



## Detection and Estimation of Platelet- Derived Microparticles Quantity during Storage Time

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### Abstract

**Background:** Platelet microparticles (MPs) are produced automatically during platelet storage. Their size is about 0.1 – 1  $\mu\text{m}$  and have ability for sub- endothelium adhering, strengthen of platelet aggregation and also platelet pre- coagulation. In fact, the release of membrane particles helps to signal to the neighbor cells or to seclude target cells from apoptosis. The purpose of this study is to determine the effect of time passing on platelet MPs production.

**Methods:** In this experimental study, 12 units of PC from healthy donors were obtained. Sampling was done at 2, 3, 5 and 7 days after storage. After separating of MPs, they were prepared for flow cytometry analyses by anti- CD41a FITC and assessed their concentration through Bradford methods. Paired sample T-test and ANOVA were used to compare the results and investigate the probable significant differences of this experiment.

**Results:** There was no significant difference in platelet MPs concentration between PC stored in day 2 and 3. ( $P > 0.05$ ) On the other hand, we could claim significant differences in platelet MPs concentration between PC stored in day 3 and day 5 and also between day 5 and day 7. ( $P < 0.05$ )

**Conclusions:** We showed that the production or releasing of platelet MPs were considerable by passing time. Also by increasing of storage time, platelet component included more MPs which were able to signal and produce interaction with other cells in patient receiving bodies. As a result, it was possible to interfere in their immune responses, inflammatory or coagulation processes after transfusion.

**Keywords:** Platelet, Microparticle, Hemostasis, Thrombosis, Inflammatory.

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are approximately 0.1-1  $\mu\text{m}$  in size and can adhere to the sub-endothelium, strengthen platelet aggregation, and cause platelet pre-coagulation. In general, platelets are shed from cells under physiological or pathological conditions. In addition to platelets, other cells including endothelial cells, monocytes, granulocytes, and red blood cells (RBCs) release MPs. Depending on the original cell or production mechanism, the MPs differ in both quantity and quality. Platelet MPs are reported as the most abundant MP subtypes in circulation and are considered to contribute to inflammatory diseases such as arthritis and atherosclerosis.<sup>3,4</sup>

The purpose of this study was to determine the effect of time storage on platelet MP release.

### Materials and Methods

In this study, 16 units of PC from healthy donors were obtained. Sampling was conducted at days 2, 3, 5, and 7 after storage. Although there was no specific blood group selection goal, all of the samples belong to the O blood group. To separate the platelet MPs, the platelets were pelleted by centrifugation at 4500 $\times$ g for 10 min. The supernatant was collected and centrifuged at 16000 $\times$ g for 20 min. The supernatant was discarded and sterile PBS was added to the precipitate and gently inverted and again centrifuged at 16000 $\times$ g for 20 min. MPs were prepared for flow cytometry using anti-CD41a FITC to determine the status and origin. The concentrations of MPs were determined using the Bradford method and a spectrophotometer set at 595 nm. Bovine serum albumin was used as the standard, as previously described.<sup>5</sup> Paired sample T-test and ANOVA were used to compare the results and determine the significance of the experiment.

### Results

The criteria of platelet MPs detection is their size in the range of 100-400 nm and the CD41 antigen in the surface as the CD marker of platelets. The MPs flow cytometry revealed an abundant population of MPs with a diameter of approximately 100-400 nm accompanied with CD41a presentation in the surface of them approved their platelet origin. The amount of MPs liberated from the platelets significantly increased as time passed. The mean and standard deviation of platelets MPs protein concentration at days 2, 3, 5, and 7 were 514.3 $\pm$ 216.1, 515.1 $\pm$ 210.4, 708.3 $\pm$ 325.8, and 1230.6 $\pm$ 660.4  $\mu\text{g/ml}$ , respectively. A significant difference was considered at  $P < 0.05$ . No significant difference in platelet MPs concentration was observed between PC stored at day 2 and day 3 ( $P > 0.05$ ). In contrast, significant differences were observed in platelet MPs concentration between PC stored at day 3 and 5 and between day 5 and 7 ( $P < 0.05$ ; Table 1).

