GLOBAL CARDIOLOGY SCIENCE & PRACTICE
A Qatar Foundation Academic Journal

OPEN ACCESS

Review article

# Microparticles: Biomarkers and effectors in the cardiovascular system

Haissam A Saleh*, Basirudeen S Kabeer

Qatar Cardiovascular Research Center, Doha, Qatar
*Email: hasaleh@qf.org.qa

**ABSTRACT**

Microparticles, in the context of this review, are defined as plasma membrane derived-particles shed by various types of vascular and blood cells in response to different stimuli. They were first described as products of platelet activation or "platelet dust", however microparticles are now ascribed prominent roles in cardiovascular diseases and contribute to the regulation of pathophysiological processes including, endothelial function, inflammation, coagulation, angiogenesis, and cellular remodelling. Furthermore, microparticles serve as cell-cell messengers by transfer of biological information to target cells in pathophysiological settings and have proven to be prominent biomarkers for health and physiology assessments for both diagnostic and risk stratification purposes. This review describes the mechanisms of microparticles formation, release and clearance, and their detection by currently available and applicable methods. It also discusses the role of microparticles in the development of cardiovascular diseases, as well as their role as biomarkers and cell effectors in the cardiovascular system.

http://dx.doi.org/10.5339/gcsp.2015.38

Submitted: 12 April 2015
Accepted: 30 June 2015


QSCIENCE.com
An Initiative of Qatar Foundation

Cite this article as: Saleh HA, Kabeer BS. Microparticles: Biomarkers and effectors in the cardiovascular system, *Global Cardiology Science and Practice* 2015:38
http://dx.doi.org/10.5339/gcsp.2015.38

## INTRODUCTION

Cardiovascular diseases (CVDs) are the leading cause of mortality worldwide killing over 17 million individuals each year, representing 30% of all global deaths.[1] In spite of advances in assessment and treatment of common cardiovascular risk factors, including hypertension, obesity, diabetes, hyperlipidemia, the world health organization estimates the number of death from CVDs will increase to almost 24 million by 2030.[1,2] This is thought to be, at least in part, due to inadequate knowledge of the contribution of the different pathways implicated in the pathophysiology of the condition of each patient, and the lack of accurate prognostic indicators, which could alert the treating physician.

Numerous investigations have indicated that the molecular mechanisms underlying the pathophysiology of CVDs could be monitored using a variety of biological parameters or biomarkers.[3,4] Recent progress in genomics, proteomics, and metabolomics has offered unique opportunities to identify a panoply of biomarkers including the expression of genes, proteins, miRNAs, metabolites, and the release of circulating extracellular vesicles. The latter are released by different cells under basal or stress conditions. Depending on their size, extracellular vesicles are classified into exosomes (40–100 nm), microparticles (0.1–1 μm) and apoptotic bodies (1–5 μm). So far, microparticles have been extensively studied due to their biological function and the availability of techniques for their isolation and quantification. This review focuses on the role of microparticles as biomarkers and effectors in the cardiovascular system.

## BIOLOGY OF MICROPARTICLES

Microparticles (MPs) are micron-sized vesicular structures with a diameter of 0.1 and 1 μm (Figure 1). They were first identified in 1967, in association with platelets in human plasma and were considered as "platelet dust" with procoagulant activity.[5] However, in the past decade, we have witnessed an unprecedented interest in the role of MPs in biomedicine. Currently, MPs are regarded as prominent biomarkers and intercellular mediators in a variety of diseases, including CVDs, autoimmune diseases, neurological disorders, and cancer. In the cardiovascular system, MPs are released from different cell types, mainly from platelets, but also from monocytes, erythrocytes, endothelial cells, cardiomyocytes, and fibroblasts.[6,7] They represent a heterogeneous population of microvesicles differing in number, size, origin, structure, and functional properties. During their formation, they retain membrane receptors from their parental cells, as well as part of their cytoplasmic content. Circulating MPs expose adhesion membrane proteins (e.g., integrins, selectins, and immunoglobulins) which can interact with their counter-receptors on the surface of target cells. This can activate intracellular signaling cascades and cause noxious responses including inflammation, endothelial dysfunction, thrombosis, extracellular matrix degradation, and vascular remodelling. Moreover, MPs can transfer proteins, bioactive lipids, and genetic materials (DNA, RNA, miRNA) to target cells by either fusion or internalization. The target cells could then undergo structural and/or functional changes and, therefore,

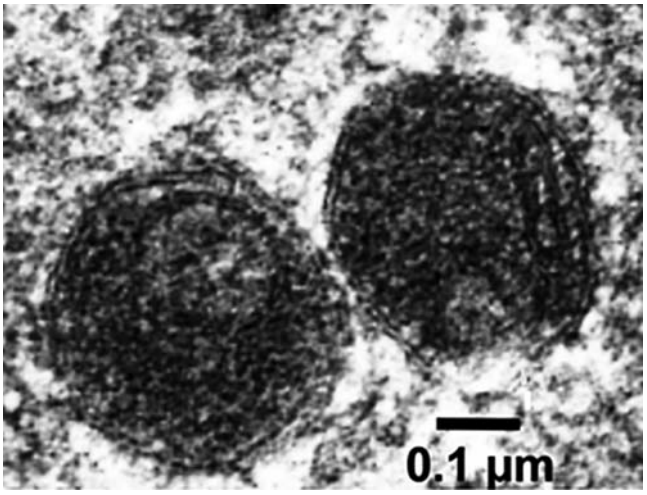


**Figure 1. MPs are micron-sized vesicular structure with a diameter between 0.1 and 1 μm when measured by transmission electron microscopy (courtesy of Dr. Ziad Mallat *et al.*).**

acquire new biological properties. However, apart from their deleterious effects, MPs may be employed as pharmacological tools and protect against cellular and vascular damage.[6]

## Formation of microparticles

The formation of MPs is a physiological process that occurs continuously throughout cell's life cycle in response to various environmental stimuli (Figure 2).[8–10] However, the release of MPs is significantly increased under stress conditions, including cell activation or apoptosis, hypoxia, oxidative damage, and shearing stress.[6,11] Although the exact mechanism governing the shedding of MPs is not yet fully understood, it is thought to involve the redistribution of membrane phospholipids between the two leaflets of the membrane bilayer, followed by cytoskeleton reorganization and cell contraction. The membrane bilayer is a fluid mosaic structure made essentially from phospholipids with embedded proteins. In resting state, phosphatidylcholine, and sphingomyeline are mainly located in the external leaflet whereas, phosphatidylserine (PS) and phosphatidylethanolamine are confined to the inner one.[12] The distribution of phospholipids is controlled by three phospholipid transporters: flippase, floppase, and scramblase. The flippase/floppase dyad governs either the inward (flip) or the outward (flop) translocation of phospholipids while the scramblase controls the unspecific distribution of phospholipids across the bilayer.

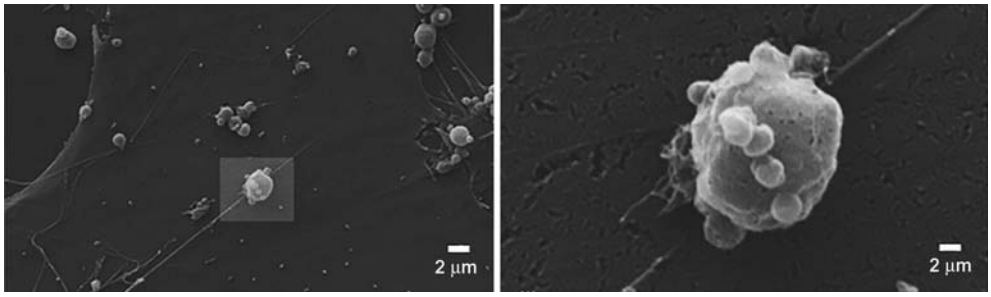


**Figure 2. Scanning electron micrograph of activated human platelets releasing microparticles. Adapted from David M. Lee *et al.*, Science, 2010; 327:588.**

Cell activation/apoptosis is accompanied by a rise in intracellular $Ca^{2+}$($[Ca^{2+}]_i$) that enhances the activity of the floppase and the scramblase and inhibits the activity of the flippase. As a result, the asymmetrical distribution of phospholipids is disrupted, and PS exposes on the cell surface leading to membrane vesiculation and release of MPs. Concomitantly, the activation of numerous intracellular signaling proteins and downstream effectors contribute to the remodelling of the cytoskeleton and subsequent MPs formation (Figure 3).

