

# Microparticles – messengers of biological information

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## Abstract

Endothelial cell apoptosis is a pivotal step in the development of atherosclerotic disease. Regeneration of the damaged endothelium is an attractive therapy option in the prevention and treatment of atherosclerotic disease. Apoptosis is associated with the release of microparticles (MP). Besides their role as marker of cell damage, recent reports have underlined their role as signalling elements in cell–cell communication. In this review, we focus on the emerging role of circulating MP as transmitters of biological information in cardiovascular disease.

**Keywords:** microparticles • atherosclerosis • molecular signalling • endothelial cells

## Introduction

Endothelial cell apoptosis is a crucial step in the development of endothelial dysfunction and atherosclerosis. Prevention of endothelial cell death or regeneration of the damaged endothelium is an interesting option in the prevention and treatment of atherosclerotic disease. Little is known about the pathways by which endothelial regeneration in atherosclerosis is concerted.

Apoptosis is associated with the release of microparticles (MP). MP are defined as vesicles shed from the cell surface of damaged cells. They carry membrane and cytoplasmic constituents from their parent cells they are released from. In addition to vessel wall derived cells such as endothelial cells, circulating cells including lymphocytes, monocytes and red blood cells release MP. Besides their role as markers of cell damage, recent reports have underlined their role as signalling elements in cell–cell communication. In this review, we focus on the emerging role of circulating cell derived MP as signalling element and transport molecule for proteins, nucleic acids and receptors in cardiovascular disease.

## Microparticles

Physiologically, cells form MP during activation and aging but MP formation also occurs in pathological conditions, *e.g.* in

response to hypoxia, irradiation, oxidative stress, exposure to proteins of the complement cascade and shear stress [1–8]. MP circulate within the peripheral blood with an average concentration of 5–50  $\mu\text{g/ml}$ . Under steady-state conditions, roughly 80% of MP are platelet derived, 10% are endothelial derived, and another 10% are leucocyte derived [9, 10]. MP differ in size from 100 nm up to 2  $\mu\text{m}$  [11]. MP from red blood cells are even smaller in size than MP from other cell types with approximately 0.15  $\mu\text{m}$  in diameter [12]. Biochemically, the phospholipid content and asymmetry of cell membranes is of tremendous importance for the formation and understanding of MP. The cell membrane consists of phosphatidylcholine and sphingomyeline on the external membrane layer, whereas the inner layer consists of phosphatidylserine and phosphatidyl-ethanolamine. The maintenance of this pattern is essential for the stability of the cell membrane and changes in this asymmetry are a key step for MP generation.

For a long time, the focus in MP biology was on understanding their role as an important factor in thrombosis (for review see [13]). More recently, MPs were described to be an important mirror of the disease status and were used as a marker to quantify vessel wall damage in cardiovascular, immunological and inflammatory diseases [14].

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## Methodological aspects

Fluorescence activated cell sorting (FACS) analysis of platelet-poor plasma represents the most frequently used method to characterize MP. Annexin V typically binds negatively charged phospholipids expressed on MP but further surface antigens are exerted depending on the releasing parent cell. Thus, different markers specific for the endothelial cell lineage have been described and initiated a more detailed subdivision. Moreover, enzyme-linked immunosorbent assays are used to characterize MP. Annexin V coating a plate captures MP by binding phosphatidylserin. Additional antibodies allow categorizing the MP. Because of the lack of a reference method and the huge spectrum of antibodies current studies have to be evaluated and compared with caution. Moreover, it has been shown that the kind of stimulus for MP generation and the cell culture medium influence the protein content in the membrane of leucocyte-derived MP [15] making it difficult to refer specific surface antigens to defined pathophysiological processes. On the other hand the physiological process itself, *e.g.* apoptosis or activation, impacts the pattern of surface antigens on endothelial cells [16].

## Understanding microparticles – from biomarker to messenger

MP play a role in a variety of clinical conditions and the role as a pro-coagulant factor is well established. It was reported in Table 1 that MP are involved in various autoimmune and inflammatory conditions, *e.g.* sickle cell disease, paroxysmal nocturnal haemo-

globinuria and thrombotic thrombocytopenic purpura. In general, in these conditions, the procoagulant and inflammatory process is followed by endothelial damage and subsequent detachment of endothelial cells thereby exposing tissue factor to the circulation. MP derived from endothelial cells, platelets and monocytes with defined surface antigens were detected in diseased patients. Endothelial-derived MP (EMP) express CD105 (ICAM-1) and CD144 (VE-cadherin) on their surface. Disease aggravation is generally associated with increasing levels of circulating MP [14]. C-reactive protein, which is synthesized during various inflammatory conditions, induces the generation of endothelial MP *via* BH4-dependent nitric oxide formation [17]. Thus, disease aggravation propagates enhanced MP liberation. In this context it has been shown that not only CRP but also platelet-derived MP reflect the vascular inflammatory status after percutaneous coronary intervention and was an independent predictor of late lumen loss [18]. Elevated levels of circulating MP are also seen in septic patients [6], which might explain the procoagulant disposition of septic patients. However, the meaning of MP in septic and autoimmune conditions is not fully understood because recent research suggests that increased MP levels might be a sign of a more favourable outcome, *e.g.* in severe sepsis [19]. In a clinical study, Soriano and coworkers investigated whether the inflammatory status in patients with severe sepsis is associated with the outcome. They observed that almost all markers of activation and inflammation including MP were significantly higher among survivors. Whether MP only occur as epiphenomenon or also have a pathophysiological meaning in this setting remains unclear.

