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Original Article

The Air Bubbles and Foam in the Bag of Platelet: Effects on the Quality of the Final Product

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ABSTRACT

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Key words
Air bubbles
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Background and Aims: The quality of platelets (PLT) during storage is influenced by various factors. The purpose of this study was to determine the potential effects of the air bubbles and foam in the bag of PLT and its effects on the quality of the final product.
Materials and Methods: In this paired-study, the air bubbles and foam, which develops after the preparation of the PLT units were excluded from the control units (n=10), but not from the test units (n=10). Various *in vitro* variables after the 5-day storage was evaluated, including measurements of PLT counts, mean PLT volume (MPV), Lactate dehydrogenase (LDH), PH, swirling, aggregation response, and the expression of CD62P.
Results: PLT count was lower (p=0.001) and LDH was higher (p=0.006) in the test vs. control units. PH was maintained at >7 (day 5) and swirling remained at the highest level (score=2) for all units throughout storage. No significant difference in MPV and CD62P expression was detected between the groups.
Conclusions: It was found that the storage of PLTs in bags containing air bubbles and foam after five day storage period increased disintegration of the PLTs.

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Introduction

Platelet (PLT) transfusion is widely used to prevent hemorrhage in different hematological diseases. Currently, one of the most significant discussions in transfusion practice is changes in PLT during storage. There is evidence that these changes can be associated with decreased post-transfusion survival and adverse events [1-3]. The quality of the PLTs can be affected by different factors such as method used for preparation [4], storage containers [5] and the transport systems [6]. It is also demonstrated that random occurrence of aggregates in PLT units leads to higher activation levels and increased release of immunomodulatory factors [7]. However, so far, there has been little discussion on the effect of air bubbles and foam on the PLT quality [8, 9]. Therefore, the aim of this study was to determine the effects of the air bubbles and foam in the bag of PLT and its effects on the quality of the final product, as measured by *in vitro* parameters after 5 days storage period.

Materials and Methods

PLTs were collected from healthy blood donors who visited a blood bank of Tehran, Iran. Initially, a total of 450 mL of whole blood (WB) were drawn into blood bag. After storage at room temperature for 2 to 6 hours, WB units were centrifuged at 22ºC and the separation of PLTs was done by PLT rich plasma (PRP) method [10]. A total of ten units was allocated to the test group (n=10) due to the presence of air bubbles and foam. PLT concentrates without air bubbles and foam were used as controls (n=10). All units were then stored on a flatbed agitator at 22±2ºC. On day 5 of storage, samples (10 ml) were aseptically collected from each unit. All the samples were tested for contamination by a microbiological culture performed at day 5. Cellular and metabolic *in vitro* parameters were evaluated, including measurements of PLT counts, mean PLT volume (MPV) and PH. The assessment of swirling was performed by inspection and grading according to Bertolini and Murphy [11]. PLT aggregation was measured by laboratory aggregometer and the maximum level of aggregation, expressed as % of aggregation. The extra cellular lactate dehydrogenase (LDH) activity (% of total), a marker for disintegration of PLTs, was assayed spectrophotometrically. Samples of PLT were assessed for PLT activation markers using a direct standard flow cytometry. The monoclonal antibody (Becton Dickinson) used were FITC*CD62P (P-selectin). The fluorescence of stained PLTs was analyzed (Cell Quest software, Becton Dickinson) to obtain the percentage of positively stained cells.

Statistical analysis

Results were expressed as mean±standard deviation (SD). Values of parameters were compared with a Student's *t* test for paired data. A p value of ≤0.05 was considered statistically significant. Statistical analyses were performed using SPSS 16.0 software.

Results

Bacterial contamination was not detected in any of the units (PLTs stored with (Test) or without (Control) air bubbles/foam) on day 5. We found statistically significant differences in a variety of parameters (Table 1).

Table 1. In vitro bubble (n=10) and control (n=10) platelet variables

Variables	Test platelet (Mean±SD)	Control platelet (Mean±SD)	P value	Normal range
Platelet count(×10^3/µL)	602.20±257.11	1031±254.28	0.001	-
Volume(ml)	56±8.5	60±5.6	0.221	40-70(ml)
PH (22º C)	7.05±0.135	7.20±0.125	0. 19	≥6.2
Swirling	2.5±0.85	3.10±0.7	0.109	≥2
LDH(U/per/bag)	727.71±1037	322.60±69.26	0.006	-
CD62P (%Positive)	33.74±19.07	27.41±14.07	0.408	14.1%
ADP %	4.70±3.30	7.60±9.32	0.366	76.4%
Ristocetin %	84.90±25.82	94.30±23.67	0.407	74.5
MPV (fL)	9.0±0.54	8.7±0.67	0.337	9.4-12.3

LDH= lactate dehydrogenase ; ADP= adenosine diphosphate ; MPV= mean platelet volume