## Composition of microparticles

The biological properties of MPs are largely determined by their protein and lipid composition, which, in turn, depend on the cells they stem from and the stimulus leading to their production. For instance, MPs that derive from endothelial cells, namely endothelial-MPs (EMPs) contain a high proportion of metabolic enzymes, proteins involved in cell extravasation, and cytoskeleton-associated proteins.[13] In the other hand, platelet-MPs (PMPs) are enriched in surface proteins (integrins, P-selectin, etc.) while MPs from activated neutrophils have a high density of the integrin Mac-1.[14,15] The proteins exposed on MPs surface can serve to determine their cellular origin by using antibodies directed against specific epitopes. Studies have shown that the molecular "fingerprint" of MPs is influenced by the type of stimulus that causes their production. For example, cell apoptosis is characterized by the nucleus condensation and fragmentation, suggesting that MPs from apoptotic cells may contain a high proportion of genetic and nuclear material. Thereby, it is entirely possible to differentiate, within a subpopulation, the MPs released by activation from those shredded by apoptosis.

The lipid content of MPs is made essentially of cholesterol and amino phospholipids. In addition to their crucial role in the collapse of membrane asymmetry, lipids can modify the biological properties of MPs. For example, monocytes enriched with cholesterol may contribute to the development of atherothrombosis through the generation of highly procoagulant MPs.[16]

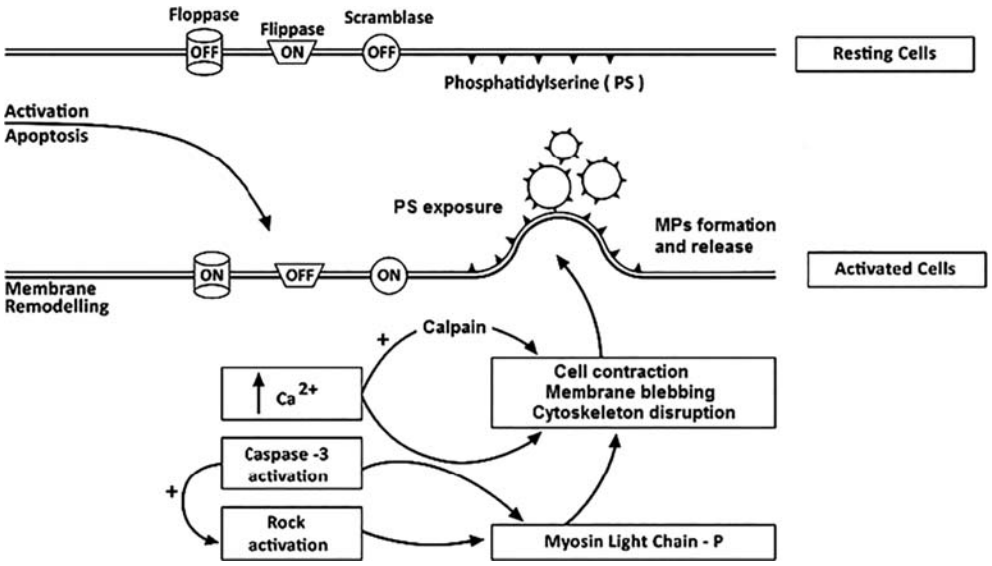


**Figure 3. Possible pathways leading to microparticles formation and release.**

### Clearance of microparticles

As yet, little is known about the fate of MPs in the circulation. When infused, MPs have a relatively short life span (< 10 minutes) as they are cleared rapidly following their introduction into circulation.[10] Nevertheless, the mechanism of clearance of MPs from the circulation is not well understood. Clearance of MPs is thought to occur mainly by phagocytosis in the spleen. Circulating MPs expose "removal signals" such as PS, tissue factor (TF), immunoglobulins, and complement that are recognized by phagocytic cells. Furthermore, studies on mice have reported a role of the spleen and the PS-binding protein lactadherin in the clearance of PMPs. They showed that splenectomized or lacadherin-deficient mice had an increased number of circulating MPs compared to their wild-type (WT) littermates.[17,18]

## ANALYSIS OF MICROPARTICLES

A growing body of evidence supports the implication of MPs in the pathophysiology of CVDs, and their analysis may readily enter the clinical scale. The analysis of MPs includes four essential steps: isolation, detection, subtyping, and quantification. However, accurate measurement of MPs is hampered by the lack of standardisation, and results vary between individual laboratories using different protocols. This is not only due to the discrepancy in the pre-analytical conditions like the anti-coagulant, centrifugation, and storage but also in the analytical methods used for their measurement.

### Pre-analytical procedures

The pre-analytical conditions represent a vast source of variability in the analysis of MPs. In most protocols, MPs are measured in the plasma obtained by fractionation of peripheral blood. Blood is usually withdrawn from the antecubital vein and mixed with anticoagulant in plastic tubes. It is recommended to use a large diameter needle (19-21G) without placing a tourniquet around the vein to avoid ex vivo activation of blood caused by the shear stress. In general, anticoagulants like sodium citrate, Ethylene Diamine Tetra Acetic (EDTA), and CTAD (a mixture of citrate, theophylline, adenosine and dipyridamole) are routinely used for blood collection. The centrifugation speed applied for plasma preparation differ between laboratories and vary from 1500 to 20.000 x g, yielding to platelet-poor plasma or platelet-free plasma solutions. Note that high centrifugation speeds (> 3000 x g) result in a significant decrease in MPs count.[19,20] Plasma samples are then stored at a temperature below − 80°C until further analysis. It is recommended to measure MPs in freshly prepared plasma (or ideally in fresh whole blood) to prevent any loss or damage that may occur during the storage process. Therefore, methods of direct measurement of MPs in unfractionated blood have been proposed.

### Measurement of microparticles by flow cytometry

Methods for MPs detection include flow cytometry, ELISA, fluorescence-based antibody array system, and functional coagulative assays.[21–24] In particular, ELISA and flow cytometry are widely used in clinical settings to measure MPs in plasma samples. Further, ELISA represents an easy and reproducible method to measure MPs with equal sensitivity to flow cytometry. This method is based on the binding of MPs by fluorescent monoclonal antibodies in a multi-well plate system and subsequent detection by a plate reader. However, the majority of studies have employed flow cytometry as the gold standard for quantification of MPs due to its simplicity and the wealth of information that can be collected from the population under study.[25–29] Flow cytometry enables the analysis of different MPs subtypes in one single run with the use of fluorescent antibodies directed against specific cell surface markers.

Technically, a MPs gate is set initially by fixing an absolute minimum threshold in forward scatter (FSC-A) or side scatter (SSC-A) parameter and the utilization of a mixture of size-calibrated fluorescent beads (Figure 4). For instance, a threshold of 200 is set in log scale SSC-A to exclude the electronic background noise, and a blend of fluorescent beads (200 nm – 3 μm) in a fixed numerical ratio (2:1, 0.5/0.9 μm beads, respectively) is used to determine the size distribution of MPs (Figure 4A).[30,31] The nominal size of MPs is distributed between the lower limit of the 200 nm beads (blue beads) and the upper limit of the 900 nm beads (black beads). Using an FSC-A vs. SSC-A setting (log scale), an MPs gate with an upper limit of approximately 1μm is defined (Figure 4B).[30]

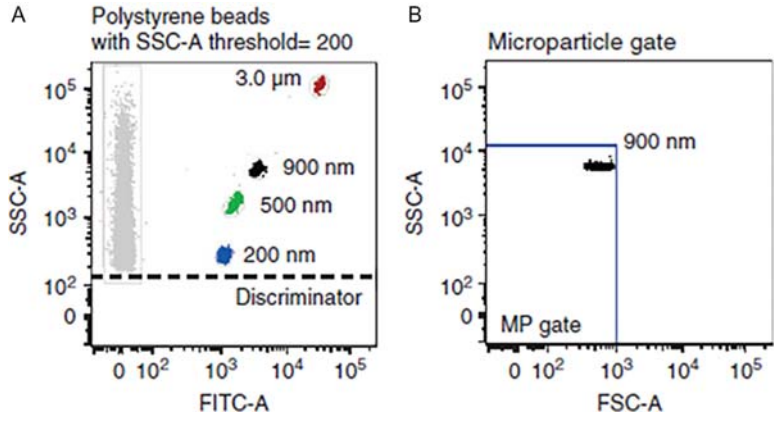


**Figure 4. A) Determination of MPs gate using traceable size beads from 200 nm to 3 μm of diameter with a set of SSC-A threshold of 200. B) Construction of MPs gate with an upper limit set just above the size distribution of the 0.9 μm beads in FSC-A vs. SSC-A setting. A logarithmic scale was used for all channels. Adapted from Aase Handberg *et al.*, Journal of Extracellular Vesicles, 2014; 3:20795.**

