**	97.99±0.42	3.63	<0.05
CD62p (↑)	-218.31±27.67**	-164.79±15.83**	-611.27±75.47	26.563	<0.001
CD42b (↓)	4.28±0.51**	6.30±0.89 **	12.56±0.75	34.689	<0.001

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Data are expressed as mean percent change where the percent change was calculated from the difference between results of day 1 and 8 divided by day 1. * $p < 0.05$ significant difference than group 3.
** $p \leq 0.01$ highly significant difference than group 3.

Table 3: Correlation between pH versus different studied parameters in different groups on day 8 storage.

Day 8	Group I PCs suspended in 100% autologous plasma (n=12)		Group II PC suspended in 30% autologous plasma &70% PAS (n=12)		Group III PCs suspended in 100% PAS (n=12)	
	R	*P value*	*R*	*P value*	*R*	*P value*
Plt yield	-.716**	.009	.131	.685	.375	.230
MPV	.409	.186	-.454	.138	.592*	.042
Glucose	.716**	.009	.058	.858	-.117	.716
LDH	-.628*	.029	.208	.517	-.002	.995
PO_2	.458	.134	-.095	.770	-.446	.146
PCO_2	-.570	.053	.201	.530	.057	.861
CD62p	.201	.531	-.427	.166	.600*	.039
CD42b	.000	.999	.659*	.020	-.523	.081

r= correlation coefficient. p= p value. *= Correlation is significant at the 0.05 level (2-tailed)
**= Correlation is highly significant at the 0.01 level (2-tailed)

Table 4: Correlation between CD62P versus different studied parameters in different groups on day 8 storage.

Day 8	Group I PCs suspended in 100% autologous plasma (n=12)		Group II PC suspended in 30% autologous plasma &70% PAS (n=12)		Group III PCs suspended in 100% PAS (n=12)	
	r	*P value*	*R*	*P value*	*R*	*P value*
PLT yield	-.254	.427	-.047	.885	.076	.814
MPV	.801**	.002	.585*	.046	.862**	.000
Glucose	.038	.906	.170	.597	.143	.657
LDH	.294	.354	-.308	.330	-.152	.638
PO_2	.730**	.007	-.113	.727	.138	.669
PCO_2	.391	.208	-.177	.583	-.453	.140
CD42b	.525	.080	-.273	.390	-.798**	.002

r= correlation coefficient. p= p value.
*= Correlation is significant at the 0.05 level (2-tailed)
**= Correlation is highly significant at the 0.01 level (2-tailed)

DISCUSSION

The introduction of PAS technology possibly allows extension of PLT shelf life. Nowadays, PAS can be considered as a 'designer solution where ingredients can be added to specifically influence certain characteristics of platelet storage [10]. In this study, we aimed to investigate the effect of the extended storage on the in vitro quality of PLTs in SSP+ PAS. It has been reported that three fundamental quality standard parameters, namely PLT counts, PLT activation and metabolic alterations, must be considered for a proper evaluation of the effect of prolonging PLT shelf-life [11].

The obvious reduction in PLT counts at the studied time-points of storage, in the 3 groups, indicates an increase in platelet elimination with storage, which could be attributed to platelet senescence, as the platelets' life span is 7-10 days [12]. However, The PLT counts in PAS containing units were slightly below the acceptable range on day 8. Here in, it is well known that they were actually administered in pools of 4-6 units rather than random donor PC [13]. The obvious reduction of basal PLT count in PAS containing units compared to group I (100% plasma containing units) on day 1, can be referred to PLT loss with partial or complete plasma removal during processing. It is well known that PCs containing high PLT yield show high rate of metabolic activity resulting in obvious reduction of pH [14]. This fact can explain the significant negative correlation which was observed between PLT yield and pH in our results.

PLT indices namely mean PLT volume (MPV) and PLT distribution width (PDW) evaluated in conjunction with PLT counts constitute further indicative parameters in assessment of the quality of PC and the extent of PSL [11,15]. Our results revealed an increase in MPV and PDW in all groups, in respective to SSP+ addition, in relation to our base line results. Equivalent results were found in studies conducted by [14,3,2]. These changes in MPV and PDW values during storage were attributed to the gradual change in PLT shape from discoid to spherical shape [16]. Taken together MPV and PDW can thus provide a more reliable description of the PLT volume distribution than if MPV is considered alone [14], as the presence of a mixture of large and small PLTs may give a normal MPV but a high PDW, this being indicative of active PLT release and consequent unsuitability of the product.

