THOUSAND WORDS

# A thousand words about microparticles in cardiology

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ABSTRACT

Microparticles (MPs) are membrane vesicles of 0.1–1 μm in diameter produced mainly by platelets, vascular endothelium and blood cells in response to cell activation and stress factors. MPs can be also released during malignant transformation or apoptosis. The essential step in MP formation is the loss of the cell membrane asymmetric phospholipid distribution as response to the increased intracellular calcium levels. MPs contain, proteins and genetic material (DNA, miRNA, mRNA) which enables them to interact and influence target cell. MPs are considered to be markers of ongoing pathophysiological processes in cardiovascular system, due to their role in inflammation and coagulation.

**Key words:** laboratory diagnostics, extracellular microvesicles, endothelial dysfunction, platelet activation.

Microparticles (MPs) are membrane vesicles of 0.1–1 μm in diameter shed from vascular endothelium and blood cells (e.g.: lymphocytes T and B, macrophages) or platelets in response to cell activation, various forms of stress (e.g. hypoxia, mechanical trauma or inflammation). MPs can be also released during malignant transformation or apoptosis [1]. The mechanism of MPs formation can be studied by stimulating cultured cells *in vitro* with numerous cytokines and apoptotic stimuli [2]. The role of MPs in various diseases can be explored by their isolation from peripheral blood [3]. So far the endothelial MPs and platelet (PMPs) microparticles have been studied extensively in cancer, atherosclerosis, sepsis, diabetes and cardiovascular diseases [1, 3].

Additionally to MPs, exosomes (Ex) and apoptotic bodies are sometimes considered members of „microparticles family". However, MPs have different size, biological properties release mechanism [4, 5]. Apoptotic bodies are cell fragments formed in the terminal phase of apoptosis during cell fragmentation, they are larger than MPs and, like exosomes, exhibit lower clotting capacity comparing to MPs. While Ex are produced in the endocytic-lysosomal system and released from cell by the fusion of multivesicular bodies (MVB) with plasma membrane, MPs are formed by cell membrane shedding [6, 7] (**Figure 1**). The essential feature of this process is the loss of the cell membrane asymmetric phospholipid distribution in response to the increased intracellular calcium levels. This in turn regulates membrane flippase, floppase and scramblase activity, leading to phosphatidylserine (PS) and phosphatidylethanolamine (PE) exposure on the outer membrane leaflet, and the activation of contractile proteins (**Figure 2**). PS is a negatively charged phospholipid and, in the presence of calcium ions, it assembles the prothrombinase complex and activates coagulation *in vivo* [3].

PMPs are the most abundant circulating MP subtype [1]. They are released from platelets after their activation by thrombin, ADP plus collagen, calcium ionophore A23187 and high shear stress. Endothelial cells, monocytes and vascular smooth cells can release MPs after activation by bacterial lipopolysaccharide, inflammatory cytokines (e.g. interleukin-1α, IL-1α), complement complex C5b-9 or reactive oxygen species, hyperhomocysteinemia, hyperglycemia, hypoxia and tumor necrosis factor α [8, 9, 10]. MPs

contain enzymes and genetic material which enables them to interact and influence target cell (**Figure 3**) [7]. Endothelial MPs transfer cell adhesion protein VE-cadherin, T-cadherin, E-selectin and sialomucin (CD34). Endothelial MPs are also the carrier of many active enzymes, including matrix metalloproteinases and their inhibitors acid sphingomyelinase, nitric oxide synthases (eNOS), NADPH oxidase. These microparticles also regulate intercellular lipid transfer (PS, PA, sphingomyelin, arachidonic acid). Additionally, MPs contain DNA, mRNA, microRNA- and, possibly, other non-coding nucleic acid chains, indicating that MPs may play a role in the cell-to-cell transfer of genetic contents [11].

MPs play role in inflammation, coagulation and vascular function and are considered to be markers of ongoing pathophysiological processes. All of these processes contribute to cardiovascular risk factors (e.g.: diabetes and hypertension) or disorders (e.g.: atherosclerosis, coronary artery disease, stroke, cardiomyopathy or thromboembolism) [2, 3].