## LABELLING OF MICROPARTICLES POPULATIONS

Platelet-MPs are detected with either single color staining using antibodies directed against platelet-CD41 or CD42b antigens or dual color staining using a combination of anti CD41/CD42b (Figure 5). Note that CD41 and CD42b antigens are exclusively expressed on platelets. Sometimes, anti CD31 antibody is used along with either anti CD41 or anti CD42b antibody for PMPs detection.[32–35] However, CD31 is also expressed on endothelial cells and other myeloid cells such as monocytes, granulocytes, and B cells but is most abundant in endothelial cells.[36]

The most common method for EMPs labelling is the dual color combination of anti-CD31 and anti-CD41 or anti-CD42b antibodies.[32,33,35,37–39] Because CD31 is a common marker for platelets and endothelial cells, PMPs appear as a $CD31^+/CD41^+$ population whereas EMPs appear as a $CD31^+/CD41^-$ population, giving both counts in a single run (Figure 5). This combination of markers has the advantage of being quite bright (abundant) and very specific. However, other combination of markers such as platelet CD61[40,41] and endothelial CD144[33,42] could be also employed.

Detection of leukocyte-MPs (LMPs) is hampered by the variability of surface markers expressed by different populations of leukocytes. In fact, LMPs may originate from neutrophils, monocytes, and B- and T-lymphocytes. However, antibodies directed against pan-leukocyte markers have been used.

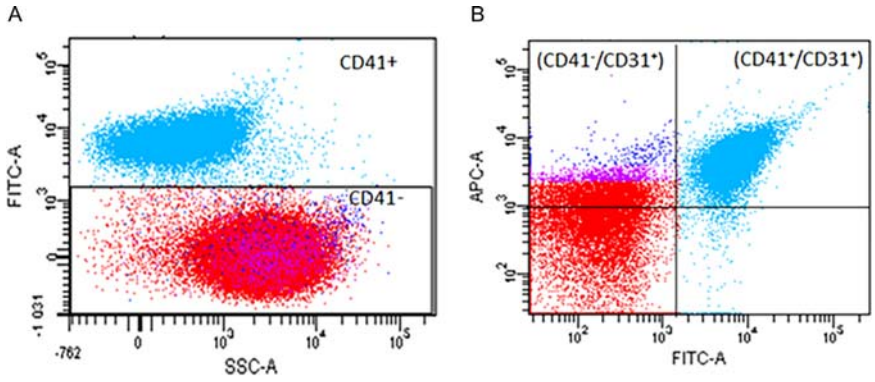


**Figure 5. A) Gating of human MPs stained with platelet CD41a antibody showing populations of MPs-CD41$^{+}$ (blue) and MPs-CD41$^{-}$ (red). B Double staining for MPs populations showing platelet-MPs (CD41$^{+}$/CD31$^{+}$) and endothelial-MPs populations (CD41$^{-}$/CD31$^{+}$).**

This includes antibodies directed against the surface markers CD11b/CD18,[15] CD11a/CD18,[43] CD66,[15], CD62 L,[44,45] or the Annexin A1.[44]

## ABSOLUTE MICROPARTICLES COUNT

Absolute MPs count is acquired using flow cytometry technique by adding a specific amount of fluorescent counting microbeads (with determined concentration) to a certain volume of sample, so that the volume of sample and microbeads is known. The number of MPs and microbeads events can be used with the sample volume to determine MPs concentration following the below equation:

$$(A/B) \times (C/D) = Number\ of\ MPs\ per\ \mu l$$

A = Number of MPs events
B = Number of beads events
C = Assigned bead count (beads/50 μl)
D = Volume of Sample (μl)

## MICROPARTICLES IN CARDIOVASCULAR DISEASES

Mounting evidence supports the role of MPs in the development of CVDs. In fact, MPs are detected in the peripheral blood of healthy individuals but their levels and phenotypes change in numerous disease conditions (Table 1).

**Table 1. Changes in level of microparticles in cardiovascular disease associated conditions.**

| Conditions | Changes in microparticles levels | Type of microparticles | References |
|---|---|---|---|
| Hypertension | Increase | EMPs, PMPs, LMPs | 46 |
| Hypercholesterolemia | Increase | PMPs | 47 |
| Diabetes mellitus | Increase | EMPs, PMPs, LMPs | 48 |
| Obesity | Increase | EMPs, PMPs | 49 |
| Coronary artery disease | Increase | EMPs, PMPs | 38,50 |
| Acute coronary syndrome | Increase | EMPs, PMPs, LMPs | 38 |
| Peripheral artery diseases | Increase | PMPs | 47 |
| Stroke | Increase | EMPs, PMPs | 51 |
| End stage renal disease | Increase | EMPs | 32 |

### Microparticles in atherosclerosis

Atherosclerosis is a life course disease that begins with the evolution of risk factors that contribute to endothelial dysfunction, inflammation, and vascular injury. Formation of MPs has been considered as a hallmark of endothelial activation and dysfunction in many earlier studies,[52–54] that indicate the

association between atherosclerosis and MPs. Stress, disturbed blood flow, and other plaque associated factors have been shown to stimulate endothelial cell apoptosis that results in excessive release of EMPs.[55,56] Detection of MPs in the atherosclerotic plaques evidences their involvement in the plaque formation. Erythrocyte-derived MPs and LMPs are abundantly present in atherosclerotic plaques, reflecting their importance as vascular biomarkers in atherogenesis and plaque rupture.[57] Adhesion of monocytes and neutrophils to the endothelium is a crucial step in the development of atherosclerosis.[58–60] In this perspective, PMPs were found to induce the expression of CD11a and CD11b on monocytes and enhance their adhesion to the endothelial cells.[61]

In patients with hypercholesterolemia, hypertension, and diabetes mellitus, circulating MPs correlate with the level of endothelium dysfunction and impairment of vasodilatation. A study showed an association between the levels of EMPs ($CD144^{+}$) and the presence of coronary non-calcified vulnerable plaques in type 2 diabetes mellitus patients.[62] The EMPs, defined as CD31+/CD42−, were found to be 2.5 fold greater in patients with high risk lesion and 3 fold greater in patients with high risk lesion and thrombi than the patients with low risk lesions.[63] A study by Nozaki *et al.*, reported that EMPs were found to be independent predictors of future cardiovascular events and could add significant value to the Framingham risk factors.[64]

Two studies assessed the association between the levels of MPs and presence of atherosclerotic plaques in asymptomatic healthy subjects. A study conducted by Chironi *et al.*, quantified various subtypes of MPs and correlated them with the presence of subclinical atherosclerosis evidenced by ultra sound.[54] Circulating LMPs, defined as $CD11a\text{-}MPs^{+}$, were higher in subjects with ultrasound evidence of subclinical atherosclerosis. LMPs differed between the 3 grades of CRP with greater value in subjects with higher CRP than in those with low CRP. LMPs levels positively related with increasing number of components of metabolic syndrome and tertiles of plasma fibrinogen. However, Annexin $V\text{-}^{+}$, PMPs- and- EMPs levels did not show a significant association with the presence of atherosclerotic plaques. A study by Amabile *et al.*, identified and quantified circulating $MP\text{-}CD144^{+}$- and $MPs\text{-}CD31^{+}/CD41^{-}$ in individuals without a history of CVDs in the Framingham Offspring cohort.[65] This study reported that hypertension, elevated triglycerides, and metabolic syndrome were associated with EMPs. Additionally, T-cadherin-exposing EMPs were reported to be elevated in patients with subclinical atherosclerosis.[66] In diabetic patients, increased pro-coagulant activity of PMPs was associated with the risk of atherosclerosis.[57]

Overall, the findings suggest that MPs may act as potential predictors of atherosclerosis. They can add a useful predictive value to that of Framingham risk score to identify the risk of developing CVDs.

