Review

Microparticle and Atherothrombotic Diseases

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Microparticles (MPs) are small membrane vesicles that are released from many different cell types by exocytotic budding of the plasma membrane in response to cellular activation or apoptosis. MPs may be involved in both physiological processes and clinical treatments because they express phospholipids, which function as procoagulants. Elevated levels of platelet-derived MPs, endothelial cell-derived MPs, and monocyte-derived MPs are observed in almost all thrombotic diseases occurring in venous and arterial beds. Several studies have shown that the quantity, cellular origin, and composition of circulating MPs depend on the type of disease, the disease state, and medical treatment. Although MPs were initially thought to be small particles with only procoagulant activity, they are now known to have many different functions. An increasing number of studies have identified new implications of elevated MPs in clinical disorders. On the basis of evidence available till date, the present review suggests that MPs may be a useful biomarker in identifying atherothrombosis.

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Microparticle

Microparticles (MPs) are small membrane vesicles that are released from many different cell types by exocytotic budding of the plasma membrane in response to cellular activation or apoptosis¹⁻³. MPs are generally measured using flow cytometry, and they can range in size from 0.1 μm to 1.0 μm ^{1,2}. MPs disseminate various bioactive effectors originating from the parent cells. Therefore, MPs can alter vascular function and may induce biological responses involved in vascular homeostasis². Most MPs in human blood originate from platelets (platelet-derived MPs, abbreviated PDMPs). PDMPs contain various glycoproteins (GPs) such as GPIIb/IIIa or GPIb/IX^{1,2}. MPs are also released from leukocytes, erythrocytes, endothelial cells, smooth muscle cells (SMCs), and cancer cells¹⁻⁴. Elevated MP levels are documented in almost all thrombotic diseases occurring in venous and arterial beds⁵⁻⁷. Elevated levels of MPs have also been associ-

ated with inflammation, cellular activation and dysfunction, angiogenesis, and cellular transport^{2,8-12}.

MPs contain functional cytoadhesions, bioactive phospholipids, cytoplasmic components, and various antigens that are characteristic of the state of the cell from which they originated and also of the type of stimulus¹³. Some studies have analyzed the proteome of MPs and identified hundreds of proteins^{14,15}. Proteins from MPs may be useful biomarkers for various disease processes¹⁵. MPs are constitutively released from the cell surface, but their formation can be upregulated by cellular activation or apoptosis^{16,17}. After cellular activation or apoptosis is triggered, there is a rise in cytosolic calcium concentrations followed by cytoskeletal changes. Plasma membranes of cells contain different types of phospholipids. Although uncharged phospholipids are generally present in the outer leaflet of the membrane bilayer, the inner leaflet contains negatively charged aminophospholipids, such as phosphatidylserine (PS). During activation or apoptosis of the cell, there is a change in the membrane with alteration in the normal lipid bilayer, flip-flopping the internal PS to the external surface. Thus, PS-exposing MPs may be released from the cells¹⁸.

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Shear Stress and MPs

High shear stress can initiate platelet aggregation and shedding of procoagulant-containing PDMPs^{19, 20}. Chow *et al.*²¹ suggested that thrombin that is formed in the vicinity of primary hemostatic plugs in areas of elevated shear stress plays a key role in the propagation of thrombi by potentiating shear-induced PDMP generation. Furthermore, platelet GPs and specific receptors can be involved in high shear stress-induced PDMP formation^{19, 22-24}. Miyazaki *et al.*¹⁹ examined the mechanisms involved in PDMP production induced by high shear stress, and they showed that binding of von Willebrand factor to GPIb, influx of extracellular calcium, and activation of platelet calpain were required to generate PDMPs under high shear stress conditions. In addition, Reininger *et al.*²⁴ reported that the GPIb receptor mediates adhesion to von Willebrand factor, and under hydrodynamic flow, stretching of the platelet membrane occurs, followed by separation of areas of tethered membranes and production of MPs. Shear stress is also involved in the generation of endothelial cell-derived MPs (EDMP) because it is a major determinant of endothelial apoptosis^{25, 26}. Flow-induced increase in laminar shear stress is a potent physiological stimulant, which exerts atheroprotective effects in the vasculature²⁷. Exercise-mediated wall shear stress increases mitochondrial biogenesis in vascular endothelium, decreasing the level of released EDMP^{28, 29}.