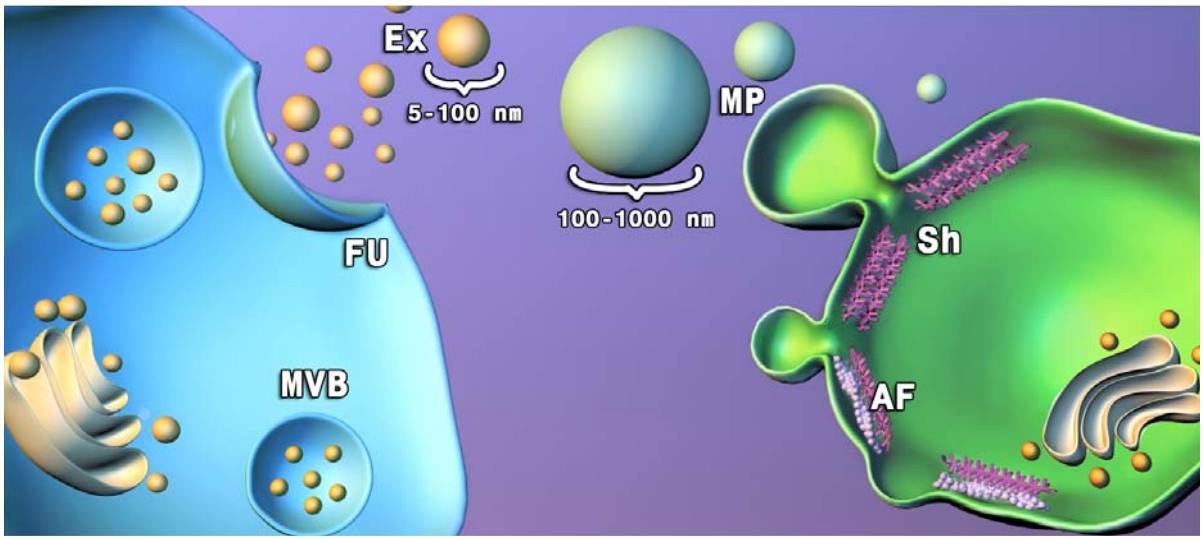


**Figure 1.** The comparison of exosomes (Ex) and microparticles (MPs). AF – actin filaments, FU – fusion of cell and vesicle membrane, MVB – multivesicular body, Sh – membrane shedding

