

# An Overview on Platelet-derived Microparticles in Platelet Concentrates: blood collection, method preparation and storage

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## Abstract

Preparations of platelet concentrates (PCs) that are stored under blood bank conditions and used for transfusion purposes, appear to be enriched in platelet derived-microparticles (PMPs) with high coagulant activity that may change platelet efficacy and safety issues. High shear stress could cause shedding of PMPs from the platelet plasma membrane, platelet aggregation, and activation of the coagulation cascade by increasing the catalytic phospholipid surface. These stresses may be prompted by processing and storage of blood and platelet rich plasma through various variables that has been fully described in this review. Depending on different rates of shear stress during processing and storage of PC, different quantities of MPs might be shed from platelets. On the other hand, the therapeutic effect of high levels of PMPs in PC has been reported for some patients. By using more sensitive and standardized methods for PMP measurement and change of platelet preparation process, further studies are required to monitor PMP generation during blood collection, processing and storage of PC to improve quality of PC and also in recipient's reactions to transfusion.

**Keywords:** Platelet-derived microparticle, Platelet concentrate, Blood collection, Platelet storage

## Introduction

Membrane fragments of various cells such as platelets, leukocytes, erythrocytes, and endothelial cells, are shed into the circulation. There is increasing evidence that these submicron fragments, defined as microparticles, have significant physiological roles<sup>1</sup>. Platelet microparticles (PMPs) are the most abundant microparticles in the bloodstream, constituting approximately 70% to 90% of circulating microparticles<sup>2</sup>. Platelets have been demonstrated to release microparticles during storage of whole blood<sup>3</sup>. Similar results were reported for platelet concentrates (PCs)<sup>4</sup>. The presence of microparticles in platelet-rich plasma has first been described in the late 60s, where these were called as platelet-dust<sup>5</sup>. Platelet microparticles are released from platelets upon activation or apoptosis. Platelet concentrates contain PMPs in the supernatant, reflecting activation during collection, and/or

storage. In general, the loss of platelet quality over storage is known as the platelet storage lesion, or the platelet storage deficit<sup>6</sup>. Platelet storage lesion include activation, proteolysis and changes in morphology, membrane glycoproteins and surface receptor expression<sup>7</sup>. A clinically important issue is that PMPs may have pro-coagulant activities and during blood component storage may have unintended consequences when transfused<sup>8</sup>, because, it has been demonstrated that PMPs have from 50 up to 100-times higher pro-coagulant activity than platelets<sup>9</sup>. These PMPs pro-coagulant properties are attributed to the presence of anionic phospholipid, particularly phosphatidylserine (PS), and the procoagulant protein tissue factor, which is the major cellular activator of the clotting cascade<sup>10</sup>. In addition, phospholipid surface enables the binding of coagulation factors and the formation of

tenase and prothrombinase complexes<sup>10,11</sup>. This hypercoagulability state of PMP is associated with an increased risk of fibrin deposition in blood vessels, which could result in thrombus formation<sup>12</sup>. Besides their role in coagulation, PMPs are involved in intercellular transport and transfer of bioactive molecules, cell activation and inflammation<sup>13,14</sup>.

Besides coagulation, PMPs play a significant role for immune regulation as well<sup>15,16</sup>. It is well-known that side effects of platelet transfusion include fever and transfusion-related acute lung injury<sup>16</sup> and increased PMP levels in platelet units were shown to be correlated with various allergic transfusion reactions<sup>4</sup>. The ability of PMPs to interact with leukocytes via P-selectin binding to PSGL-1<sup>4,17</sup> as well as their potential to induce CD154 (CD40L)-mediated B-cell activation<sup>18</sup>, confirms their ability to affect the immune system. The presence of various signaling molecules on PMPs makes it even more likely that they contribute to the transfusion burden<sup>19</sup>. While most circulating PMPs are derived from megakaryocytes<sup>20</sup> P-selectin+ PMPs constitute an essential part of the PMPs storage burden, and probably have a distinct biological function<sup>4,21</sup>. Furthermore, PMPs can cooperate with platelets to transport RNA, cytokines, or chemokines to other cells and tissues, as they are small enough to exit the circulation and to enter the surrounding tissues<sup>22</sup>. In addition, these microparticles may participate in cell-to-cell interactions with the platelets, monocytes, leucocytes and endothelial cells as well<sup>23</sup>.

