



VERSITA

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Review

## What about microparticles? Perspectives and practical aspects

### Microparticulele. Perspective și aspecte practice

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#### Abstract

*The first description of microparticles dates back to 1967, when Wolf reported platelet membrane fragments in human plasma and called them "platelet dust". These vesicles were later called microparticles and the knowledge about their characterization and function has advanced since then. The generation of microparticles represents a mechanism of intercellular communication, playing various roles in both physiological and pathological conditions. Besides other multiple roles in pathology such as inflammation, atherogenesis and cancer spreading, platelet-derived microparticles are involved in thrombogenesis. Tissue factor and phosphatidylserine are both exposed on the outer membrane of platelet-derived microparticles, providing catalytic procoagulant surfaces. The evaluation of microparticles may represent a possible investigation and diagnostic tool. Their enumeration and characterization is challenging and flow cytometry remains the most widely used method for the analysis of microparticles. The aim of the authors is to review the most relevant information on the main properties, mechanisms of generation, and clinical relevance of platelet-derived microparticles, since their evaluation is increasingly considered as a diagnostic biomarker.*

**Keywords:** microparticles, haemostasis, flow cytometry

#### Rezumat

*Prima descriere a microparticulelor datează din 1967, când Wolf a comunicat prezența fragmentelor membranare trombocitare în plasma umană, numindu-le "praf plachetar". Aceste vezicule au fost ulterior denumite microparticule, iar cunoștințele despre caracterizarea și funcția lor au avansat de atunci. Generarea de microparticule reprezintă un mecanism de comunicare intercelulară, având diferite roluri, atât în statusul fiziologic, cât și în diverse afecțiuni. În afară de participarea lor în patogenia unor procese precum inflamația, aterogeneza sau metastazarea neoplaziilor, microparticulele trombocitare sunt implicate în trombogeneză. Factorul tisular și fosfatidilserina sunt ambele expuse pe membrana externă a microparticulelor trombocitare,*

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oferind suprafețe catalitice procoagulante. Evaluarea microparticulelor constituie un potențial instrument de investigare și diagnostic. Enumerarea și caracterizarea lor reprezintă o provocare, iar metoda de analiză cea mai utilizată este citometria în flux. Având în vedere tendința actuală de a considera microparticulele ca fiind un adevărat marker de diagnostic, autorii își propun să prezinte o imagine de ansamblu asupra celor mai relevante informații cu privire la proprietățile principale, mecanismele de producție și importanța clinică a microparticulelor trombocitare.

**Cuvinte cheie:** microparticule, hemostază, citometrie în flux

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## Introduction

Microparticles (MPs) represent sub-micron sized vesicles released from the surface of activated or apoptotic cells as a result of membrane remodeling (1). All cell types shed MPs, therefore they are present in various body fluids such as plasma, synovial fluid, and urine (2).

MPs constituents include cell-surface receptors, cytosolic proteins, DNA, mRNA, and micro RNA derived from their cell of origin. The size of microparticles ranges between 0.1 and 1 micrometer and the phospholipid and protein content depends on the type and state of the parental cell. This high heterogeneity in dimensions and composition distinguishes clearly the MPs from exosomes, which are smaller in size and more homogenous in composition and act as conveyors of immune responses (3).

Besides the surface proteins and the cytoplasmic components emerged from the parental cells, microparticles are able to acquire proteins from other cell types through a fusion process between MPs and the membrane of a different cell (4).

Microparticles shed from different cell types including leukocytes, platelets, erythrocytes, endothelial cells, and various cancer cells have been identified in the circulating blood.

Furthermore, syncytiotrophoblast derived microparticles were found into maternal circulation during pregnancy. (5)

## MPs roles

According to Nieuwland and Sturk (6), the roles of microparticles have been reported to

fall into the following three categories: intercellular communication, MPs being able to transfer specific parental cell receptors to the surface of other cells (e.g., tissue factor (TF) transfer from the surface of leukocyte-derived microparticles to the surface of activated platelets (4); protection, enabling cells to discard toxic metabolites or noxious modified cellular components (e.g., chemotherapeutics, oxidized phospholipids, caspase3); intercellular exchange of genetic information due to mRNA, microRNAs and DNA content (the uptake of tumor derived microparticles containing microRNA may modify gene expression in host cells) (7, 8).

Therefore, MPs from various cells are implicated both in physiological and pathological conditions. Recent reports suggest that MPs can regulate inflammation, stimulate coagulation, affect vascular functions, can also play a role in immuno-modulation, stem cell engraftment, angiogenesis, cell proliferation or differentiation, and tumor metastasis (9-14). Moreover, MPs are incriminated in spreading of infective virus (HIV) and prions to target cells (15).