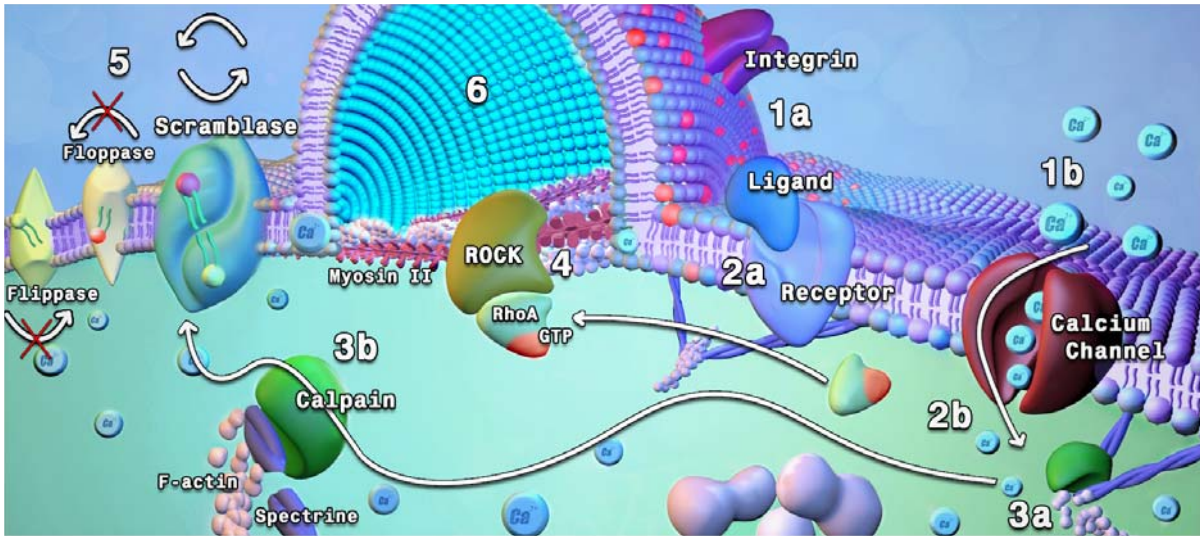


**Figure 2.** The regulation and physiology of cell membrane budding and microparticle formation. This model describes the stages of cell activation leading to membrane phospholipid asymmetry. The first step is cell stimulation via ligand-to-receptor binding (1a) or calcium channel activation (1b). A receptor transmits a signal towards the cell (2a) and/or calcium influx occurs (2b). Increased calcium activates calpain to brake F-actin and release spectrin from submembrane compartments (3a, 3b) or evokes RhoA phosphorylation (4). Membrane lipid asymmetry is regulated by the cooperative activities of three transporters: (flippase) the ATP-dependent aminophospholipid-specific translocase, which rapidly transports PS and PE from the cell's outer-to-inner leaflet; the ATP-dependent nonspecific lipid floppase, which slowly transports lipids from the cell's inner-to-outer leaflet; and the Ca2+-dependent nonspecific lipid scramblase, which allows lipids to move randomly between both leaflets (5). Finally, a microvessicle is formed and myosin II contraction controls its release (6)