Platelet-derived microparticles generated during storage may be an important contributor to adverse reactions to transfusions, because it has been described that PC contain PMPs, which may be related to the increased incidence of venous thrombosis and embolism following platelet transfusion<sup>24</sup>. These PMPs have been detected in PC prepared from whole blood<sup>25</sup> and in apheresis concentrates<sup>26</sup> and have been related to harvest conditions and storage stress. Due to platelet microparticle involvement in hemostasis and thrombosis, removal of these active small vesicles from transfused platelets speculatively could decrease prothrombotic complications of recipients. In general, this study wants to highlight the factors that may affect in levels of PMPs during

blood collection, method preparation and storage of PCs with regarding to standardize and improve platelet quality for transfusion.

## Blood collection

### Venipuncture and needles

Previous studies have shown that, the method of blood collection, including the needle diameter, application of a tourniquet and the use of a vacutainer to withdraw blood may all affect the PMP concentration in the sample. In addition, the type of anticoagulant used has a profound effect on observed PMP numbers, as exemplified by the apparent loss of PMP and endothelial cell-derived MP in chelating anticoagulants<sup>27,28</sup>. Needles ranging from 19 to 22 Gauge (G) are commonly used for venipuncture in haemostasis laboratories<sup>29</sup>. Prolonged placement of a tourniquet is avoided during venepuncture due to the change of several hematologic parameters<sup>30</sup>. The impact of preanalytical steps, like the collection of blood samples, has already been analyzed and discussed by other investigators<sup>28,31</sup>. It is recommended to collect samples in an atraumatic fashion with larger needles to avoid sheer stress and endothelial activation. During blood collection, the first 3–7.5 ml of blood is discarded to avoid the effects of the vascular damage caused by the venepuncture for the sample analysis in the lab<sup>32</sup>. Similarly, in blood transfusion services the diversion of the first 10-50 mL of donated blood with the aim of preventing microbes or skin fragments from entering the collection bag, has been recommended by some investigators<sup>33,34</sup>. Sensibly, the presence of these unexpected substances in PC may activate platelets and possibly enhance PMPs generation.

### Blood volume and time of blood donation

According to the technical manual guidelines of AABB, blood collection volumes must be within the manufacturer's specified range to ensure the correct anticoagulant-to-WE (whole erythrocyte) ratio. High-volume allogeneic collections should be discarded. Low-volume allogeneic collections should be relabeled as "RBCs Low Volume". Plasma and platelets from low-volume units should not be used. The average time to collect 500 mL of blood is less than 10 minutes. A draw time longer than 15 to 20 minutes may not be suitable for collecting platelets or plasma for transfusions.

The collection bag should be periodically mixed during the collection to ensure uniform distribution of anticoagulant<sup>35</sup>. Previous studies have shown that the type of anticoagulant and its concentration<sup>36-39</sup>, platelet concentration in the bag<sup>40</sup> may help to limit platelet activation during blood collection and platelet preparation, because platelet activation will result in release of alpha and dense granules and PMPs<sup>41</sup>. Interestingly, blood collected in heparin gave significantly higher levels of annexin V-positive microparticles than blood collected in sodium citrate<sup>28</sup> and EDTA alters the expression of GP IIb/IIIa<sup>42</sup>. Rationally, long time of blood donation and reduction of blood flow may increase the platelet activation<sup>29</sup> and so may enhance PMP generation.

