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Platelet Metabolism and Function of Buffycoat Pooled Cold Stored Platelets vs Conventional Room Temperature Platelets - An Invitro Study

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BACKGROUND

- Conventional room temperature storage of platelets have some inherit problems like: short shelf life, risk of bacterial contamination and studies are showing limited functional viability of platelets post transfusion for 3 days of Room Temperature Storage because of storage lesions.
- Cold stored platelets have a longer shelf life, easy transport along with red cell and lesser chances of bacterial contamination and studies are showing better haemostatic control in bleeding patients.

AIM AND OBJECTIVES

Aim

- To study platelet metabolism and function of cold stored platelet at 4°C up to 21 days as compared to conventional room temperature platelets for five days of storage.

Objectives

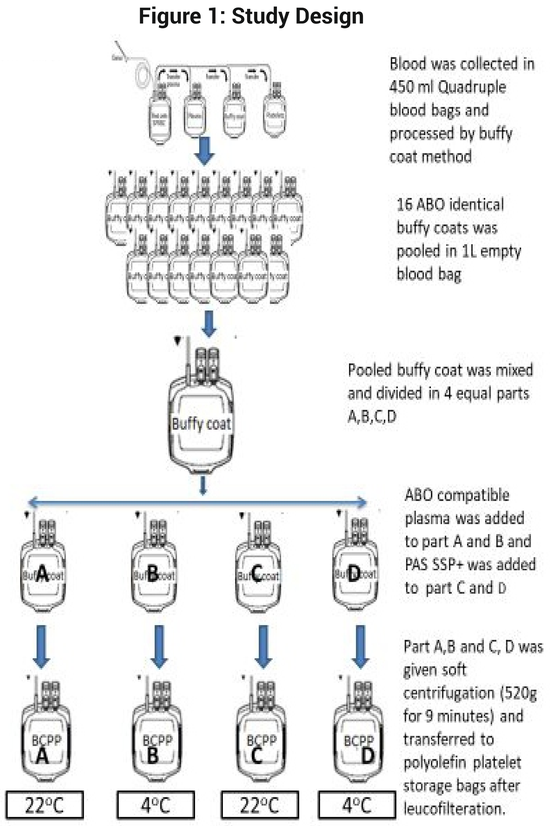
1. To compare biochemical activation markers, aggregation properties, clot strength and sterility for cold stored platelets for 21 days vs. room temperature stored platelets for five days.
2. To study the effect of platelet additive solution (PAS) suspended platelets vs plasma suspended platelets on biochemical activation markers, aggregation properties, clot strength in cold-stored platelets and room temperature platelets.

MATERIALS AND METHODS

- We pooled 16 ABO compatible Buffycoat to prepare four final buffycoat platelet bags: part A, B, C and D. Part A & B were suspended in plasma, and part C & D were suspended in PAS.
- Part A and C were kept at Room temperature(RT) for five days in an agitator, and Part B and D were kept at 4°C (Cold Storage (CS)) without agitation for 21 days.
- The Final platelet product A and C (Stored at 22°C) were serially sampled on Day 1,3,5, and B and D (Stored at 4°C) were sampled on Day 1,3,5,7,10,14,21.
- Each sample was tested for parameters like platelet count, WBC count, Swirling, pH, Glucose, Lactate, CD-62, IL-6, TNF-α, Aggregation studies with platelet agonists: ADP and Epinephrine, Clot strength tested by SonoClot® (Sienco®, Inc, Arvada, CO, USA) and sterility by culture on Day 5 for Part A &C and Day 10 and 21 for part B&D.
- Five such experiments were done.

STUDY DESIGN

Figure 1: Study Design



RESULTS

- **Physical Appearence:** There were no precipitates, discolouration or gas formation in any part during storage from days 1 to 21.
- **Volumes:** Mean volumes in part A and B was 200±10 ml and in Parts C and D was 190±10 ml on day of preparation. The Plasma to PAS ratio was 32:68 in parts B and D.
- **Swirling:** maintained in RT platelets till day 5(Part A and C). Disappeared in CS platelets (part B and D) within few hours of placing at cold and no swirling seen through Day 1-21.
- **WBC count:** As all platelet components were initially leukofiltered and these have WBC count < $1X10^6$ / bag.