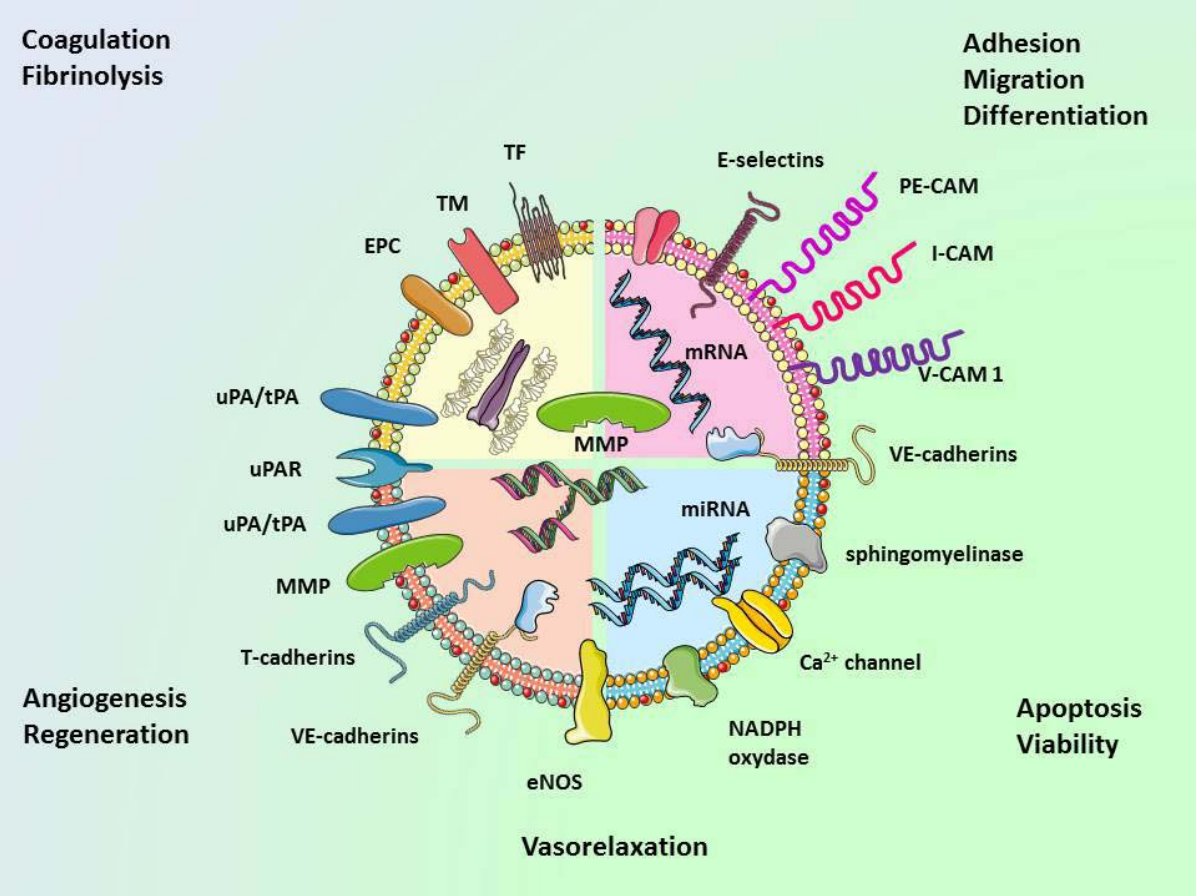


**Figure 3.** The scheme of typical EMP content. CAM – cell adhesion molecules, eNOS – endothelial nitric oxide synthase, EPC – endothelial protein C, MMP – matrix metalloproteinase, TF – tissue factor, TM – thrombomodulin, uPA/tPA – urokinase/tissue plasminogen activator, uPAR – urokinase plasminogen activator receptor. Based on Stępień and Targosz-Korecka 2013 [7], with agreement and modified

Atherosclerosis is an inflammatory process caused largely by transendothelial migration and accumulation of macrophages and neutrophils in the arterial wall [12]. The endothelial MPs stimulate endothelial cells to secrete cytokines responsible for activation and chemotaction of leukocytes [13]. Platelet MPs, by delivering arachidonic acid to endothelial cells, increase adhesion of monocytes to human umbilical vein endothelial cells (HUVEC), following upregulation of cellular adhesion molecules (ICAM-1) on endothelium and CD11a/CD18 and CD11b/CD18 on monocytes [14]. Leukocyte to leukocyte adhesion is also mediated by platelet MPs thorough the up-regulation of CD11b on cell surface. After the arterial wall is infiltrated with leukocytes, they start to secrete cytokines and growth factors that activate the proliferation of vascular smooth muscle cells, and initiates formation of atherosclerotic plaque [15].

Coagulation (thrombogenesis) is an important part of hemostasis. Cardiovascular patients are at high risk of thrombosis and they are predisposed to develop serious adverse cardiovascular events such as myocardial infarction, stroke, and acute lower limb ischemia. MPs have a large impact on coagulation mainly from the exposure of negatively charged PS on the outer membrane leaflet [5]. In the presence of calcium ions PS forms the prothrombinase complex assembly and increases thrombogenesis. Moreover, activated factor V (a clotting activator) can be found at the MPs surface. In the presence of calcium and phospholipids, an activated factor V merges with an activated factor X to produce thrombin (protrombinase activity). In turn, to catalyze factor X conversion to its active form, the complex of tissue factor (TF) with factor VIIa is necessary. That again leads to MPs because TF is present in the surface of platelet-derived microvesicles [16]. In patients with myocardial infarction the higher coagulation plasma potential due to MPs can be assumed (**Figure 4**).

Boulanger et al. (2001) showed the MPs impact on the vascular function [17]. Authors exposed rat aortic rings (with endothelium) for 24 hours to circulating MPs isolated from 7 non-ischemic patients and 19 patients with acute myocardial infarction. They found