### Platelet concentrate preparation

#### *Preparation methods*

Platelet concentrates could be prepared through an automated process of blood cell selection, called apheresis, or from whole blood centrifugation such as platelet-rich plasma (PRP) method or even buffy coat (BC). Variables involved in these procedures cause changes in morphology, activation, aggregation and fragmentation of the PC<sup>43</sup>. In Europe, the routine removal of BC allows the use of more intense primary centrifugation, which improves plasma recovery. The platelets are sedimented onto the BC, and if the separated BC is then diluted with plasma, it is possible to separate platelets by differential centrifugation<sup>44,45</sup>. There is a remarkable quality improvement in laboratories marking platelets using buffy coat (BC-PC) method relative to platelets produced by PRP (PRP-PC) method<sup>46,47</sup>. The second centrifugation in the preparation of PC in PRP-PC method is associated with reversible platelet aggregation and contact with non-biological surface, which probably is induced by activation due to the close contact between platelets in the platelet pellet while, BC-PC method does not involve significant close cell contact between platelets in a pellet. In addition, PRP-PC method may stimulate more degranulation or activation during hard spin centrifugation without the presence of WBC and RBC as a barrier "biologic cushion" to lower these stresses. It seems that buffy coat platelets possibly experience less stress during production than PRP platelets<sup>48</sup>. The quality of apheresis platelets may

be dependent on the type of cell separator used<sup>49</sup>. As a whole, centrifugation and mechanical stimulation may induce platelet activation<sup>50</sup>, MP aggregation and PS exposure<sup>51</sup>, and in that way also influence biological activity of PMP.

#### **Leucocyte depletion methods**

The leucocytes present in PC can cause febrile transfusion reactions, HLA alloimmunisation and CMV transmission to recipients. Prestorage whole blood leukofiltration was demonstrated to significantly reduce initial PMP and platelet counts, consistent with other blood product and filter combinations previously reported<sup>52</sup>. In addition, Sugawara and et al. also indicated that PMP and platelet-gated events increase by 2 log or more during storage of unfiltered whole blood<sup>3</sup>. Besides of filtration, light spin centrifugation at the second step of BC method can significantly remove WBC and provide lower residual WBC in PC in comparison with PRP method<sup>46,53,54</sup>. On the other hand, an increasing number of studies indicate that PMP play a significant role in cell-to-cell interactions with neutrophils, monocytes and endothelial cells<sup>55</sup>. Aggregation formation followed by these interactions may facilitate the removal of PMP during filtration or centrifugation.

#### **Storage conditions**

##### *Storage containers*

The plastic material of the storage bag 40 and the ability of bags to exchange gas across its surface<sup>56</sup> are the variables that may influence the quality of platelets during storage. Moreover, storage-dependent PMP generation is possibly the result of platelets activation that is enhanced by interaction of platelets with the bag walls<sup>25</sup>. It was observed that platelet reactivity is better maintained in the storage bags manufactured without the plasticizer di-(2-ethyl-hexyl) phthalate (DEHP) which is slowly leached from the wall of the PVC blood bags into the blood products<sup>57</sup>. Previous studies have shown that polyvinyl chloride (PVC) plastic material with a butyryl-n-trihexyl citrate (BTHC) versus tri-(ethylhexyl)-tri-mellitate as a reference container had no statistically significant difference for the 5-day storage of platelets<sup>58</sup>. Albeit, this reference container material made of polyvinyl chloride has been previously demonstrated to be safe for platelet transfusion practice<sup>59</sup>. These

observations confirms that inner wall of the plastic container play a significant role in the platelet quality and may induce complement activation and/or platelet activation and thereby could augment PMP generation.