## Introduction

Platelets play a crucial role in hemostasis and thrombosis. If a transfusion is inevitable, physicians need platelets of dependable quality for treating their patients. Progresses in surgical method have led to an increase in the use of platelets. In Europe, platelet concentrates (PC) are prepared by buffy-coat extraction from several units of whole blood (Pooled platelets) or obtained through apheresis (Single-donor platelets) and have to be kept at room temperature (22 $^{\circ}\text{C}$ ).<sup>1</sup>

Wolf described releasing membrane pro-coagulant vesicles from activated platelets in 1967.<sup>2</sup>

It has been reported that platelet microparticles (MPs) are produced automatically during platelet storage. Platelets MPs

**Table 1. The mean of protein concentration related to platelet micro particles of PC sample in 2, 3, 5 and 7 day of storage.**

	2 <sup>nd</sup> day (Mean±SD)	3 <sup>th</sup> day (Mean±SD)	5 <sup>th</sup> day *(Mean±SD)	7 <sup>th</sup> day *(Mean±SD)
The concentration of platelet micro particles (µg/ ml)	514.286±216.11	515.1±210.35	708.33±325.82	1230.56±660.35

\*P&lt;0.05

## Discussion

The presence of pro-coagulant or pre-inflammatory vesicles in plasma that was centrifuged at high speed has been reported and distinguished many years ago. However, Wolf described the release of membrane pro-coagulant vesicles from activated platelets as “platelet dust” in 1967.<sup>2,4</sup> Since then, various studies have been conducted on the shed particles of activated or apoptotic cells and their presence in human plasma. There are three types of membrane shedding fragments including exosome, apoptotic bodies and MPs. Among them, MPs are particles with a diameter of 100 nm and are derived from the cytoplasmic cell membrane. Exosomes are derived from the endoplasmic membranes and are smaller than MPs (40-100 nm). Larger vesicles with a 1.5 µm diameter involving nuclear material are considered “apoptotic bodies.”<sup>3,4,6</sup> In this study, we investigated the effect of storage time on platelet MPs concentration. We observed that there was no significant difference in platelet MPs concentration between PC stored at day 2 and day 3 (P>0.05). On the other hand, we could claim significant differences in platelet MPs concentration between PC stored in day 3 and day 5 and also between day 5 and day 7 (P<0.05). Although time storage had not been influenced in the platelet MPs production at days 2 and 3 – there was significant difference between MPs production in days 3,5,7. That means the more time passed, the more MPs were produced.

MPs cause the anionic phospholipid phosphatidylserine to be in the outward form, which plays an important role in coagulation and complement stimulation processes. Many studies have discussed the significance of MPs derived from different cells as both a marker and an activator of numerous diseases. In addition, the potential predictive importance of plasma MPs to estimate health problem risk has been reported.<sup>2,7,8</sup> They are also considered as membrane shedding that establishes a signaling being to other cells such as phagocytes or nearby cells, and consequently, initiates their interaction by regulating inflammation, immune responses, and mending processes. It is considered platelet MPs act in signaling and produce interaction between cells; for example by signaling to cells like phagocytes, they starts regulating of inflammation and immunomodulatory. Additionally the cells may shed MPs in order to release the cellular complex of apoptosis and finally continue to live or save from cellular death. Therefore platelet MPs may transfer and enter there signaling bodies to patients. In fact, the release of membrane particles helps to signal the neighbor cells or to seclude target cells.<sup>2</sup>

Platelet MPs are cell membrane vesicles derived from apoptotic or activated cells and contain many specific platelet antigens including CD41 or CD61.<sup>2,3</sup> Platelet MPs could not be detected by common counting techniques used for blood cells. However, they could be detected by flow cytometry and using the detection of protein concentration methods.<sup>3,5,9</sup> It has been reported that MPs participate in the pathophysiology of many

disorders including cardiovascular, pulmonary problems, or even renal failures. The amount of platelet MPs increase in various types of thrombosis diseases including heparin induced thrombocytopenia, immune thrombocytopenic purpura, diabetes mellitus, cardiovascular diseases, and current abortion.<sup>2,5,9</sup> These findings show the effect of platelet MPs in hemostasis and thrombosis.<sup>3</sup> Different characteristics of platelet MPs have been extensively studied including their pro-coagulation functions and specific surface antigens. A study conducted by Yari (2011) showed no significant differences in platelet MPs quantity stored in the plasma or composol. However, the generation of platelet MPs increased significantly in the plasma compared to the composol after day 7 of storage. Therefore, there was a benefit of composol rather than a PC storage medium.<sup>5</sup> In addition, we showed that the production or release of platelet MPs increased as time passed and the platelet component had more MPs with increased storage time, since one of the functions of MPs considered signal transduction. MPs of platelets component are able to signal and interact with other cells in patient receiving bodies. Therefore, MPs can interfere with the immune responses and inflammatory or coagulation processes.

## Conflict of Interest

The authors declare that they have no conflict of interests.

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