## Microparticles in myocardial infarction

A growing body of evidence supports the role of MPs in the development of myocardial infarction (MI). Various case controlled studies evidence the elevated levels of MPs in patients with ST-segment elevation myocardial infarction (STEMI). More specifically, it has been shown that LMPs stimulate endothelial cells cytokine release and TF induction and are associated with venous thrombus formation. Jung *et al.*, showed that the number of circulating EMPs correlates with the myocardium at risk and infarct size.[27] In a study by Min *et al.*, the levels of MPs were found to be significantly higher in patients with STEMI patients than in healthy controls.[67] When compared between the culprit coronary arteries and peripheral arteries, the level of MPs was significantly higher in the culprit coronary arteries. The number of EMPs progressively increases in the blood of patients with unstable angina and MI as compared with stable angina or non-coronary patients.[68]

Platelet-MPs levels often correlate with other markers of platelet activation, such as soluble P-selectin (CD62P), membrane bound P-selectin, CD63, and CD40L. The increase in PMPs levels is related to the severity and the burden of the acute MI. In fact, both PMPs and EMPs levels were significantly higher in the intracoronary than in the aortic blood samples in STEMI patients undergoing PCI, supporting their role as markers of acute thrombosis. Thus, it is likely that an abnormal MPs profiles and particularly an augmentation of the number of circulating pro-coagulant and pro-inflammatory MPs will help in the diagnosis and prognosis of acute MI.

## Microparticles in vascular remodelling and heart failure

Accumulating evidence indicates the existence of a strong relationship between MPs and vascular remodelling process predisposing to heart failure (HF). In fact, it has been reported that circulating MPs are the major factor associated with carotid artery remodelling[69] and contribute to the extra cellular

matrix degradation mainly through the matrix metalloproteinases (MMPs) activity that they harbor.[70–73]

Recently, it has been reported that small-size MPs (below 0.5 μm) could be potentially implicated in the modulation of the post-acute coronary syndrome reparative response to injury, with prognostic implications.[28] Specifically, LMPs activate endothelial cells and stimulate the release of cytokines and TF.[74,75] EMPs, on the other hand, are acquiring emerging roles in the pathogenesis of cardiac remodelling, and its plasmatic levels are elevated in acute coronary syndrome,[38,76] acute MI,[26,27,43] and HF[77]. In fact, EMPs carry oxidized phospholipids at their surface and trigger the release of chemokines from endothelial cells, which in turn attracts leukocytes and induces their adhesion to the endothelium.[78] Moreover, EMPs can activate neutrophils, provoke thrombin generation, and induce extra cellular matrix degradation.[79]

A study by Nozaki *et al.*, reported that high EMPs level is an independent predictor of future cardiovascular events in HF patients.[77] They measured the plasma CD144+ - EMPs levels in HF patients with New York Heart Association (NYHA) class I or more and found that EMPs were significantly increased with NYHA functional class. The level of plasma EMPs were higher in NYHA class III followed by NYHA class II, I and healthy controls. During the 30 months follow up, patients in the high EMPs group frequently developed cardiovascular events compared with those in the low EMPs group. Berezin *et al.*, reported that the number of circulating EMPs independently predicted all-cause mortality, chronic HF related death, and chronic heart related re-hospitalization.[80]

Collectively, these data reflect the importance of MPs in acute MI and suggest that assessment of MPs in conjunction with other bioactive markers may significantly improve risk stratification and determination of adverse remodelling in acute MI.

### Microparticles in cardiac-related diseases

Obstructive sleep apnoea is independently associated with various CVDs, including MI and stroke. Obstructive sleep apnoea may promote atherosclerosis risk factors such as endothelial dysfunction, hypertension, diabetes, and dyslipidemia and may have direct pro-atherogenic effects on the vascular wall.[81] Case controlled studies have shown that platelet-, endothelium- and leukocyte derived-MPs levels are increased in obstructive sleep apnoea.[82–85] Experimental evidence has demonstrated that MPs from patients with obstructive sleep apnoea induce endothelial dysfunction, inflammation, and vascular hyper-reactivity when injected into mice.[86]

Endothelial-MPs were reported to be an independent predictor of severe cardiovascular outcome in end-stage renal failure patients.[87] Endothelial-MPs increase in patients with stable chronic pulmonary diseases and increases further in patients with exacerbated chronic pulmonary diseases than the patients with non-chronic pulmonary diseases.[88] Levels of MPs in those patients correlate with lung destruction and airflow limitation. Psoriasis is a risk factor for a cardiometabolic disease apart from the traditional risk factors, and studies evidence the higher concentration of MPs in patients with psoriasis compare to controls.[89,90]

## MICROPARTICLES AS CELL EFFECTORS

The role of MPs as cell effectors has been witnessed by their ability to transfer biological information between cells. Mostly, MPs exchange information at the levels of endothelium and significantly influence their physiological role. Furthermore, various in vitro studies, in which MPs were isolated from biological fluids or generated in vitro conditions using cell lines, demonstrate that MPs are involved in several conditions such as coagulation, thrombosis, inflammation, and vascular dysfunction.

### Coagulation

Plaque rupture followed by thrombosis is the cause of most of the acute coronary syndromes. There is a strong evidence of the involvement of MPs in the activation of the coagulation cascade.[51] In fact, MPs expose PS, which is an essential anionic phospholipid for surface amplification of thrombin generation. MPs also display activated factor V on their surface and contribute to the activation of clotting.

Tissue factors are present in MPs derived from platelet, monocytes, and smooth muscle cells (SMCs). These tissue factors trigger factors VII-mediated thrombin generation. Pericardial blood of cardiac surgery patients was shown to have a higher amount of TF-exposed MPs antigen levels when compared to healthy individuals.[91] Pericardial-MPs were highly pro-coagulant in vitro and highly

thrombogenic in a rat model.[92] Inhibitory antibody against human TFs abolished their thrombogenicity, suggesting that MPs exert their pro-coagulant and thrombogenic effect via TF activity. Platelet deposition on damaged arteries was found to be higher in platelet-enriched blood samples. Characterization of atherosclerotic plaques also shows that it contains highly pro-coagulant MPs of monocytic and lymphocytic origins, which expose TF and exhibit pro-coagulant activity.[93]

### Inflammation

Circulating MPs exert varieties of pro-inflammatory activities by stimulating platelets, leukocytes, and endothelial cells. Microparticles released by apoptotic Jurkat cells are shown to have the complement components $C_3$ and $C_4$ on their surface. These complement factors mediate pro-inflammatory effects and cause vascular injury. The oxidized phospholipids present in the MPs are released due to the oxidative stress causes the adhesion of monocytes and neutrophils to the endothelium.[94]

Microparticles also stimulate the secretion of several inflammatory cytokines and thereby contribute to the propagation of inflammatory reactions associated with CVDs.[75,95–97] Microparticles released from lymphocytes and monocytes stimulate fibroblasts to secrete MMPs, cytokines and chemokines.[98] They also induce the secretion of interleukin-6 (IL-6) and monocyte chemoattractant protein-1 (MCP-1) by endothelial cells.[75] Platelet-MPs are shown to enhance the secretion of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-$\alpha$), IL-1b, and IL-6.[99] Platelet-MPs also contain large amounts of the inflammatory chemokine RANTES ($CCl_5$) which they deposit on activated or atherosclerotic endothelium.[100] On the other hand, one study showed that MPs released by activated neutrophils attenuate cellular activation in macrophages and may help to down-regulate the inflammatory response.[101]

### Angiogenesis

Recently, the modulation of vascular tone by MPs has been reported. Circulating MPs may modulate both Nitric Oxide (NO) and Prostacyclin ($PGI_2$) pathways in endothelial cells and also produce thromboxane $A_2$ ($TXA_2$) to act directly on SMCs.[102] Upon activation, platelets release MPs that promote the proliferation and survival, migration, and tube formation in human umbilical vein endothelial cells (HUVEC).[71] Lipid components of MPs handle this activity, and the signaling is dependent on different kinases. Another study by Brill *et al.*, showed a dose dependent proangiogenic effect of PMPs.[72] In the rat aortic ring models, MPs generated from thrombin-activated platelets showed a dose dependent proangiogenic effect. This effect was mediated via PI3-kinase, Src kinase, and ERK signaling pathways. Also, PMPs were proved to induce angiogenesis in the in vivo model. These effects were mediated by vascular endothelial growth (VEGF) factor and other growth factors such as basic fibroblast growth factor (bFGF) and platelet-derived growth factor (PDGF). The proangiogenic activity of EMPs was also reported. Endothelial-MPs were shown to contain MMPs responsible for proteolysis of the basement membrane and thus facilitating neovascular structure formation and/or cell invasion.[103]