### Platelet additive solutions

In order to improve PC quality and clinical effectiveness, storage in synthetic media has been recommended. The practical advantages of platelet additive solutions (PASs) include: i) the reduction of platelet storage lesions caused by the rapid accumulation of lactate and the consequent pH fall; ii) the removal of some plasma components which could contribute to thrombin, plasmin, complement and kallikrein/kinin generation; and iii) the partial removal of cell-released enzymes and microparticles, in particular soluble HLA<sup>60</sup>. In addition, PASs can be produce sterile and pathogen-free, have a standardized composition in contrast to donor plasma variation, more plasma for fractionation, possible using pathogen reduction technologies and reduced protein content and antibodies that may decrease allergic reactions and better toleration of ABO-incompatible transfusions<sup>61</sup>. In spite of these advantages, PASs may require at least 30% residual plasma in the PC to maintain platelet quality and in the buffy coat method, the centrifugation of the buffy coat pool is more difficult due to the lower viscosity of PAS, and therefore the platelet yield is generally 10–15% lower as when plasma is used<sup>61</sup>. Many attempts have been made to use different additive solutions to improve platelet quality and long-term storage of PC<sup>62-65</sup>. Magnesium is considered to reduce platelet activation in vitro and both potassium and magnesium decrease glucose consumption<sup>62</sup>. The effect of glucose<sup>64</sup> and bicarbonate<sup>65</sup> in PASs have been recently investigated in PC. Unfortunately, there is not much evidence regarding the effect of the various composition of PASs on PMP generation in PC. Yari and coworkers have shown that the binding of PMPs to vWF was affected from the storage media of PC (plasma and Composol) but PS exposure was not affected from the type of storage media<sup>66</sup>. These findings indicate that PASs may change PMP biological activity during PC storage.

### Agitation

Between the years 1982 and 1988, George and coworkers<sup>7,67</sup> isolated and partially characterized membranous MP in supernatant preparations of fresh frozen plasma, serum, and platelet concentrates. They observed a population of MP bearing platelet membrane glycoprotein (GP) IIb that increased over storage time in the supernatant plasma of PCs at a rate dependent on the type of agitation used. The effects of rotary and flat shakers<sup>68</sup>, interruption of agitation<sup>69</sup>, agitation and resting combination<sup>70</sup> and interval manual agitation<sup>71</sup> on the platelet quality have been demonstrated by previous studies. Increased gas exchange capacity and uniform platelets distribution in the bag are the reasons that may explain platelet pacification during agitation. In contrast, platelet oxygen transfer may not be efficient during the period that the PC remain stationary (“resting”)<sup>72</sup> which may drop the pH. Interestingly, recent studies have shown that strong agitation of blood samples during transportation may stimulate MP generation and effects on MP measurement<sup>51,73</sup>. Little research has focused on the PMP release by platelets in the PC during agitation and needs to be further investigated.

### Storage temperature

Platelet concentrates have a short period of life of 5 days and are relatively susceptible to environmental changes. Platelets are stored between 20 °C to 24 °C with constant agitation which is necessary for the maintenance of platelet viability<sup>40,68,74</sup>. There are two main reasons that platelets shelf life is limited to a small number of days. The first one is the risk for bacterial contamination and the second one is loss of quality that raises concerns about the efficacy of a transfusion. On the other hand, lower temperature keeping of PC may have detrimental effects. Since, cold activation of citrated blood samples has been reported to result in falsely elevated levels of (activated) factor VII and factor VIII and this may lead to the loss of von Willebrand factor, and platelet disruption<sup>75</sup>. Moreover, storage at low temperature has long been deliberated unsuited with platelet transfusion because cold activation led to alterations in platelet shape<sup>76</sup> and P-selectin expression<sup>77-79</sup>. In addition, refrigeration of platelets increases cytosolic calcium, actin

polymerization and shape change<sup>80</sup>. In contrast, it has been demonstrated the platelets stored at 4° C retain their in vitro characteristics better than those stored at 22° C, except for parameters that reflect changes in shape<sup>63</sup>. It has been reported that annexin V(+) and tissue factor (+) microparticles may significantly reduce upon sample storage at low temperatures<sup>28</sup>.