Platelet Counts

- Platelet content in the bags in all parts ranges from $2X10^{11}$ to $2.6X10^{11}$ per bag on Day 1 of storage.
- No significant fall of counts in RT stored platelets from Day 1-5 (p=0.67) but significant fall in CS platelets from Day 1-21 (p=0.01)
- No significant difference in fall of platelet counts in Plasma vs PAS from Day 5(p=0.19) to Day 21(p=0.43)

Table 1: Platelet Counts during storage in part A,B,C,D

	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14	Day 21
Part A ($X10^{11}$) (Plasma, RT)	2.6 ±0.5	2.4 ±0.4	2.1 ±0.5				
Part B ($X10^{11}$) (Plasma, CS)	2.6 ±0.4	2.4 ±0.3	2.2 ±0.4	1.9 ±0.3	1.6 ±0.4	1.5 ±0.4	1.3 ±0.3
Part C ($X10^{11}$) (PAS, RT)	2.0 ±0.4	1.9 ±0.4	1.7 ±0.4				
Part D ($X10^{11}$) (PAS, CS)	2.2 ±0.5	1.9 ±0.5	1.8 ±0.5	1.6 ±0.6	1.4 ±0.5	1.2 ±0.5	1.1 ±0.5

pH

- pH maintained from 6.8-7.7 in all parts during storage.
- No significant difference of pH between cold stored vs RT storage platelets in plasma(p=1) and PAS(p=0.29)

Table 2: pH levels during storage in part A,B,C,D

	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14	Day 21
Part A (Plasma, RT)	7.6 ±0.3	7.5 ±0.3	7.3 ±0.3				
Part B (Plasma, CS)	7.4 ±0.3	7.4 ±0.2	7.4 ±0.2	7.4 ±0.3	7.3 ±0.4	7.1 ±0.2	6.8 ±0.2
Part C (PAS, RT)	7.7 ±0.3	7.7 ±0.3	7.6 ±0.3				
Part D (PAS. CS)	7.4 ±0.2	7.3 ±0.3	7.3 ±0.3	7.3 ±0.4	7.2 ±0.3	7.0 ±0.4	6.9 ±0.3

Aggregation response to ADP

- we used 5µL of ADP and responses were recorded over 5-6 mins. The percent aggregation to ADP in Parts A, B, C, D was 66±17, 57±11, 56±20, 53±20 on Day1 which steadily decreased over further days of storage.
- The decrease of aggregation to ADP in room temperature platelets was noticeably more than cold-stored platelets at five days of storage when kept in plasma.

Table 3 : Aggregation response to ADP over 21 days of storage

	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14	Day 21
Part A (Plasma, RT)	66 ±17	44 ±16	35 ±10				
Part B (Plasma, CS)	57 ±11	52 ±10	51 ±18	38 ±9.6	28 ±9.4	18 ±8.6	10 ±12
Part C (PAS, RT)	56 ±20	48 ±18	42 ±24				
Part D (PAS. CS)	53 ±20	43 ±15	42 ±16	36 ±15	19 ±6	13 ±9	10 ±7

Aggregation response to Epinephrine

- We tested our samples with 10µmol of Epinephrine and the mean aggregation to 10µL of Epinephrine in individual parts over days of storage is shown in Table 4.
- The decrease of aggregation to Epinephrine in room temperature platelets was noticeably more than cold-stored platelets at five days of storage when kept in plasma.

Table 4: Response to Epinephrine over 21 days

	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14	Day 21
Part A (Plasma, RT)	36 ±13	24 ±11	16 ±6				
Part B (Plasma, CS)	36 ±15	29 ±8	23 ±8	19 ±8	12 ±8	7 ±7	2 ±6
Part C (PAS, RT)	26 ±11	20 ±8	16 ±7				
Part D (PAS. CS)	27 ±13	17 ±8	16 ±7	13 ±8	10 ±6	5 ±6	2 ±3

Platelet Function Analysis by Sonoclot

- The platelet function by Sonoclot was mostly maintained in all parts until the last days of storage.
- Platelet function was better maintained in Plasma stored platelets as compared to PAS stored platelets.

Table 5: Platelet Function by sonoclot for 21 days

	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14	Day 21
Part A (Plasma, RT)	4.8 ±0.5	4.6 ±0.4	4.2 ±0.5				
Part B (Plasma, CS)	4.7 ±0.6	4.4 ±0.5	4.3 ±0.5	±4.2 ±0.4	3.8 ±0.8	3.2 ±1.0	3 ±1.0
Part C (PAS, RT)	3.6 ±0.7	3.3 ±0.8	3 ±0.9				
Part D (PAS. CS)	4 ±0.5	3.9 ±0.4	3.8 ±0.4	3.7 ±0.2	3.3 ±0.6	3 ±0.8	2.7 ±0.9

Soluble P-selectin/CD-62 Analysis

- CD-62 is an activation marker present on the platelets surface and levels increases during platelet activation.
- The levels of CD-62 were increasing all parts during storage but the increase in room temperature platelets was steeper than cold-stored platelets.