## MICROPARTICLES AS THERAPEUTIC TOOLS

Besides their role as mediators of noxious responses, MPs may have a therapeutic role by reducing deleterious signaling in target cells and inducing tissue repair, endothelial function, and survival of apoptotic cells.[104,105] Benameur *et al.*, reported that circulating MPs harboring the peroxisome proliferator-activated receptor alpha (PPAR$\alpha$) promote angiogenesis by inducing differentiation of endothelial progenitor cells (EPCs) via a PPAR$\alpha$/Akt/NF-$\kappa$B dependent pathway.[106] Similarly, another study using MPs derived from in vitro activated platelets showed that PMPs can improve the regenerative potential of EPCs by promoting their proangiogenic profile.[107] Major mechanisms involve the upregulation of endothelial markers and induction of EPCs recruitment, migration, differentiation, and release of proangiogenic factors. Enhancement of EPCs differentiation may represent a useful strategy for vascular regeneration. Moreover, MPs can be engineered to overexpress different proteins following induction/activation of their parental cells. For instance, various studies demonstrated that MPs generated from activated/apoptotic human T lymphocytes, and harboring the morphogen sonic hedgehog (Shh), improve endothelial function by inducing NO release and promoting angiogenesis through upregulation of adhesion protein and release of proangiogenic factors.[73,108,109] Taken together, these studies suggest that engineered MPs could represent a therapeutic tool to correct impaired functions associated with CVDs. Finally, MPs contains biological information carried by DNA,

RNA, and miRNA. The cytoplasmic pool of MPs confers an appropriate biochemical environment which protects the genetic material from degradation and favors the delivery of biological messages between cells.[110–112]

## CONCLUSIONS AND FUTURE DIRECTIONS

Formerly considered as inert particles or by-product of platelet activation, MPs have emerged as key players in the pathogenesis of numerous CVDs as they can induce endothelial dysfunction, inflammation, coagulation, and cardiovascular remodelling. In contrast, MPs could play an important role in cellular homeostasis, intercellular communication, and serve as indices for health and physiology assessments for both diagnostic and risk stratification purposes. Furthermore, engineered MPs are acquiring emerging role as therapeutic tools to correct cardiovascular pathologies and MPs-associated miRNA prone to be a potential vector for gene therapy.

The ubiquitous role of MPs in the cardiovascular system necessities further investigations on their cell lineage and the molecular mechanisms underlying their formation, release, and clearance. More in depth studies should be focused on further characterization of MPs composition (proteins, lipids, mRNA, microRNA) and the message they transport to the targeted cells and tissues. This will significantly improve our understanding of the biological effects they can induce and the extent of their contribution to the pathophysiological processes. The challenges rely on the refinement and validation of the methodology of detection of MPs. This implies standardization of the pre-analytical handling of samples and analysis of MPs by biophysical techniques including flow cytometry. Given the fast-developing field of biotechnology and the increasing research interest on MPs, significant advances are expected in a near future. Such advances will expand the current knowledge on the pathophysiological role of MPs in CVDs and ultimately set the ground for the application of MPs in personalized medicine.

## REFERENCES

[1] Alwan A. Global status report on non-communicable diseases 2010. *World Health Organization*. Geneva 2011.
[2] Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS medicine*. 2006;3:e442.
[3] Ahmad T, Fiuzat M, Felker GM, O'Connor C. Novel biomarkers in chronic heart failure. *Nature reviews. Cardiology*. 2012;9:347–359.
[4] Amodio G, Antonelli G, Di Serio F. Cardiac biomarkers in acute coronary syndromes: A review. *Current vascular pharmacology*. 2010;8:388–393.
[5] Wolf P. The nature and significance of platelet products in human plasma. *British journal of haematology*. 1967;13:269–288.
[6] Martinez MC, Tual-Chalot S, Leonetti D, Andriantsitohaina R. Microparticles: Targets and tools in cardiovascular disease. *Trends in pharmacological sciences*. 2011;32:659–665.
[7] Helal O, Defoort C, Robert S, Marin C, Lesavre N, Lopez-Miranda J, Riserus U, Basu S, Lovegrove J, McMonagle J, Roche HM, Dignat-George F, Lairon D. Increased levels of microparticles originating from endothelial cells, platelets and erythrocytes in subjects with metabolic syndrome: Relationship with oxidative stress. *Nutrition, metabolism, and cardiovascular diseases: NMCD*. 2011;21:665–671.
[8] Hong Y, Eleftheriou D, Hussain AA, Price-Kuehne FE, Savage CO, Jayne D, Little MA, Salama AD, Klein NJ, Brogan PA. Anti-neutrophil cytoplasmic antibodies stimulate release of neutrophil microparticles. *Journal of the American Society of Nephrology: JASN*. 2012;23:49–62.
[9] Oehmcke S, Morgelin M, Malmstrom J, Linder A, Chew M, Thorlacius H, Herwald H. Stimulation of blood mononuclear cells with bacterial virulence factors leads to the release of pro-coagulant and pro-inflammatory microparticles. *Cellular microbiology*. 2012;14:107–119.
[10] Rand ML, Wang H, Bang KW, Packham MA, Freedman J. Rapid clearance of procoagulant platelet-derived microparticles from the circulation of rabbits. *Journal of thrombosis and haemostasis: JTH*. 2006;4:1621–1623.
[11] Barteneva NS, Fasler-Kan E, Bernimoulin M, Stern JN, Ponomarev ED, Duckett L, Vorobjev IA. Circulating microparticles: Square the circle. *BMC cell biology*. 2013;14:23.
[12] Creutz CE, Hira JK, Gee VE, Eaton JM. Protection of the membrane permeability barrier by annexins. *Biochemistry*. 2012;51:9966–9983.
[13] Banfi C, Brioschi M, Wait R, Begum S, Gianazza E, Pirillo A, Mussoni L, Tremoli E. Proteome of endothelial cell-derived procoagulant microparticles. *Proteomics*. 2005;5:4443–4455.
[14] Garcia BA, Smalley DM, Cho H, Shabanowitz J, Ley K, Hunt DF. The platelet microparticle proteome. *Journal of proteome research*. 2005;4:1516–1521.
[15] Pluskota E, Woody NM, Szpak D, Ballantyne CM, Soloviev DA, Simon DI, Plow EF. Expression, activation, and function of integrin alphambeta2 (mac-1) on neutrophil-derived microparticles. *Blood*. 2008;112:2327–2335.
[16] Liu ML, Reilly MP, Casasanto P, McKenzie SE, Williams KJ. Cholesterol enrichment of human monocyte/macrophages induces surface exposure of phosphatidylserine and the release of biologically-active tissue factor-positive microvesicles. *Arteriosclerosis, thrombosis, and vascular biology*. 2007;27:430–435.
[17] Dasgupta SK, Abdel-Monem H, Niravath P, Le A, Bellera RV, Langlois K, Nagata S, Rumbaut RE, Thiagarajan P. Lactadherin and clearance of platelet-derived microvesicles. *Blood*. 2009;113:1332–1339.

[18] Ait-Oufella H, Kinugawa K, Zoll J, Simon T, Boddaert J, Heeneman S, Blanc-Brude O, Barateau V, Potteaux S, Merval R, Esposito B, Teissier E, Daemen MJ, Leseche G, Boulanger C, Tedgui A, Mallat Z. Lactadherin deficiency leads to apoptotic cell accumulation and accelerated atherosclerosis in mice. *Circulation*. 2007;115:2168–2177.

[19] Chandler WL. Microparticle counts in platelet-rich and platelet-free plasma, effect of centrifugation and sample-processing protocols. *Blood coagulation & fibrinolysis: an international journal in haemostasis and thrombosis*. 2013;24:125–132.

[20] Ayers L, Kohler M, Harrison P, Sargent I, Dragovic R, Schaap M, Nieuwland R, Brooks SA, Ferry B. Measurement of circulating cell-derived microparticles by flow cytometry: Sources of variability within the assay. *Thrombosis research*. 2011;127:370–377.

[21] Jy W, Horstman LL, Jimenez JJ, Ahn YS, Biro E, Nieuwland R, Sturk A, Dignat-George F, Sabatier F, Camoin-Jau L, Sampol J, Hugel B, Zobairi F, Freyssinet JM, Nomura S, Shet AS, Key NS, Hebbel RP. Measuring circulating cell-derived microparticles. *Journal of thrombosis and haemostasis: JTH*. 2004;2:1842–1851.

[22] Enjeti AK, Lincz LF, Seldon M. Detection and measurement of microparticles: An evolving research tool for vascular biology. *Seminars in thrombosis and hemostasis*. 2007;33:771–779.

[23] Lal S, Brown A, Nguyen L, Braet F, Dyer W, Dos Remedios C. Using antibody arrays to detect microparticles from acute coronary syndrome patients based on cluster of differentiation (cd) antigen expression. *Molecular & cellular proteomics: MCP*. 2009;8:799–804.