Some publications indicate that MPs differ in concentration, composition and function in various diseases compared to healthy subjects, consequently they could represent important biomarkers.

## Platelet derived Microparticles (PMPs)

Described for the first time 45 years ago (16), PMPs represent the majority of the pool of circulating microparticles, constituting between 70% and 90% of the total number of

MPs, whereas less than 10% originate from granulocytes and less than 5% from endothelial cells, red blood cells and monocytes (17).

### **PMPs formation**

PMPs are continuously formed in the circulation through an active and highly organized process triggered by platelet activation or apoptosis, involving cytoskeletal reorganization and distinct domains of the cell membrane shedding (18, 19).

In "resting" cells each of the two leaflets of the plasma membrane bilayer has a specific lipid composition: the inner leaflet contains primarily phosphatidylserine (PS) and phosphatidylethanolamine (PE), whereas the external leaflet is rich in phosphatidylcholine and sphingomyelin. This asymmetric distribution is essential for the membrane function and is under the control of a complex transmembrane enzymatic balance that involves: gelsolin (present only in platelets, involved in the cleavage of the actin capping proteins), a flippase (an inward directed pump specific for PS and PE), a floppase (an outward directed pump), a lipid scramblase (which promotes bidirectional translocation across the bilayer), and calpain (hydrolyzes actin-binding proteins inducing the cleavage of cytoskeletal filaments).

Cellular activation or apoptosis induces subsequent increase of calcium concentration in the cytosol, followed by calcium-dependent activation of scramblase and floppase and inhibition of flippase activity, determining the loss of phospholipid asymmetry with the exposure of PS on the outer cell surface. Activation of calpain facilitate MPs shedding by cleaving cytoskeletal filaments. As a result blebbing (outward protrusion) and shedding of membrane MPs into circulating blood occur (20, 21).

A second physiologic source for circulating CD41+ MPs represented by megakaryocytes has been proposed by Flaumenhaft et al. Megakaryocyte-derived microparticles form as beads along the length of slender, unbranched micropodia and also form from continuous blebbing of the

megakaryocyte surface. Unlike platelet-derived microparticles, they contain full length filamin A and do not express CD62P or lysosome-associated membrane protein-1 (LAMP-1). The degraded filamin A from PMPs represents a marker of cytoskeletal degradation, while CD 62P and LAMP-1 are markers of granule fusion (22).

### **PMPs clearance**

The mechanism of clearance of PMPs from the circulation is not well understood. Recent evidence indicates the role of developmental endothelial locus-1 (Del-1) as a mediator of clearance of PS expressing PMPs by the endothelium. Del-1, a 52-kDa glycoprotein secreted by endothelial cells acts as a bridging molecule between endothelial cells and PS-containing microparticles. For homeostatic reasons, additional PS-dependent clearance mechanisms must certainly exist (23).

### **PMPs properties**

PMPs express various antigens including GP IX (CD42a), GPIb (CD42b), GPIIb/IIIa (CD41/CD61), TF, platelet activation dependent P-Selectin and contain various cytosolic proteins, mRNA, microRNA, depending on the triggering stimulus. Through the transfer of functional receptors or other compounds from the platelet, PMPs are able to amplify certain functions of the cells exposed to them.

Increasingly evidence shows that PMPs play important roles in blood coagulation, inflammation, atherogenesis, angiogenesis and cancer metastasis (2, 24-27).

### **PMPs in hemostasis**

The central role of PMPs in hemostasis and coagulation is illustrated on the one hand by the bleeding syndromes associated with decreased number of microparticles in patients with Scott's and Castaman's syndromes (due to a defect in

MPs production) and on the other by the high levels found in thrombotic diseases (28-30).

Although some were reported to carry anticoagulant proteins (thrombomodulin, TFPI, Protein C), PMPs are generally considered to be procoagulant and are believed to be key players in thrombus formation (31,32).

Their procoagulant function relies on the expression of phosphatidylserine (PS) and on the presence of TF on the outer surface of their membrane, which are the main initiators of the coagulation cascade (33). As a result, the microparticle surface possesses approximately 50- to 100-fold higher procoagulant activity than the platelet surface (34).

PS is a negatively-charged aminophospholipid. In the vascular territory, exposed PS serves as a "catalytic template" enabling the assembly of the tenase and prothrombinase complexes and thrombin generation, thus promoting *in situ* hemostasis (20). This procoagulant role is enhanced by the capacity of PS to activate TF (35). In addition to these procoagulant features, PS represents a critical ligand for MPs clearance (36).