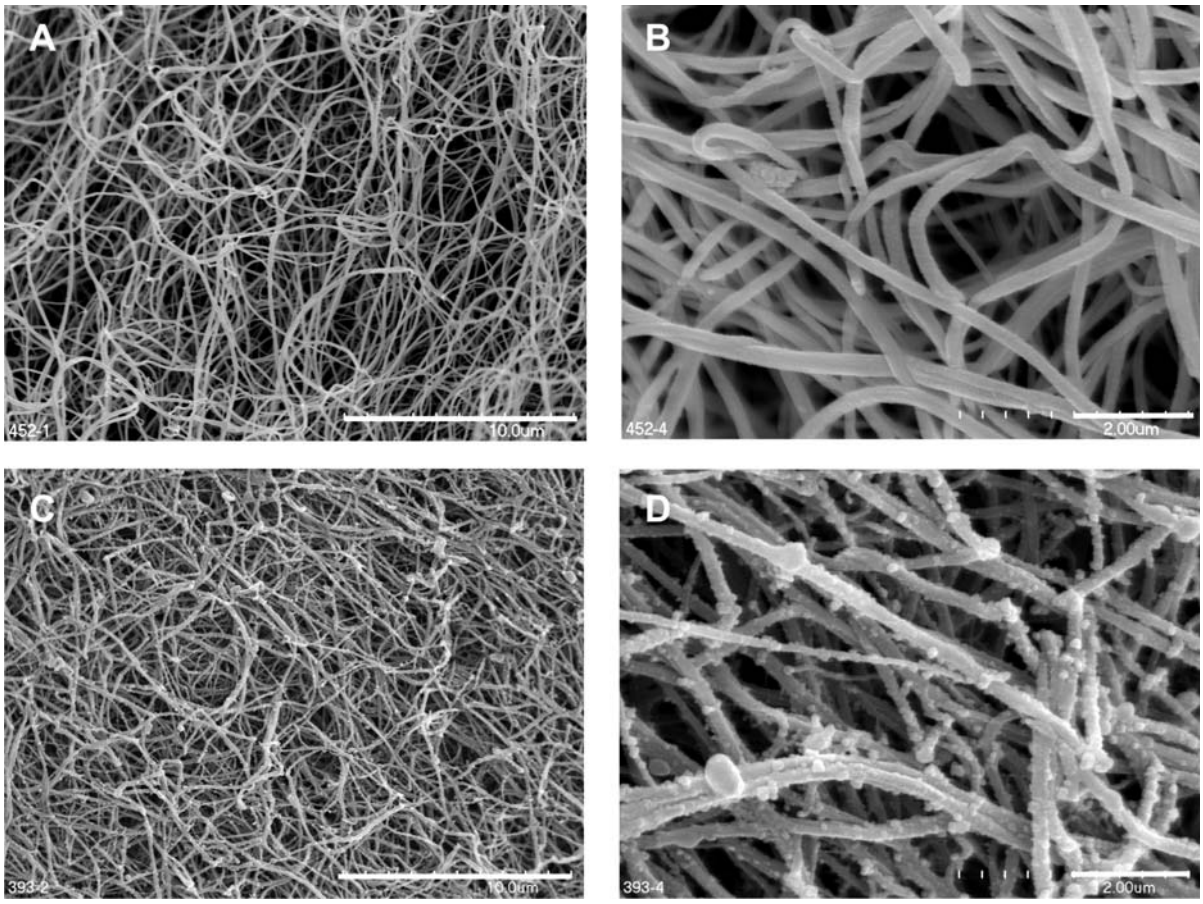


**Figure 4.** Microphotographs of fibrin clots obtained from acute cardiovascular patient plasma, incrusted with MPs emerging from fibrin fibers. Micrographs were taken using scanning electron microscopy (SEM) at 5000 (A, C) and 20000 (B, D) times magnification in collaboration with Mrs. Jadwiga Faber from Department of Cell Biology and Imaging, Institute of Zoology, Faculty of Biology and Earth Sciences, Jagiellonian University, Kraków, Poland. Bar represents 10 µm (A, C) and 1 µm (B, D)

that endothelium-dependent relaxations to acetylcholine were reduced in samples exposed to MPs from patients with myocardial infarction but not from non-ischemic patient [17].

Many in vivo studies regarding MPs in cardiovascular diseases, concerning their overall amount and composition, have been published in recent years, e.g.: endothelial MP levels were elevated comparing to control in deep vein thrombosis or pulmonary embolism [18], in acute coronary syndrome [19], in acute ischemic stroke [20], in diabetes [21, 22] or in the severe hypertension with systolic pressure correlation [23]. Very interesting study was carried out in 2005 by Koga et al. who proved that elevated endothelial MP levels were predictive for the presence of coronary artery lesions in diabetic patients referred for coronary angiography these microparticles were the strongest risk factor for CAD irrespective of other risk factors such as length of diabetic disease, lipid concentration, and hypertension. The elevated EMP were particularly useful in identifying patients with angiographically confirmed coronary artery disease and without typical angina symptoms [24].

In summary, MPs are diverse biological objects with various structure and function. Their impact on coagulation, inflammation and vascular function is important for endothelial function [25]. Precise knowledge of their release and activity *in vivo* may help to identify patients with increased cardiovascular risk in the future and perhaps apply appropriate therapies before acute complications occur.

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