[24] Nomura S, Shouzu A, Taomoto K, Togane Y, Goto S, Ozaki Y, Uchiyama S, Ikeda Y. Assessment of an elisa kit for platelet-derived microparticles by joint research at many institutes in japan. *Journal of atherosclerosis and thrombosis*. 2009;16:878–887.

[25] Biasucci LM, Porto I, Di Vito L, De Maria GL, Leone AM, Tinelli G, Tritarelli A, Di Rocco G, Snider F, Capogrossi MC, Crea F. Differences in microparticle release in patients with acute coronary syndrome and stable angina. *Circulation journal: official journal of the Japanese Circulation Society*. 2012;76:2174–2182.

[26] Porto I, Biasucci LM, De Maria GL, Leone AM, Niccoli G, Burzotta F, Trani C, Tritarelli A, Vergallo R, Liuzzo G, Crea F. Intracoronary microparticles and microvascular obstruction in patients with st elevation myocardial infarction undergoing primary percutaneous intervention. *European heart journal*. 2012;33:2928–2938.

[27] Jung C, Sorensson P, Saleh N, Arheden H, Ryden L, Pernow J. Circulating endothelial and platelet derived microparticles reflect the size of myocardium at risk in patients with st-elevation myocardial infarction. *Atherosclerosis*. 2012;221:226–231.

[28] Montoro-Garcia S, Shantsila E, Tapp LD, Lopez-Cuenca A, Romero AI, Hernandez-Romero D, Orenes-Pinero E, Manzano-Fernandez S, Valdes M, Marin F, Lip GY. Small-size circulating microparticles in acute coronary syndromes: Relevance to fibrinolytic status, reparative markers and outcomes. *Atherosclerosis*. 2013;227:313–322.

[29] Skeppholm M, Mobarrez F, Malmqvist K, Wallen H. Platelet-derived microparticles during and after acute coronary syndrome. *Thrombosis and haemostasis*. 2012;107:1122–1129.

[30] Dey-Hazra E, Hertel B, Kirsch T, Woywodt A, Lovric S, Haller H, Haubitz M, Erdbruegger U. Detection of circulating microparticles by flow cytometry: Influence of centrifugation, filtration of buffer, and freezing. *Vascular health and risk management*. 2010;6:1125–1133.

[31] Robert S, Poncelet P, Lacroix R, Arnaud L, Giraudo L, Hauchard A, Sampol J, Dignat-George F. Standardization of platelet-derived microparticle counting using calibrated beads and a cytomics fc500 routine flow cytometer: A first step towards multicenter studies? *Journal of thrombosis and haemostasis: JTH*. 2009;7:190–197.

[32] Amabile N, Guerin AP, Leroyer A, Mallat Z, Nguyen C, Boddaert J, London GM, Tedgui A, Boulanger CM. Circulating endothelial microparticles are associated with vascular dysfunction in patients with end-stage renal failure. *Journal of the American Society of Nephrology: JASN*. 2005;16:3381–3388.

[33] Boulanger CM, Amabile N, Guerin AP, Pannier B, Leroyer AS, Mallat CN, Tedgui A, London GM. In vivo shear stress determines circulating levels of endothelial microparticles in end-stage renal disease. *Hypertension*. 2007;49:902–908.

[34] Chirinos JA, Heresi GA, Velasquez H, Jy W, Jimenez JJ, Ahn E, Horstman LL, Soriano AO, Zambrano JP, Ahn YS. Elevation of endothelial microparticles, platelets, and leukocyte activation in patients with venous thromboembolism. *Journal of the American College of Cardiology*. 2005;45:1467–1471.

[35] Arteaga RB, Chirinos JA, Soriano AO, Jy W, Horstman L, Jimenez JJ, Mendez A, Ferreira A, de Marchena E, Ahn YS. Endothelial microparticles and platelet and leukocyte activation in patients with the metabolic syndrome. *The American journal of cardiology*. 2006;98:70–74.

[36] Minagar A, Jy W, Jimenez JJ, Sheremata WA, Mauro LM, Mao WW, Horstman LL, Ahn YS. Elevated plasma endothelial microparticles in multiple sclerosis. *Neurology*. 2001;56:1319–1324.

[37] Garcia S, Chirinos J, Jimenez J, Del Carpio Munoz FC, Canoniero M, Jy W, Jimenez J, Horstman L, Ahn Y. Phenotypic assessment of endothelial microparticles in patients with heart failure and after heart transplantation: Switch from cell activation to apoptosis. *The Journal of heart and lung transplantation: the official publication of the International Society for Heart Transplantation*. 2005;24:2184–2189.

[38] Bernal-Mizrachi L, Jy W, Jimenez JJ, Pastor J, Mauro LM, Horstman LL, de Marchena E, Ahn YS. High levels of circulating endothelial microparticles in patients with acute coronary syndromes. *American heart journal*. 2003;145:962–970.

[39] Wang JM, Huang YJ, Wang Y, Xu MG, Wang LC, Wang SM, Tao J. Increased circulating cd31+/cd42− microparticles are associated with impaired systemic artery elasticity in healthy subjects. *American journal of hypertension*. 2007;20:957–964.

[40] Blann AD, Woywodt A, Bertolini F, Bull TM, Buyon JP, Clancy RM, Haubitz M, Hebbel RP, Lip GY, Mancuso P, Sampol J, Solovey A, Dignat-George F. Circulating endothelial cells. Biomarker of vascular disease. *Thrombosis and haemostasis*. 2005;93:228–235.

[41] Tan KT, Tayebjee MH, Lynd C, Blann AD, Lip GY. Platelet microparticles and soluble p selectin in peripheral artery disease: Relationship to extent of disease and platelet activation markers. *Annals of medicine*. 2005;37:61–66.