Some studies have looked at preanalytical variables, including thawing temperature<sup>31</sup>, increased annexin V-positive microparticles after storage at -80°C for 24 hours<sup>81</sup> and increase PMP storage at 4°C<sup>82</sup>. Recently, pre-analytical issues such as sampling, storage and freezing have been well reviewed by Mullier and coworkers<sup>73</sup> in the measurement of circulating microparticles. Information on the effect of storage temperature on PMPs in PCs is often lacking in studies and should be further investigated. It may be assumed that the change of temperature during storage of PC might led to platelet shape change, activation and probably PMP generation.

## Conclusion

It is well described that PCs contain MPs, which may be related to the increased incidence of venous thrombosis and embolism following platelet transfusion<sup>24</sup>. Microparticles released from platelets may contribute in the normal hemostatic responses to vascular injury because they demonstrate prothrombinase activity<sup>83</sup>. Meanwhile PMPs accumulate in PC during storage, transfusion of more or "older" units will offer to the recipient higher number of PMPs. The transfused PMPs could increase the risk of adverse reactions, by inducing a hypercoagulable state leading to thromboembolic complications. Inversely, in other situations requiring blood transfusion, a hypercoagulable state may be useful to reduce or even helping to stop the bleeding.

Observed haemostatic advantage in clinical trial from transfusion of older stored platelets may be due to increased PMP generation during storage<sup>84</sup>. The functional significance of PMP as an infusible platelet membrane (IPM) product in vivo was indicated by previous and recent studies<sup>85-88</sup>. Rank and coworkers reported in a recent investigation that most of the MPs of apheresis of PC originated from unstimulated platelets and their levels did not change during storage<sup>21</sup>. The PMPs are an

essential part of the platelet product, which is not detected commonly used by routine blood count analyzers and are not routinely implemented by blood transfusion services and may needs to be considered as a quality marker for monitoring of PC. Interestingly, single platelet units may contain higher concentrations of PMPs compared to double platelet units, while the platelet concentration of both products did not differ significantly<sup>89</sup>. Platelet concentrates contain MPs from resting platelets, activated platelets which accumulate during storage and also white blood cells and endothelial cells that can contribute in cell-to-cell interactions in the PC. Regardless of the cause of PS+ MP release, the presence of these particles may facilitate these interactions and transfer of signals and receptors between different cell types, inducing thus signalling and response in a variety of cells that may start positive loop of further PS+MP release from previously resting endothelial cells and platelets<sup>90</sup>. It is noteworthy that shedding of procoagulant microparticles can occur by unstimulated platelets as well<sup>91</sup>.

In summary it can be concluded that preparations of platelet concentrates (PC) that are stored under blood bank conditions and used for transfusion purposes, appear to be enriched in platelet derived-microparticles ( PMPs) with high coagulant activity that may change the platelet efficacy and safety issues. High shear stress could cause shedding of PMPs from the platelet plasma membrane, platelet aggregation, and activation of the coagulation cascade by increasing the catalytic phospholipid surface. Shear stress of platelets and other blood cells is prompted by processing and storage of blood and platelet rich plasma through prolonged placement of tourniquet, the needle diameter, entering of skin fragments, microbes and released-tissue factor into the collection bag by excessive probing with the needle, longer donation time, blood volume to anticoagulant ratio, inappropriate mixing during blood collection, methods of PC preparation (centrifugation speed, hard or light brake), type of additive solution, type of cell separator, material and gas exchange capacity of the plastic bag wall, type of extractor, single or pooled platelet preparation, prestorage leukofiltration of whole blood, rotatory or flat shaker (vertical or horizontal), continuous or interruption of agitation, rate of agitator, storage



temperature and transportation status. Depending on different rates of shear stress during processing and storage of PC, different quantities of MPs might be shed from platelets and other cells as well. On the other hand, the therapeutic effect of high levels of PMPs in PC has been reported for some patients. By using more sensitive and standardized methods for PMP measurement and change of platelet preparation process, further studies are required to monitor PMP generation during blood collection, processing and storage of PC that may affect quality of PC and also in recipient's reactions to transfusion.

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