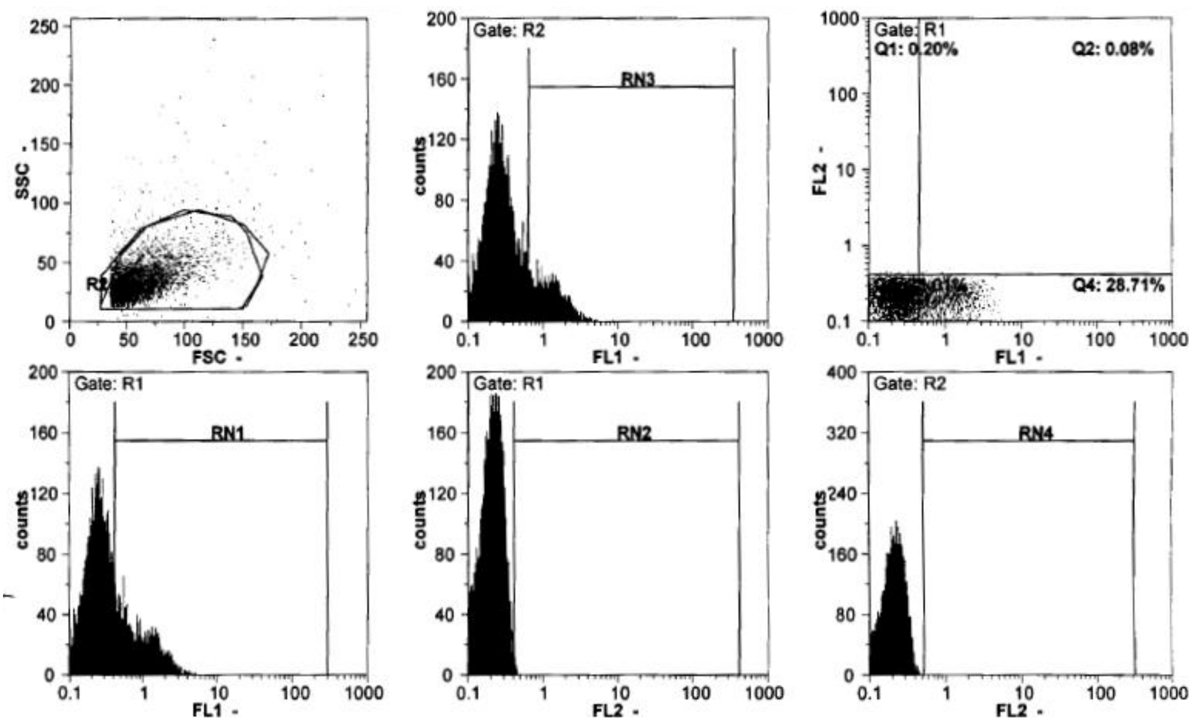

Fig. 1. The percentage of platelets expressing the activation marker CD62P (FITC mouse anti human CD62P)

There were statistically significant differences in platelet counts between the groups on day 5. PLT count in the control and test groups were 1031×10^3 and 602×10^3 PLTs/µL, respectively (p=0.001). LDH concentration increased in the test units (1037 U/L) and was significantly higher than the control units (322 U/L) on day 5 (p=0.006). Throughout the entire storage period, the PH values were within the established range of PLT products for clinical use. No significant difference in MPV and swirling was detected between the groups. Swirling remained at the highest level (score=2) for all units. The percentage of PLTs expressing the CD62P increased slightly in the test units on day 5. But no significant differences were detected (p=0.4) (Fig. 1). Stimulation of PLTs with 25 µM adenosine diphosphate on day 5 in the control and test groups was 7.6 and 7.4, respectively, no statistical significance was seen (p=0.366). The mean percentage of aggregation with 150 µg/mL ristocetin in the control and test PLTs were 94.30 and 84.90, respectively, which was not significantly different (p=0.4).

Discussion

Air bubbles and foam produce during agitation PLT units. These changes can interact with the PLTs, causing activation and release reactions. The effect of air bubbles/foam on PLT behavior is poorly characterized. In this study, we found significant differences in a variety of parameters between PLTs stored in the presence or absence of air bubbles/foam. Disintegration of PLTs caused by exposure to air bubbles and foam seems to be significantly enhanced. PLT counts were lower (p=0.001) and LDH was higher (p=0.006) in the test vs. control groups. LDH is a potential marker of cell damage. PH was maintained at >7 (day 5) and swirling remained at the highest level (score=2) for all units throughout the storage. The percentage of PLTs expressing the activation marker CD62P showed a slight increase, with not statistical significance (p=0.4). Our results also indicated that the presence or absence of air bubbles/foam has no effect on the PLT aggregation.

These findings are consistent with a previous report by Sandgren et al, [8]. They showed the decrease in PLT counts after 7 days and increase LDH and CD62P expression after 5 days in the units with air bubbles and foam. In another study, Oikawa et al, [9] compared the influence of the storage conditions on the *in vitro* PLT quality, including single-bag storage without air bubbles/foam and double-bag storage with air bubbles/foam. They found that air bubbles/foam induced the reversible activation of PLTs. Single-bag without air bubbles/foam had better quality for 24 h on a flatbed agitator. It is also reported that bubbles containing different gases (N2, He, Ne, Ar, or an O2-CO2-N2 mixture) are equally potent PLT agonists [12].

Conclusion

We found that the storage of PLTs in bags containing air bubbles/foam after the 5-day storage period increased disintegration of the PLTs. Therefore, due to the low quality of these

PLT bags, it is recommended that their use should be avoided, particularly in the patients requiring frequent transfusions of PLTs.

Conflict of Interest

The authors declare no competing interest.

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