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REVIEW

Platelet microparticles: Detection and assessment of their paradoxical functional roles in disease and regenerative medicine

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ABSTRACT

There is increasing research on and clinical interest in the physiological role played by platelet microparticles (PMPs). PMPs are 0.1–1-μm fragments shed from plasma membranes of platelets that are undergoing activation, stress, or apoptosis. They have a phospholipid-based structure and express functional receptors from platelet membranes. As they are the most abundant microparticles in the blood and they express the procoagulant phosphatidylserine, PMPs likely complement, if not amplify, the functions of platelets in hemostasis, thrombosis, cancer, and inflammation, but also act as promoters of tissue regeneration. Their size and structure make them instrumental in platelet–cell communications as a delivery tool of platelet-borne bioactive molecules including growth factors, other signaling molecules and micro (mi)RNA. PMPs can therefore be a pathophysiological threat or benefit to the cellular environment when interacting with the blood vasculature. There is also increasing evidence that PMP generation is triggered during blood collection, separation into components, and storage, a phenomenon potentially leading to thrombotic and inflammatory side effects in transfused patients. Evaluating PMPs requires strict pre-analytical and analytical procedures to avoid artifactual generation and ensure accurate assessment of the number, size repartitioning, and functional properties. This review describes the physical and functional methods developed for analyzing and quantifying PMPs. It then presents the functional roles of PMPs as markers or triggers of diseases like thrombosis, atherosclerosis, and cancer, and discusses the possible detrimental immunological impact of their generation in blood components. Finally we review the potential function of PMPs in tissue regeneration and the prospects for their use in therapeutic strategies for human health.

1. Introduction: scientific and clinical relevance of platelet microparticles (MPs, PMPs)

Much is already known—and much still remains to be discovered—about the roles played by platelets in human health. Platelets are quantitatively and qualitatively important blood cells that play key roles in hemostasis, and are also critically involved in physiological processes influencing tumor metastasis, vascular biology, angiogenesis, immunity, and tissue regeneration. Platelets are sensitive health markers that are prone to activation under physiological conditions involving stimulation of blood coagulation, as occurs as a result of trauma or pathologies like cancer. Therefore, platelets fulfill wide roles in balancing health and disease.

Recently, much interest has been devoted to cellular MPs that are fragments shed from plasma membranes of virtually all cell types that are undergoing apoptosis or are being subjected to various types of stimulation or stress. They are distinct from exosomes which are shed from intracellular membranes. In general, they are collectively referred to as microvesicles [1]. As characterization methods are still under

Abbreviations: AFM, atomic force microscopy; BDNF, brain-derived neurotrophic factor; bFGF, basic fibroblast growth factor; CAC, circulating angiogenic cell; CSF, cerebrospinal fluid; DLS, dynamic light scattering; ELISA, enzyme-linked immunosorbent assay; EGF, epithelial growth factor; EOC, endothelial outgrowth cell; FVIII, factor VIII; FX, factor X; FACS, fluorescence-activated cell sorting; FC, flow cytometry; GP, glycoprotein; HUVEC, human umbilical vein endothelial cell; IL, interleukin; ITP, immune thrombocytopenic purpura; MCAO, middle cerebral artery occlusion; MP, microparticle; MS, mass spectrometry; NTA, nanoparticle tracking analysis; PDGF, platelet-derived growth factor; PMP, platelet microparticle; PPL, phospholipid; PS, phosphatidylserine; TEM, transmission electron microscopy; TGF-β1, transforming growth factor-β1; TRPS, tunable resistive pulse sensing; TF, tissue factor; VEGF, vascular endothelium growth factor; VTE, venous thromboembolism.

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development, care should be taken when using terms for specific subsets [2]. Several types of MPs, with a heterozygous size estimated to range from approximately 0.10 μm (100 nm) to 1 μm, circulate in the blood at an estimated concentration of 5–50 μg/ml [3]. These MPs are circulating phospholipid (PPL)-enclosed vesicles that originate from either endothelial cells, erythrocytes, leukocytes, megakaryocytes, or platelets. The external membrane layer of the cell membrane consists of phosphatidylcholine and sphingomyelin, while the inner layer contains phosphatidylserine (PS) and phosphatidyl-ethanolamine, an asymmetrical structural arrangement that confers stability to MPs [3]. MPs express membrane markers characteristic of cells from which they originate. Exposure to PS, an anionic PPL, promotes the formation of procoagulant protein complexes. For instance, an increase in the cytosolic calcium concentration is one of the factor that triggers the release of MPs [4], eventually leading to an enzymatically controlled (floppase and scramblase) remodeling of plasma membranes involving the translocation of anionic PPLs from the inner membrane to the outer membrane [5] and MP release [6].