TF, a transmembrane glycoprotein belonging to the cytokine receptor class II family, represent a key player in the initiation of blood coagulation, being the main trigger of the coagulation cascade (37, 38). MPs constitute the main pool of blood-borne TF, which could be either in "encrypted" form without coagulant activity or in "de-encrypted" active form (15, 18). MPs express TF at different degree and with different characteristics under physiological and pathological conditions (39). The role of TF-bearing MPs as biomarker in prethrombotic states prediction and diagnosis is under evaluation (40-42).

### **Why MPs evaluation is needed?**

The screening tests in hemostasis evaluate the coagulation according to the classic cascade model. The paradigm shift to the "cell based" model of coagulation requires that the hemostasis evaluation should consider also cells and microparticles (43, 44).

Furthermore, there is a discrepancy between the results of screening tests and the bleeding or thrombotic complications of patients and the prediction of these complications is difficult because of the lack of laboratory markers.

### **Methods of MPs analysis**

Because of their small size and heterogeneity, MPs detection and quantification is a difficult task. MPs analysis can be performed through various methods including flow cytometry, dynamic light scattering, atomic force microscopy, electron microscopy and new techniques such as impedance-based flow cytometry and nanoparticle tracking analysis (45). In addition, the procoagulant activity of MPs, can be evaluated using different functional assays. Each method has advantages and disadvantages: flow cytometry allows enumeration and determination of the cell origin but with a high level of variability, dynamic light scattering is efficient in size determining and quantification but unable to offer information on the properties of MPs, while atomic force microscopy and electron microscopy permit precise estimation of size and shape but have limitations due to the insufficiency of available equipment, routine analysis being excluded (46, 47).

### **Flow cytometric analysis of MPs**

Flow cytometry remains the most widely used method for MPs analysis in clinical samples, although their enumeration and characterization is challenging. The small size of MPs places them at the limit of detection of flow cytometry devices, which is determined by the wavelength of laser light. Therefore, conventional flow cytometry detects only the MPs larger than 0.5µm (48).

MPs are sensitive to pre-analytical procedures including blood sampling, type of collection tube, transportation, time before processing, centrifugation steps and long term stor-

age conditions. Cells can be easily activated during these steps, minor protocol changes leading to significant differences in MPs levels. According to Lacroix et al., there are three major pre-analytical sources of variability: agitation of the tubes during transportation, the delay before first centrifugation and the protocol of centrifugation. Blood collection should be done on plastic-citrated tubes, using large size needles, without applying venostasis and discarding the first milliliters of blood. It is recommended to transport the sample tubes in vertical position, avoiding agitation. Samples should be processed optimal in the first hour postdrawing, but an interval of 2 hours between blood sampling and the first centrifugation is acceptable. Serial centrifugation is performed in order to obtain platelet free plasma, which will be stored at -80 °C (49-51).

A working definition proposed by Robert S. et al. for flow cytometric determination of MPs includes: events that are < 1µm in size, express phosphatidylserine (PS), which is usually detected by binding of labeled AnnexinV, express antigens that characterize the cell of origin (e.g. CD41 for PMPs), and may be obtained by high speed centrifugation of platelet-poor plasma to deplete small platelets and cell debris (52).

Recent studies suggest two possible causes for unspecific antibody binding: one is the existence of Fc receptors on microparticles and the other is represented by the insoluble complexes, which appear to have overlapping biophysical properties (53-55).

The analytical phase is also a source of variability, due to differences in instrument settings and resolution (56). In an attempt to standardize these settings and to determine the MPs analysis region a blend of fluorescent beads of three diameters (0.5, 0.9 and 3 µm) in a fixed numerical ratio (Megamix, Biocytex, Marseille) was created. Fine optical adjustments and both fluidics and optics cleanness are essential for a good resolution between background noise and microparticles.

In response to the heterogeneity of protocols which leads to difficulties in data comparison,

the Scientific Standardization Committee of the International Society on Thrombosis and Haemostasis is in the process of developing guidelines to standardize the evaluation of microparticles (57).

## Conclusions

In recent years, the study of microparticles has received increasing interest and some progress has been made in understanding their multiple roles. Platelet-derived microparticles may possess diagnostic significance as biomarkers in hypercoagulable state, constituting a possible hemostasis parameter. However, the flow cytometric evaluation of MPs needs urgent standardization in order to obtain reliable and reproducible results.

Also, further research should be directed to the development of pharmacological agent targeting MPs production.

## Conflicts of interests

The authors state that they have no conflict of interest.

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