In the current study, on performing the intergroup comparison, we estimated the mean percent change from the difference between results of day 1 and day 8 divided by day 1results in order to eliminate the changeable irrelevant factors such as variability of donor's criteria, as baseline levels, and discrepancy in unit's volume. We found no significant percent change between different studied groups regarding PLT count. However the

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reduction in PLT count was more obvious in group III (~30%) compared to the other two groups (~26%). Regarding MPV and PDW, data derived from the present study demonstrated that group II showed the least mean percent increase about (10 % and 7% respectively) followed by group I (17 % and 15% respectively) then group III (30 % and 45% respectively). However, no significant difference was noticed between group I and II.

pH level is considered a global indicator of the PLT environment, demonstrating the balance between PLT metabolism and the buffer capacity of the medium with an acceptable range of 6.4 - 7.4 at 22°C in Europe and > 6.2 in USA in order to retain PLT function according to UK specification 2005 and AABB 2008 [16,14].

The current study recorded a significant decrease in pH level in the all studied groups, during storage time on day 8 versus day 1. This decrease in pH could be attributed to the production of lactic acid and carbon dioxide by PLT metabolisms during storage [17]. Meanwhile, this decline in pH was within the acceptable range, and therefore had no detrimental effect on PLT viability and activation except at the end of the storage period where a significant positive correlation was found between pH and both MPV and CD62p in group III (100% PAS containing units). Equivalent results were found in studies conducted by [18, 3, 2]. There might be three reasons for this limitation in pH decline. Firstly, absence of bacterial contamination as demonstrated by negative culture in all studied PCs, and secondly, the stabilizing effect of plastic PLT storage bags permitted a constant gas exchange of the PCs over the testing period [14,3]. Thirdly, the efficacy of the buffering capacity of the storage media which mainly depends on bicarbonate in plasma and acetate in PASs [3]. However the data obtained from the current study revealed no significant difference in the mean percent change of pH between all studied groups; group II, PLTs suspended in a mixture of SSP+ and plasma, showed the highest buffering capacity with the least change in pH throughout the storage period. This finding can be attributed to the combined buffering effect of acetate and bicarbonate. Equivalent results were found in the study conducted by Christine [3].

Regarding glucose level, our results showed significant reduction of glucose levels during the storage period on comparing day 8 versus day 1 and 5 in all groups. This is in agreement with [19,3,2]. PLTs are living cells, their metabolism requiring the take up of glucose to maintain ATP, major energy carrier, in an anaerobic process called glycolysis. In glycolysis the glucose is converted into lactic acid. High levels of lactic acid content in the PCs lower the pH values and render the PCs unsuitable for transfusion. This process continued even in the presence of adequate oxygen [3]. This fact explains the noticed significant positive correlation between pH and glucose at the end of the storage in group I (100% plasma containing units). This significant reduction in glucose level can be attributed not only to PLT metabolism but also to leukocyte contamination and high PLT yield in some units. This is in accordance with the finding of [20, 2].

Many studies indicated that, only a very small amount of glucose from plasma carryover is sufficient to support PLT metabolism for up to 5 days. Furthermore, the complete depletion of glucose is associated with PLT dysfunction despite normal pH [21,3]. These previous findings explain the highest mean percent change noticed in group III (100% PAS containing units) and the accelerated deterioration of the studied parameters on day 8 in the same group where the small amount of glucose remaining as a result of incomplete removal of plasma rapidly consumed throughout the first 5 days, taking in consideration the lack of glucose in SSP+ and therefore, PLT metabolism could no longer be supported. Data obtained from the intergroup comparison of the mean glucose values revealed that, SSP+ containing units (groups II & III) consumed a lower amount of glucose compared with group I. This can be attributed to the availability of acetate in these units which reduce the glucose requirement as a source of energy. However, the noticed higher PLT yield in group I must be taken in consideration when comparing it with the other two groups.