The roles that PMPs, previously known as "platelet dust" [7], play in complementing the various physiological functions of platelets are the subject of active research and clinical interest. *In vivo*, PMPs can be released from platelets under normal physiological conditions or as a result of activation, stress, or apoptosis. From a study of surface markers, PMPs are probably the most abundant MPs in healthy subjects, accounting for 70%–90% of those circulating in the blood [8], with a range of approximately 100–1000/μL [9]. Megakaryocytes can also release MPs [10], but determining their proportion requires further studies. The remaining MPs are released by endothelial cells, leucocytes, and red blood cells. The evaluation of PMPs continues to require the development of strict pre-analytical and analytical procedures to ensure the most accurate assessment of the number, size repartitioning, and functional properties. The number of PMPs increases as a result of activation of the coagulation cascade or of the complement system, or when under the influence of apoptotic signals or shear forces. The PMP number increases in several prothrombotic and inflammatory disorders, and some cancers, as discussed below [11]. As PMPs can express functional receptors from platelet membranes and are PPL-based nanoparticles, they are increasingly regarded as being instrumental in platelet–cell and cell–cell communication [12]. They contain bioactive molecules capable of transferring messages to neighboring or target cells [13]. As such, PMPs can potentially be a threat or benefit to the cellular environment that is interacting with the blood vasculature as they expose a procoagulant PPL surface and act as a conveyer of a range of bioactive molecules, including growth factors and other signaling molecules, and genetic materials, including miRNAs [1,14,15]. PMP generation is also triggered *in vitro* during blood cell processing and storage [16], a phenomenon that can potentially induce transfusion side effects [9]. Whereas PMPs can amplify the actions of activated platelets in promoting coagulation and negatively influencing pathological events [17], it is increasingly suspected, paradoxically, that they may have beneficial effects in enhancing vascular tissue repair and regeneration. This paper reviews the methods currently available for characterizing PMPs, then discusses the paradoxical physiological roles they are thought to play in human health.

2. Detection methods

Accurate characterization of PMPs requires rigorous handling of blood and other samples of biological fractions to avoid experimental artifacts. In addition, a judicious choice of analytical methods, combining techniques characterizing the cellular origin, number, size, and functional activity should be made in PMP assessments [2,18]. In some assays, risks of interference with protein aggregates or other particles sharing biophysical characteristics with PMPs can occur but can be minimized by differential lysis using a non-ionic detergent such as 0.1% Triton X-100 [19].

2.1. PMP isolation

When assessing PMPs, very rigorous pre-analytical handling and processing of blood and plasma samples (*e.g.*, phlebotomy, transport, and plasma preparation and storage) are needed to avoid artifactual generation of PMPs [20–22], and facilitate intra- and inter-laboratory standardization and data interpretation [23,24]. Important experimental factors to consider include (a) using a large needle size (21-G minimum); (b) discarding the first few milliliters of blood collected that are prone to artifactual activation; (c) using a citrate anticoagulant to limit vesiculation; (d) limiting agitation and horizontal transportation of blood tubes to avoid PMP generation; (e) delaying by a 2-h maximum between blood collection and the first centrifugation to eliminate most of the platelets; and (f) carefully controlling centrifugation procedures [22]. Several-fold artifactual increases in PMPs were found to occur as a result of a freeze/thaw cycle of plasma not fully depleted of platelets [19,21]. Therefore, ensuring extensive removal of platelets prior to freezing is required for an accurate PMP analysis [20]. Platelet removal can be achieved by centrifuging freshly collected blood at 2500 *g* for 15 min at room temperature twice to isolate PMPs in the plasma [22]. Any procedure used should be validated to ensure the absence of platelets after isolation of PMPs [10]. Centrifugation at 2880 *g* for 20 min within 1 h of blood collection reduces the platelet count to approximately 1% that of the baseline blood level. One additional centrifugation at 10,840 *g* for 5 min, or centrifugation twice at 2880 *g* for 20 min, decreases the content to 0.02%. Once platelets are eliminated, plasma containing the PMPs, or isolated PMPs, can be frozen at −80 °C until being analyzed. When interpreting the data, potential contamination of PMP samples by platelet exosomes of 40–100 nm, which originate from multivesicular bodies and have a distinct molecular content, should be kept in mind [25–27]. Fig. 1 summarizes recommended PMP isolation procedure from whole-blood samples for assessing clinical samples [22].

2.2. PMP assessment

Assessing PMPs focuses on obtaining information like the phenotype (to confirm the cellular origin), number, size, structural features, and functional activity. Several methods have been developed [18], but each has limits, related in particular to their capacity to detect the smallest PMPs with a size of <400 nm. Standardization efforts are underway under the auspices of the Scientific Standardization Committee of the International Society on Thrombosis and Haemostasis. Several main categories of assays can be distinguished depending on whether they aim to characterize physical properties, *in vitro* functional activities, detailed protein contents, or cellular impacts. At this stage, only a combination of techniques, summarized in Fig. 2, and detailed below, can provide the required set of information needed for assessing PMPs [18].

2.2.1. Physical methods

The methods described below attempt to characterize the PMP cellular origin, size, population, number, and structure.

2.2.1.1. Flow cytometry (FC). FC, the most frequently used procedure to study MPs, including PMPs, detects surface antigens specific to the cellular origin [28]. Annexin-V can be used as a general marker for MPs; integrin-α2b (CD41), CD-61, CD42b, or P-selectin (CP-62P) for PMPs; CD45 for leucocyte MPs; and VE-cadherin (CD144), V-CAM 1, and E-selectin (CD62E) for endothelial cell MPs [20]. The possibility of simultaneously detecting two or more antigens on MPs by antibodies conjugated with different chromophores is an advantage of the method [29]. FC requires careful pre-analytical sample preparation, and accurate bead-based definition of gates and enumeration [19], but application to whole blood has been described [29]. Intra- and inter-laboratory method standardization necessitates a definition of the resolution