Table 6:CD-62 levels over 21 days of storage

	Day 1	Day 3	Day 5	Day 7	Day 10	Day 14	Day 21
Part A (Plasma, RT)	22.7 ±5.5	31.57 ±5.4	40.2 ±12.8				
Part B (Plasma, CS)	20.3 ±8.0	23.3 ±9.4	27.6 ±3.6	28.3 ±8.9	42.7 ±16.4	51.3 ±17.8	64.9 ±23.2
Part C (PAS, RT)	2.5 ±2.1	27 ±7.4	37.8 ±15.0				
Part D (PAS. CS)	17.8 ±3.7	19 ±3.0	20.3 ±2.6	22.2 ±7	24 ±3.9	29.6 ±5.3	36.6 ±9.5

Analysis of IL-6 and TNF-α levels

- Most of the values of the interleukins were below the level of detection of the ELISA kit used (IL-6:2ng/ml) and TNF-α (8ng/ml)
- This was because the products were leukofiltered on day of preperation and the extremely low number of WBC produced negligible amount of cytokines as compared to suspending media volume.

Sterility

- All parts were sterile except Part A(RT/plasma) platelet of second experiment. Organism was found to be Staphylococcus epidermidis. This was attributed to improper sampling technique because other parts from same pool including Part C(RT/PAS) was found to be sterile.

Figure 2: Sonoclot Signature graph

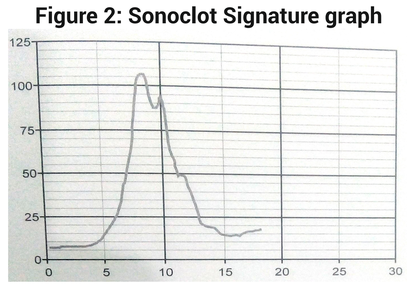
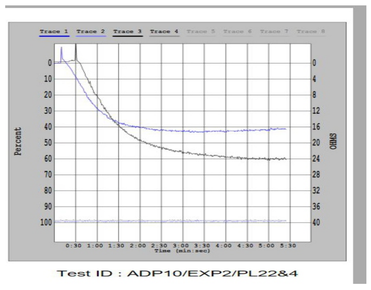


Figure 3: Aggregation graph



DISCUSSION

- Platelet storage has gone from variations in temperature to changes in shelf-life and storage container modification for the past 50 years as new research comes up and legal regulations and scientific consensus keeps on changing.
- The current modality of 5 days storage in a gas-permeable bag, suspended in plasma at 22±2°C with continuous gentle agitation has been contested by many authors.
- We have done this study to find out the optimum storage temperature and suspending media to extend the shelf life but keep platelets functionally active.
- The platelet count of Part A and B were within the guidelines for BCPP on all five days of storage.
- Cold Stored plasma suspended platelets (Part B) maintained acceptable counts until Day 7 of storage.
- The pH of the cold-stored platelets was above the required guidelines over the entire 21 days of storage in both plasma and PAS suspension. The cold-stored platelets showed better pH maintenance irrespective of suspension media up to 7 days of storage.
- Since the products were leukofiltered on the day of preparation, residual WBC was extremely low on initial days of storage and with cold exposure, WBCs were not detectable by the Nageotte chamber after 5-7 days of storage. Lesser number of WBCs also translated to a very low quantity of inflammatory cytokines in all 4 parts.
- Cold-induced activation of platelets is a known phenomenon in literature and is the main reason for not storing platelets in cold throughout the past few decades. We assessed the soluble-p Selectin (CD-62) levels to find out any major in-bag platelet activation while being kept at cold temperature but did not find any alarming level of activation of cold platelets in comparison to room temperature platelets.
- The rise in CD 62 is slower in cold platelets as compared to RT platelets. Cold platelets had CD-62 levels on Day 10 comparable to corresponding Day 5 RT platelets.
- We found a higher level of CD-62 toward the end of storage in platelets kept in plasma only indicating that PAS may have some protective effect on decreasing platelet activation.
- The functional ability of platelets was judged by aggregation response to weak agonists like ADP and Epinephrine by Optical Lumi-aggregometry. The responses to ADP were not significantly different across storage media or storage temperatures but fall in aggregation was slower in plasma stored cold platelets than plasma stored RT platelets.
- Platelets were showing above 30% aggregation to ADP till Day7 in cold-stored platelets.
- The platelet function was also assessed using the Sonoclot analyzer. Sonoclot showed no deterioration of platelet function (Level >1.5) in any individual parts during storage. Platelet function during storage using sonoclot was best maintained by plasma suspended Cold platelets and decreased in function was more rapid in PAS stored platelets than plasma stored platelets.
- The bacterial sterility of cold-stored platelets is a definite point in favour of extending the shelf life irrespective of suspending media used.

CONCLUSION

- Our study concludes that cold-stored platelets maintain invitro functional viability similar to or even better till day 7 to day 10 of storage both in plasma and PAS. PAS further helps in slowing the platelet storage lesions and we were able to demonstrate in-vitro functional viability of platelets up to 14 days.
- Further studies are required to evaluate the clinical efficiency of cold-stored platelets in patients requiring urgent hemostasis.