Tissue Factor and MPs

MPs were initially thought to be related to diseases because they express phospholipids, which are procoagulants. These MPs support generation of thrombin and could be involved in diffuse intravascular coagulation occurring in disease states. However, such a coagulation system is activated not only in disease states but also in healthy individuals. Berckmans *et al.*³⁰ reported that MPs circulate in the blood of healthy humans and support low-grade thrombin generation. Sinauridze *et al.*³¹ reported that PDMPs have 50- to 100-fold higher specific procoagulant activity than activated platelets. Exposure of PS not only facilitates formation of coagulation complexes, but it also promotes the ability of TF to initiate coagulation³². MPs support coagulation by factor (F)VII/TF-dependent and independent pathways³³. During vascular damage, blood contacts extravascular TF, resulting in extrinsic coagulation activation and formation of fibrin. Indeed, TF can be activated upon adhesion and fusion of MPs to activated platelets.

Several studies revealed that stimulation by tumor necrosis factor (TNF)- α , lipopolysaccharide, or oxidized low-density lipoprotein in cultured human umbilical vein endothelial cells results in an increase in release of EDMPs expressing surface TF³⁴⁻³⁶. The addition of increasing concentrations of these EDMPs to a coagulation assay reduces the plasma clotting time. This effect is not observed in FVII-deficient plasma, indicating that procoagulant activity of EDMPs is FVII/TF dependent in such situations. Interestingly, a subset of EDMPs bearing von Willebrand factor appears to be able to induce platelet aggregation³⁷.

Monocyte-derived MPs (MDMPs) also contribute to the development of platelets and fibrin-rich thrombus at the sites of vascular injury, through the recruitment of cells and accumulation of TF. MDMPs express the P-selectin glycoprotein ligand-1 and TF³⁸. The binding of these MDMPs to P-selectin on activated endothelial cells on activated platelets in the developing thrombus may promote TF accumulation and localized thrombin generation. Subsequently, activated platelets expose P-selectin and are capable of capturing TF-exposing MDMPs via P-selectin glycoprotein ligand-1. Consequently, MP-associated TF becomes rapidly deposited at the site of the developing thrombus. DelConde *et al.*³⁹ showed *in vitro* that fusion of membranes of TF-exposing MPs and activated platelets results in the transfer of TF in platelet membranes. This fusion results in co-localization of TF and coagulation factors, thus promoting a more efficient initiation and propagation of coagulation.

At the MP surface, the presence of proteins inhibiting coagulation, such as TF pathway inhibitor, protein C, or thrombomodulin, increases the possibility of eventual contribution of MPs to an anticoagulant pathway^{40, 41}. Although TF is exposed by MDMPs, TF activity is markedly inhibited by the MP-associated tissue factor pathway inhibitor (TFPI). In storage-induced PDMPs, 10% of which contain TF, TF-dependent thrombin generation can only be observed in plasma in which TFPI is neutralized³⁹. A balance between TF and TFPI at the MP surface is probably an important feature in the initiation of blood coagulation, and higher levels of MPs containing TF possibly overcome the TFPI anticoagulant pathway⁴⁰.

There exist a large number of target antigens for EDMP measurement compared with antigens available for identifying cells^{2, 3}. This suggests that EDMP involves functional molecules related to endothelial cells (**Fig. 1**). In particular, EDMP inherit the anticoagulant ability that is the most important function of endothelial cells^{4, 13, 17, 26}. Researchers have also inves-

Fig. 1. Target antigens for EDMP

EDMP involves functional molecules related to endothelial cells. EPCR: endothelial protein C receptor, eNOS: endothelial nitric oxide synthase, PS: phosphatidylserine, ICAM-1: intracellular cell adhesion molecule-1, VCAM-1: vascular cell adhesion molecule-1, PECAM-1: platelet endothelial cell adhesion molecule-1, MMP: matrix metalloproteinase, uPA: urokinase-type plasminogen activator, TM: thrombomodulin, APC: activated protein C, EPC: endothelial protein C, MC: mitochondria, ER: endoplasmic reticulum, miRNA: micro RNA, mRNA: messenger RNA

tigated the effect of activated protein C, which has anticoagulant and anti-inflammatory properties, on endothelial cells and EDMP formation⁴¹⁾. Cultured endothelial cells exposed to activated protein C release EDMP with membrane-bound endothelial protein C receptor. Activated protein C that is bound to this receptor retains its anticoagulant activity, that is, its ability to inhibit the formation of thrombin⁴¹⁾.