In contrast, the LDH levels in all groups showed significant increase on day 8 in relation to day 1, a finding that agrees with those conducted by [14,19, 3]. Measurement of LDH helps in evaluating out the extent of cell damage. However, the LDH cannot be considered as an indicative marker of PLT status in case of non-filtered PRP-PCs as the contaminant leucocytes have an effect which cannot be neglected and definitely contribute to remarkable LDH increase [22,2]. This notification can explain the highest mean LDH levels in all studied days of group I where PLT were suspended in 100% plasma while the lowest levels were noticed in group III where plasma was completely replaced by SSP+ with consequent removal of significant numbers of leukocytes. The significantly higher mean percent change in group III in relation to the other two groups can be attributed to glucose depletion in these units with significant cell damage.

Aerobic metabolism has been indirectly monitored in PC through the measurement of partial pressures of oxygen (pO2) and carbon dioxide (PO2 and PCO2). High O2 availability, achieved by adequate gas exchange, is required to maintain the oxidative metabolism [23,4]. Our results revealed that, significant increase of PO2 on day 8 compared to day 1 in group I and II while these results were reversed regarding PCO2. These finding are in accordance with Hornsey and his coworkers [24]. In contrast, these results did not agree with that conducted by Picker and his coworkers [23], who demonstrated that, the increase in pO2 and concurrent decrease in PCO2, interpreted as a decline in oxidative metabolism. However, there were other factors that may contribute to high PO2 including current gas-permeable storage packs, as the pressures also relate to the equilibration of O2 and pCO2 in the pack relative to atmospheric pressures [25, 3]. Regarding group III (100% PAS containing units),

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data obtained from the study of the mean values throughout the storage period showed that there was increase in the PO2 with concurrent decrease in PCO2 throughout the first 5 days, after that the results were reversed. This was concurrent with glucose depletion denoting that PLT metabolism has been terminated.

Results of CD42b, external membrane protein, showed significant decrease in all groups at the end of the storage with the highest mean percent change was noticed in group III accompanied with significant cell damage. This decrease may be related to the redistribution of glycoprotein into the open canalicular system during the storage [3]. In contrast, CD62p, granule membrane proteins, are expressed on the external membrane of the PLT upon activation [18,4]. The extent of PLT activation depends on methods of collection, processing, duration of storage and the storage medium of PCs [26,27,28,14]. This fact can explain the noticed significant increase of CD62p expression at the end of storage in relation to day one in all groups and also the noticed significant correlation between pH and CD62 on day 1 in group I (100% plasma containing units) which can be attributed to the activation induced by collection and processing. In response to activation, PLTs change shape from disks to so-called spiny spheres [29]. This fact can explain the observed positive correlation between CD 62P and MPV at the end of the storage in all groups. Our findings regarding CD62P and CD42b are in accordance with [18,24,3].

Concerning bacterial contamination, Data derived from this study showed that the 36 PCs were culture negative on day1 and 8.

To sum up, 8 days PCs storage instead of the recommended 5 days shelf life is feasible with respect to the optimum requirements of PLT quality and bacterial screening. This was almost achieved in group I where PLTs are suspended in 100% plasma, and in group II where PLTs are suspended in a mixture of plasma and PAS with respect to PLT yield, PLT activation and pH optimization. Throughout the 8 days storage period, group II was superior to the other two groups, evidenced by optimal maintenance of metabolic parameters associated with the least activated adequate PLT yield. Furthermore, better viability of PLTs was demonstrated by lower mean percent increase in MPV and PDW than the other 2 groups. Our choice between different concentrations of storage media actually should depend on a critical balance between the safety, quality and cost. In other words, the availability of usage of PAS beside rapid automated bacterial culturing system.

Conclusion:

To minimize PCs outdating that are licensed currently for a maximum of five days, we can conclude that PCs stored in a medium containing 30% plasma and 70% SSP+ could provide the highest quality possible, when coupled with a good and rapid bacterial detection system to assure the sterility of PCs that were kept at 22 – 24°C for eight days.

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