[42] Koga H, Sugiyama S, Kugiyama K, Watanabe K, Fukushima H, Tanaka T, Sakamoto T, Yoshimura M, Jinnouchi H, Ogawa H. Elevated levels of ve-cadherin-positive endothelial microparticles in patients with type 2 diabetes mellitus and coronary artery disease. *Journal of the American College of Cardiology*. 2005;45:1622–1630.
[43] Morel O, Pereira B, Averous G, Faure A, Jesel L, Germain P, Grunebaum L, Ohlmann P, Freyssinet JM, Bareiss P, Toti F. Increased levels of procoagulant tissue factor-bearing microparticles within the occluded coronary artery of patients with st-segment elevation myocardial infarction: Role of endothelial damage and leukocyte activation. *Atherosclerosis*. 2009;204:636–641.
[44] Dalli J, Norling LV, Renshaw D, Cooper D, Leung KY, Perretti M. Annexin 1 mediates the rapid anti-inflammatory effects of neutrophil-derived microparticles. *Blood*. 2008;112:2512–2519.
[45] Gasser O, Hess C, Miot S, Deon C, Sanchez JC, Schifferli JA. Characterisation and properties of ectosomes released by human polymorphonuclear neutrophils. *Experimental cell research*. 2003;285:243–257.
[46] Preston RA, Jy W, Jimenez JJ, Mauro LM, Horstman LL, Valle M, Aime G, Ahn YS. Effects of severe hypertension on endothelial and platelet microparticles. *Hypertension*. 2003;41:211–217.
[47] Nomura S, Inami N, Shouzu A, Omoto S, Kimura Y, Takahashi N, Tanaka A, Urase F, Maeda Y, Ohtani H, Iwasaka T. The effects of pitavastatin, eicosapentaenoic acid and combined therapy on platelet-derived microparticles and adiponectin in hyperlipidemic, diabetic patients. *Platelets*. 2009;20:16–22.
[48] Sabatier F, Darmon P, Hugel B, Combes V, Sanmarco M, Velut JG, Arnoux D, Charpiot P, Freyssinet JM, Oliver C, Sampol J, Dignat-George F. Type 1 and type 2 diabetic patients display different patterns of cellular microparticles. *Diabetes*. 2002;51:2840–2845.
[49] Casey RG, Joyce M, Roche-Nagle G, Cox D, Bouchier-Hayes DJ. Young male smokers have altered platelets and endothelium that precedes atherosclerosis. *The Journal of surgical research*. 2004;116:227–233.
[50] van der Zee PM, Biro E, Ko Y, de Winter RJ, Hack CE, Sturk A, Nieuwland R. P-selectin- and cd63-exposing platelet microparticles reflect platelet activation in peripheral arterial disease and myocardial infarction. *Clinical chemistry*. 2006;52:657–664.
[51] Simak J, Gelderman MP. Cell membrane microparticles in blood and blood products: Potentially pathogenic agents and diagnostic markers. *Transfusion medicine reviews*. 2006;20:1–26.
[52] Constans J, Conri C. Circulating markers of endothelial function in cardiovascular disease. *Clinica chimica acta; international journal of clinical chemistry*. 2006;368:33–47.
[53] Boulanger CM, Amabile N, Tedgui A. Circulating microparticles: A potential prognostic marker for atherosclerotic vascular disease. *Hypertension*. 2006;48:180–186.
[54] Chironi GN, Boulanger CM, Simon A, Dignat-George F, Freyssinet JM, Tedgui A. Endothelial microparticles in diseases. *Cell and tissue research*. 2009;335:143–151.
[55] Brodsky SV, Zhang F, Nasjletti A, Goligorsky MS. Endothelium-derived microparticles impair endothelial function in vitro. *American journal of physiology. Heart and circulatory physiology*. 2004;286:H1910–H1915.
[56] Densmore JC, Signorino PR, Ou J, Hatoum OA, Rowe JJ, Shi Y, Kaul S, Jones DW, Sabina RE, Pritchard KA Jr, Guice KS, Oldham KT. Endothelium-derived microparticles induce endothelial dysfunction and acute lung injury. *Shock*. 2006;26:464–471.
[57] Leroyer AS, Isobe H, Leseche G, Castier Y, Wassef M, Mallat Z, Binder BR, Tedgui A, Boulanger CM. Cellular origins and thrombogenic activity of microparticles isolated from human atherosclerotic plaques. *Journal of the American College of Cardiology*. 2007;49:772–777.
[58] Soehnlein O. Multiple roles for neutrophils in atherosclerosis. *Circulation research*. 2012;110:875–888.
[59] Swirski FK, Robbins CS. Neutrophils usher monocytes into sites of inflammation. *Circulation research*. 2013;112:744–745.
[60] Swirski FK, Nahrendorf M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. *Science*. 2013;339:161–166.
[61] Barry OP, Pratico D, Savani RC, FitzGerald GA. Modulation of monocyte-endothelial cell interactions by platelet microparticles. *The Journal of clinical investigation*. 1998;102:136–144.
[62] Tushuizen ME, Nieuwland R, Rustemeijer C, Hensgens BE, Sturk A, Heine RJ, Diamant M. Elevated endothelial microparticles following consecutive meals are associated with vascular endothelial dysfunction in type 2 diabetes. *Diabetes care*. 2007;30:728–730.
[63] Bernal-Mizrachi L, Jy W, Fierro C, Macdonough R, Velazques HA, Purow J, Jimenez JJ, Horstman LL, Ferreira A, de Marchena E, Ahn YS. Endothelial microparticles correlate with high-risk angiographic lesions in acute coronary syndromes. *International journal of cardiology*. 2004;97:439–446.
[64] Nozaki T, Sugiyama S, Koga H, Sugamura K, Ohba K, Matsuzawa Y, Sumida H, Matsui K, Jinnouchi H, Ogawa H. Significance of a multiple biomarkers strategy including endothelial dysfunction to improve risk stratification for cardiovascular events in patients at high risk for coronary heart disease. *Journal of the American College of Cardiology*. 2009;54:601–608.
[65] Amabile N, Cheng S, Renard JM, Larson MG, Ghorbani A, McCabe E, Griffin G, Guerin C, Ho JE, Shaw SY, Cohen KS, Vasan RS, Tedgui A, Boulanger CM, Wang TJ. Association of circulating endothelial microparticles with cardiometabolic risk factors in the framingham heart study. *European heart journal*. 2014;35:2972–2979.
[66] Philippova M, Suter Y, Toggweiler S, Schoenenberger AW, Joshi MB, Kyriakakis E, Erne P, Resink TJ. T-cadherin is present on endothelial microparticles and is elevated in plasma in early atherosclerosis. *European heart journal*. 2011;32:760–771.
[67] Min PK, Kim JY, Chung KH, Lee BK, Cho M, Lee DL, Hong SY, Choi EY, Yoon YW, Hong BK, Rim SJ, Kwon HM. Local increase in microparticles from the aspirate of culprit coronary arteries in patients with st-segment elevation myocardial infarction. *Atherosclerosis*. 2013;227:323–328.
[68] Zielinska M, Koniarek W, Goch JH, Cebula B, Tybura M, Robak T, Smolewski P. Circulating endothelial microparticles in patients with acute myocardial infarction. *Kardiologia polska*. 2005;62(531-542):543–544.
[69] Chironi GN, Simon A, Boulanger CM, Dignat-George F, Hugel B, Megnien JL, Lefort M, Freyssinet JM, Tedgui A. Circulating microparticles may influence early carotid artery remodeling. *Journal of hypertension*. 2010;28:789–796.