Identification of MPs

An identification method for MPs is important for clinical studies on MPs. Appropriate sampling conditions, processing, and storage of samples are essential^{1, 2)}. MPs can be directly quantified in platelet-poor plasma, obtained by serial centrifugation of citrated whole blood. Alternatively, washed MPs can be isolated from platelet-poor plasma by ultracentrifugation before resuspension and analysis. The most widely used method for studying MPs is flow cytometry because of its simplicity and the extent of informa-

tion that can be obtained from the population under study⁴²⁾.

The enzyme-linked immunosorbent assay (ELISA) is an easier and reproducible PDMP method^{43, 44)}. When using ELISA, PDMPs may be quantitated with reference to a standard curve. This method will hopefully contribute toward the understanding of participation of PDMPs in the clinical setting, if antibodies that are reactive with platelet activation markers, such as P-selectin and soluble CD40 ligand, are used. One of the problems associated with the ELISA is the possibility that it contains soluble GPs, such as the GPIb/IX/V complex. Ueba *et al.*⁴⁵⁻⁴⁸⁾ measured circulating PDMPs in healthy Japanese individuals using the ELISA, and they suggested that PDMPs were positively associated with the level of metabolic syndrome. The use of MP quantification as a clinical tool is still debatable. A large-scale clinical trial for various thrombotic diseases using ELISA kits was performed in Japan⁴⁹⁾.

Atherothrombosis and MPs

Production of PDMPs, EDMPs, and leukocyte-derived MPs can be increased by inflammatory conditions^{50, 51}. MPs formed by *in vivo* stimulation with a chemotactic peptide in healthy volunteers were able to induce IL-6 and monocyte chemoattractant protein (MCP)-1 release, as well as TF expression, by endothelial cells *in vitro*. A major feature in atherosclerosis is adhesion of monocytes to endothelial cells, followed by subendothelial transmigration. Cytokines, such as IL-1 β and TNF- α , affect this process by inducing synthesis or upregulation of leukocyte-endothelial adhesion molecules. *In vitro* stimulation of monocytes and endothelial cells by high shear stress-induced PDMPs results in significantly increased production of IL-8, IL-1 β , and TNF- α ⁵². Furthermore, treatment of endothelial cells and monocytes with PDMPs prior to co-incubation modulates monocyte-endothelial cell interactions by increasing the expression of adhesion molecules on both cell types⁵². Recently, some associations between PDMP levels and clinical outcomes in critically ill patients have also been evaluated, in addition to the importance of PDMPs in inflammation⁵³.

Circulating MPs of platelet and leukocytic origins promotes recruitment of inflammatory cells and induces cellular adhesiveness through upregulation of cytokines and cytoadhesions in endothelial cells and monocytes⁵⁴. Under high shear stress conditions, PDMP rolling enables delivery of RANTES to inflamed endothelium, thus favoring adhesion of monocytes and infiltration of plaques⁵⁵. Development and progression of atherosclerotic plaques are associated with apoptotic cell death, explaining the presence of a considerable amount of procoagulant MPs in plaques⁴. Furthermore, enhanced apoptosis or activation of leukocytes, SMCs, and endothelium contribute to accumulation of MPs^{4, 56}. Compared with their circulating counterpart, MPs trapped in the plaque are present at much higher concentrations and display higher thrombogenic potential. In plaques, most of these MPs are from activated leukocytes, a hallmark of inflammation, and from erythrocytes, indicating the occurrence of intraplaque hemorrhage, which is a marker of vulnerability of plaques⁵⁶. Atherosclerotic plaques also contain a substantial amount of SMC-derived MPs and EDMPs⁴. MPs not only contribute to plaque thrombogenicity but can also contribute to instability by mediating the recruitment of inflammatory cells. Therefore, circulating MPs can result in vascular inflammation, endothelial dysfunction, leukocyte adhesion, and recruitment. This could contribute to plaque growth or stent-induced vascular inflamma-

tion because MPs convey biological effectors⁵⁷.

Oxidative stress, caused by reactive oxygen species (ROS), plays an important role in the pathogenesis of various diseases including atherothrombosis^{58, 59}. Recent reports have also indicated that oxidative stress increases the levels of various MPs in circulation^{60, 61}. Xanthin oxidase is among the major enzymatic sources of ROS, whereas anti-xanthin oxidase inhibitor can reduce the activity of ROS⁵⁸. In addition, Nishizawa *et al.*⁶² reported that anti-xanthin oxidase inhibitor reduced PDMP levels in patients with hyperuricemia.

Cardiovascular Diseases

Procoagulant MPs have been implicated in the development and progression of atherosclerosis, the underlying cause of cardiovascular diseases^{63, 64}. In particular, EDMP levels are higher in patients with acute coronary syndrome compared with patients with stable anginal symptoms or control patients⁶⁵. Moreover, EDMP levels are higher in high-risk coronary lesions compared with low-risk lesions⁶⁶. These findings indicate the participation of procoagulant EDMPs according to the degree of acute vascular injury and inflammation at the time of measurement. However, MPs do not always play a procoagulant role in cardiovascular diseases. For example, Steppich *et al.*³⁷ reported that in acute myocardial infarction, some MPs may have an anticoagulant function through expression of TFPI and reduction of TF-dependent thrombin generation, which may help limit thrombus formation. EDMPs may also possess anticoagulant properties, dependent on the presence of thrombomodulin and endothelial protein C receptor^{4, 67, 68}.

A number of cytokines can induce procoagulant activity in leukocytes⁶⁹. A proinflammatory member of the C-C chemokine family, RANTES, is a potent chemoattractant of memory T-lymphocytes, monocytes, eosinophils, and basophils. Several previous studies have suggested that RANTES is an inflammatory mediator in cardiovascular disease^{5, 48, 70}. In addition, the presence of RANTES may predict restenosis after percutaneous coronary intervention in patients with stable angina^{71, 72}. PDMPs also relate to the levels of RANTES^{5, 44, 55} and are associated with atherosclerotic events after percutaneous coronary intervention⁷³. Postmenopausal women are at an increased risk of cardiovascular disease. One study reported a correlation between increased PDMP levels and early stage coronary artery calcification in menopausal women⁷⁴. Psoriasis is also a risk factor for cardiovascular disease because it is an immune-mediated inflammatory skin disease associated with systemic inflammation⁷⁵.

Fig. 2. Role of MPs in atherosclerosis and thrombosis in type 2 diabetes

Production of PDMPs, MDMPs, and EDMPs can be increased in type 2 diabetes. These MPs contribute to the generation of atherothrombosis in type 2 diabetes. Mac-1: β -2 integrin family in monocyte (CD11b/CD18), ICAM-1: intercellular adhesion molecule-1, VCAM-1: vascular cell adhesion molecule-1, VLA-4: very late antigen-4, AGE: advanced glycation end products, RAGE: receptor for AGEs, TF: tissue factor, RANTES: regulated on activation, normal T cell expressed and secreted, CD40L: CD40 ligand

Recently, Chandrashekar *et al.*⁷⁶⁾ reported that PDMP levels were significantly elevated in patients with psoriasis.

Diabetes Mellitus

Diabetes mellitus is the main risk factor associated with atherosclerosis. MPs are elevated in diabetic patients, and interestingly the MP phenotype may vary within the type of diabetes. Some studies have found differences in the MP profile in relation to the disease type and the presence or absence of MPs^{2, 3, 77)}. Sabatier *et al.*⁷⁷⁾ reported that in type 1 diabetes, the procoagulant potential of MPs, as measured by a prothrombinase assay, was elevated and correlated with the degree of glycemic control. However, in type 2 diabetes, although total numbers of MPs were elevated, there was no associated increase in their procoagulant potential. A few studies on the potential role of PDMPs in diabetic complications have been reported⁷⁸⁻⁸⁰⁾. In

addition, both PDMPs and MDMPs are correlated with diabetic complications, including the extent of diabetic retinopathy, which is associated with microvascular damage⁸¹⁻⁸⁷⁾. Elevated EDMP levels are predictive of coronary artery lesions and are a more significant independent risk factor than the duration of diabetic disease, lipid levels, or presence of hypertension⁸⁸⁾. Interestingly, elevated EDMP levels are predictive in identifying a subpopulation of diabetic patients without typical anginal symptoms who have angiographic evidence of coronary artery disease. Therefore, in patients with type 2 diabetes mellitus, the level of circulating EDMPs increases with the severity of the disease^{89, 90)}. In conclusion, production of PDMP, MDMP, and EDMP can be increased in type 2 diabetes, and these MPs contribute to the generation of atherothrombosis in type 2 diabetes⁹¹⁾ (**Fig. 2**).

Conclusion

This manuscript summarizes the literature till date on MPs, including a growing list of clinical disorders that are associated with elevated MP levels. MPs were initially thought to be small particles with procoagulant activity. However, increasing number of researchers are investigating the possibility that MPs evoke cellular responses in the immediate microenvironments where they are formed.

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Competing Interests

None of the authors disclose any financial or personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

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