[70] Mezentsev A, Merks RM, O'Riordan E, Chen J, Mendelev N, Goligorsky MS, Brodsky SV. Endothelial microparticles affect angiogenesis in vitro: Role of oxidative stress. *American journal of physiology. Heart and circulatory physiology*. 2005;289:H1106–H1114.
[71] Kim HK, Song KS, Chung JH, Lee KR, Lee SN. Platelet microparticles induce angiogenesis in vitro. *British journal of haematology*. 2004;124:376–384.
[72] Brill A, Dashevsky O, Rivo J, Gozal Y, Varon D. Platelet-derived microparticles induce angiogenesis and stimulate post-ischemic revascularization. *Cardiovascular research*. 2005;67:30–38.
[73] Soleti R, Benameur T, Porro C, Panaro MA, Andriantsitohaina R, Martinez MC. Microparticles harboring sonic hedgehog promote angiogenesis through the upregulation of adhesion proteins and proangiogenic factors. *Carcinogenesis*. 2009;30:580–588.
[74] Mesri M, Altieri DC. Endothelial cell activation by leukocyte microparticles. *Journal of immunology*. 1998;161:4382–4387.
[75] Mesri M, Altieri DC. Leukocyte microparticles stimulate endothelial cell cytokine release and tissue factor induction in a jnk1 signaling pathway. *The Journal of biological chemistry*. 1999;274:23111–23118.
[76] Mallat Z, Benamer H, Hugel B, Benessiano J, Steg PG, Freyssinet JM, Tedgui A. Elevated levels of shed membrane microparticles with procoagulant potential in the peripheral circulating blood of patients with acute coronary syndromes. *Circulation*. 2000;101:841–843.
[77] Nozaki T, Sugiyama S, Sugamura K, Ohba K, Matsuzawa Y, Konishi M, Matsubara J, Akiyama E, Sumida H, Matsui K, Jinnouchi H, Ogawa H. Prognostic value of endothelial microparticles in patients with heart failure. *European journal of heart failure*. 2010;12:1223–1228.
[78] Huber J, Vales A, Mitulovic G, Blumer M, Schmid R, Witztum JL, Binder BR, Leitinger N. Oxidized membrane vesicles and blebs from apoptotic cells contain biologically active oxidized phospholipids that induce monocyte-endothelial interactions. *Arteriosclerosis, thrombosis, and vascular biology*. 2002;22:101–107.
[79] Diamant M, Tushuizen ME, Sturk A, Nieuwland R. Cellular microparticles: New players in the field of vascular disease? *European journal of clinical investigation*. 2004;34:392–401.
[80] Berezin AE, Kremzer AA, Samura TA, Martovitskaya YV. Circulating endothelial-derived apoptotic microparticles in the patients with ischemic symptomatic chronic heart failure: Relevance of pro-inflammatory activation and outcomes. *International cardiovascular research journal*. 2014;8:116–123.
[81] Trzepizur W, Martinez MC, Priou P, Andriantsitohaina R, Gagnadoux F. Microparticles and vascular dysfunction in obstructive sleep apnoea. *The European respiratory journal*. 2014;44:207–216.
[82] Priou P, Gagnadoux F, Tesse A, Mastronardi ML, Agouni A, Meslier N, Racineux JL, Martinez MC, Trzepizur W, Andriantsitohaina R. Endothelial dysfunction and circulating microparticles from patients with obstructive sleep apnea. *The American journal of pathology*. 2010;177:974–983.
[83] Yun CH, Jung KH, Chu K, Kim SH, Ji KH, Park HK, Kim HC, Lee ST, Lee SK, Roh JK. Increased circulating endothelial microparticles and carotid atherosclerosis in obstructive sleep apnea. *Journal of clinical neurology*. 2010;6:89–98.
[84] Kim J, Bhattacharjee R, Kheirandish-Gozal L, Spruyt K, Gozal D. Circulating microparticles in children with sleep disordered breathing. *Chest*. 2011;140:408–417.
[85] Maruyama K, Morishita E, Sekiya A, Omote M, Kadono T, Asakura H, Hashimoto M, Kobayashi M, Nakatsumi Y, Takada S, Ohtake S. Plasma levels of platelet-derived microparticles in patients with obstructive sleep apnea syndrome. *Journal of atherosclerosis and thrombosis*. 2012;19:98–104.
[86] Tual-Chalot S, Fatoumata K, Priou P, Trzepizur W, Gaceb A, Contreras C, Prieto D, Martinez MC, Gagnadoux F, Andriantsitohaina R. Circulating microparticles from patients with obstructive sleep apnea enhance vascular contraction: Mandatory role of the endothelium. *The American journal of pathology*. 2012;181:1473–1482.
[87] Amabile N, Guerin AP, Tedgui A, Boulanger CM, London GM. Predictive value of circulating endothelial microparticles for cardiovascular mortality in end-stage renal failure: A pilot study. *Nephrology, dialysis, transplantation: official publication of the European Dialysis and Transplant Association - European Renal Association*. 2012;27:1873–1880.
[88] Takahashi T, Kubo H. The role of microparticles in chronic obstructive pulmonary disease. *International journal of chronic obstructive pulmonary disease*. 2014;9:303–314.
[89] Takeshita J, Mohler ER, Krishnamoorthy P, Moore J, Rogers WT, Zhang L, Gelfand JM, Mehta NN. Endothelial cell-, platelet-, and monocyte/macrophage-derived microparticles are elevated in psoriasis beyond cardiometabolic risk factors. *Journal of the American Heart Association*. 2014;3:e000507.
[90] Martinez-Sales V, Vila V, Ricart JM, Vaya A, Todoli J, Nunez C, Contreras T, Ballester C, Reganon E. Increased circulating endothelial cells and microparticles in patients with psoriasis. *Clinical hemorheology and microcirculation*. 2013.
[91] Nieuwland R, Berckmans RJ, Rotteveel-Eijkman RC, Maquelin KN, Roozendaal KJ, Jansen PG, ten Have K, Eijsman L, Hack CE, Sturk A. Cell-derived microparticles generated in patients during cardiopulmonary bypass are highly procoagulant. *Circulation*. 1997;96:3534–3541.
[92] Biro E, Sturk-Maquelin KN, Vogel GM, Meuleman DG, Smit MJ, Hack CE, Sturk A, Nieuwland R. Human cell-derived microparticles promote thrombus formation in vivo in a tissue factor-dependent manner. *Journal of thrombosis and haemostasis: JTH*. 2003;1:2561–2568.
[93] Schecter AD, Spirn B, Rossikhina M, Giesen PL, Bogdanov V, Fallon JT, Fisher EA, Schnapp LM, Nemerson Y, Taubman MB. Release of active tissue factor by human arterial smooth muscle cells. *Circulation research*. 2000;87:126–132.
[94] Biro E, Nieuwland R, Tak PP, Pronk LM, Schaap MC, Sturk A, Hack CE. Activated complement components and complement activator molecules on the surface of cell-derived microparticles in patients with rheumatoid arthritis and healthy individuals. *Annals of the rheumatic diseases*. 2007;66:1085–1092.
[95] VanWijk MJ, VanBavel E, Sturk A, Nieuwland R. Microparticles in cardiovascular diseases. *Cardiovascular research*. 2003;59:277–287.
[96] Cerri C, Chimenti D, Conti I, Neri T, Paggiaro P, Celi A. Monocyte/macrophage-derived microparticles up-regulate inflammatory mediator synthesis by human airway epithelial cells. *Journal of immunology*. 2006;177:1975–1980.

[97] Puddu P, Puddu GM, Cravero E, Muscari S, Muscari A. The involvement of circulating microparticles in inflammation, coagulation and cardiovascular diseases. *The Canadian journal of cardiology*. 2010;26:140–145.
[98] Distler JH, Jungel A, Huber LC, Seemayer CA, Reich CF 3rd, Gay RE, Michel BA, Fontana A, Gay S, Pisetsky DS, Distler O. The induction of matrix metalloproteinase and cytokine expression in synovial fibroblasts stimulated with immune cell microparticles. *Proceedings of the National Academy of Sciences of the United States of America*. 2005;102:2892–2897.
[99] Nomura S, Tandon NN, Nakamura T, Cone J, Fukuhara S, Kambayashi J. High-shear-stress-induced activation of platelets and microparticles enhances expression of cell adhesion molecules in thp-1 and endothelial cells. *Atherosclerosis*. 2001;158:277–287.
[100] Mause SF, von Hundelshausen P, Zernecke A, Koenen RR, Weber C. Platelet microparticles: A transcellular delivery system for rantes promoting monocyte recruitment on endothelium. *Arteriosclerosis, thrombosis, and vascular biology*. 2005;25:1512–1518.
[101] Gasser O, Schifferli JA. Activated polymorphonuclear neutrophils disseminate anti-inflammatory microparticles by ectocytosis. *Blood*. 2004;104:2543–2548.
[102] Mostefai HA, Agouni A, Carusio N, Mastronardi ML, Heymes C, Henrion D, Andriantsitohaina R, Martinez MC. Phosphatidylinositol 3-kinase and xanthine oxidase regulate nitric oxide and reactive oxygen species productions by apoptotic lymphocyte microparticles in endothelial cells. *Journal of immunology*. 2008;180:5028–5035.
[103] Taraboletti G, D'Ascenzo S, Borsotti P, Giavazzi R, Pavan A, Dolo V. Shedding of the matrix metalloproteinases mmp-2, mmp-9, and mt1-mmp as membrane vesicle-associated components by endothelial cells. *The American journal of pathology*. 2002;160:673–680.
[104] Soriano AO, Jy W, Chirinos JA, Valdivia MA, Velasquez HS, Jimenez JJ, Horstman LL, Kett DH, Schein RM, Ahn YS. Levels of endothelial and platelet microparticles and their interactions with leukocytes negatively correlate with organ dysfunction and predict mortality in severe sepsis. *Critical care medicine*. 2005;33:2540–2546.
[105] Perez-Casal M, Downey C, Cutillas-Moreno B, Zuzel M, Fukudome K, Toh CH. Microparticle-associated endothelial protein c receptor and the induction of cytoprotective and anti-inflammatory effects. *Haematologica*. 2009;94:387–394.
[106] Benameur T, Tual-Chalot S, Andriantsitohaina R, Martinez MC. Pparalpha is essential for microparticle-induced differentiation of mouse bone marrow-derived endothelial progenitor cells and angiogenesis. *PloS one*. 2010;5:e12392.
[107] Mause SF, Ritzel E, Liehn EA, Hristov M, Bidzhekov K, Muller-Newen G, Soehnlein O, Weber C. Platelet microparticles enhance the vasoregenerative potential of angiogenic early outgrowth cells after vascular injury. *Circulation*. 2010;122:495–506.
[108] Agouni A, Mostefai HA, Porro C, Carusio N, Favre J, Richard V, Henrion D, Martinez MC, Andriantsitohaina R. Sonic hedgehog carried by microparticles corrects endothelial injury through nitric oxide release. *FASEB journal: official publication of the Federation of American Societies for Experimental Biology*. 2007;21:2735–2741.
[109] Benameur T, Soleti R, Porro C, Andriantsitohaina R, Martinez MC. Microparticles carrying sonic hedgehog favor neovascularization through the activation of nitric oxide pathway in mice. *PloS one*. 2010;5:e12688.
[110] Chen TS, Lai RC, Lee MM, Choo AB, Lee CN, Lim SK. Mesenchymal stem cell secretes microparticles enriched in pre-micrornas. *Nucleic acids research*. 2010;38:215–224.
[111] Cai J, Han Y, Ren H, Chen C, He D, Zhou L, Eisner GM, Asico LD, Jose PA, Zeng C. Extracellular vesicle-mediated transfer of donor genomic DNA to recipient cells is a novel mechanism for genetic influence between cells. *Journal of molecular cell biology*. 2013;5:227–238.
[112] Loyer X, Vion AC, Tedgui A, Boulanger CM. Microvesicles as cell-cell messengers in cardiovascular diseases. *Circulation research*. 2014;114:345–353.