The pivotal role of MPs in influencing biological answers has been convincingly shown in cancer disease. MP have been implicated in tumour growth, progression and metastasis. MP released from cancer cells, platelets, monocytes and lymphocytes strongly enrich the tumour microenvironment. It has been demonstrated

**Table 1** Surface antigens of endothelial-, platelet- and leucocyte-derived MP. MP denotes microparticles. MP are detectable in a huge variety of diseases and MP of different origin are elevated in various pathological conditions. This table provides an overview of selected disorders and involved MP

MP	Endothelial-derived MP	Platelet-derived MP	Leucocyte-derived MP
Surface antigens	CD31, CD34, CD51, CD54, CD62E, CD62P, CD105, CD144, CD146	CD31, CD41a, CD42b, CD61, CD62P, CD63	CD3, CD4, CD8, CD11, CD14, CD66b
Disease			
Coronary artery disease	↑	↑	–
Deep venous thrombosis and pulmonary embolism	↑	↑	–
Sickle cell disease	↑	↑	↑
Paroxysmal nocturnal haemoglobinuria	↑	↑	↑
Antiphospholipid-syndrome	↑	–	–
Chronic renal failure	↑	↑	↑
Cancer: (breast, gastric, pancreatic)	–	↑	↑
Stroke	↑	↑	–
Sepsis	↑	↑	↑

that MP released from malignant cells induce the expression of several molecules like interleukin-11, vascular endothelial growth factor, MMP-9 and leukaemia inhibitory factor. Moreover, they influence secretion of pro-angiogenic factors by fibroblasts, chemoattraction and increase the proliferation of endothelial cells and participate in neoangiogenesis [20]. By influencing the expression of pro-apoptotic signals like Fas ligand on tumour cells, MP provide an elegant but fatal mechanism for malignant cells to encounter and evade infiltrating human lymphocytes [21]. Interestingly, it has been shown that the levels of circulating MP are associated with prognosis and metastasis in patients with gastric cancer [22]. Although, the role of MPs is still incompletely understood in cancer at present, it is conceivable that these tumour cell derived MP might be an instrument for influencing the local environment by serving as a tool for information transfer. In accordance with this assumption it has been demonstrated that MP derived from glioma cancer cells transfer oncogenic receptors like EGFRvIII between cells, thereby also transferring oncogenic activity [23] (and for review see [24]).

In studies investigating the role of MP in stable coronary artery disease it has been demonstrated that elevated levels of MP positive for CD31 are associated with endothelial function and the morphology and severity of coronary stenosis [25–27]. Multiple studies from Boulanger and coworkers confirmed these findings and demonstrated, additionally, increases of endothelial MP in end-stage renal disease patients treated with haemodialysis and an association with endothelial dysfunction and arterial stiffness [28, 29].

In patients with acute coronary syndromes, endothelial MP exerting CD31 and CD146 are significantly increased compared to stable CAD [30]. Moreover, EMP are equipped with oxidized phospholipids which enhance atherosclerotic progression. They are also capable of activating neutrophils and matrix metalloproteinases thereby leading to degradation of collagens and promoting plaque destabilization [31, 32]. MP taken from patients with acute coronary syndrome induced severe endothelial dysfunction in rat aorta, emphasizing their deleterious effect [33]. Leucocyte-derived MP (CD11a) are detectable in increasing concentrations in early stages of CAD with silent plaques [34]. Intriguingly, not EMP but leucocyte-derived CD11a MP predominantly appeared in this setting. Nevertheless, experimental data revealing that MP play a causative role in atherogenesis are currently not available.

Besides these roles of MP as markers of damage and mediators of deleterious effects, strong evidence hints towards an important signalling of MP in induction of angiogenesis and endothelial regeneration [35]. Recently, it has been shown that MP harbouring sonic hedgehog proteins promote angiogenesis through the up-regulation of adhesion proteins and pro-angiogenic factors [35]. MP equipped with sonic hedgehog induced the formation of capillary-like structure in an *in vitro* model using human endothelial cells and promoted proliferation. Moreover, adhesion molecules like RhoA which are involved in these mechanisms were up-regulated. Additionally, mRNA and protein levels of pro-angiogenic factors were increased. In accordance with these findings, Leroyer and coworkers demonstrated that MP carrying CD40 from human atherosclerotic plaques stimulate endothelial proliferation and

angiogenesis which might lead to an intraplaque neovascularization [36]. The activation of plasminogen into plasmin takes place at the surface of EMP [37]. Interestingly, EMP-induced plasmin generation modulates angiogenic properties of endothelial progenitor cells *in vitro*, which are believed to play a protective role in terms of atherosclerosis. Thus, a close and effective interaction between MP, endothelial cells and endothelial progenitor cells is highly likely. Platelet activation provides not only a deleterious mechanism regarding atherosclerosis and myocardial infarction but also is accompanied by the release of MP. Brill and coworkers demonstrated that MP derived from platelets possess angiogenic properties and promote post-ischemic revascularization *in vitro* and *in vivo* [38]. Leroyer and coworkers obtained similar results: MP taken from ischemic mice hind-limb muscles promoted the differentiation of bone marrow-mononuclear cells into endothelial cells *in vitro* and, additionally, influenced neovascularization *in vivo* [39]. Own data support the interaction between EMP and regenerating endothelial cells in terms of EMP-enhanced replenishment of the damaged vessel wall (Werner *et al.*, unpublished data).

Thus, the role of EMP in cardiovascular disease not only as a marker of disease has clearly come into the focus of research. It remains unclear whether elevated MP levels in cardiovascular disease represent just a reflection of disease status or can be understood as a biological response to damage triggering further biological signals with potential favourable and/or deleterious effects. It is controversial whether vascular-protective or maladaptive properties of MP predominate but it is conceivable that MP mediate both due to various pleiotropic effects depending on the physiological process. The presented data showing the involvement of MP in angiogenesis and endothelial cell recruitment pinpoints towards a pivotal role of MP in cardiovascular disease.

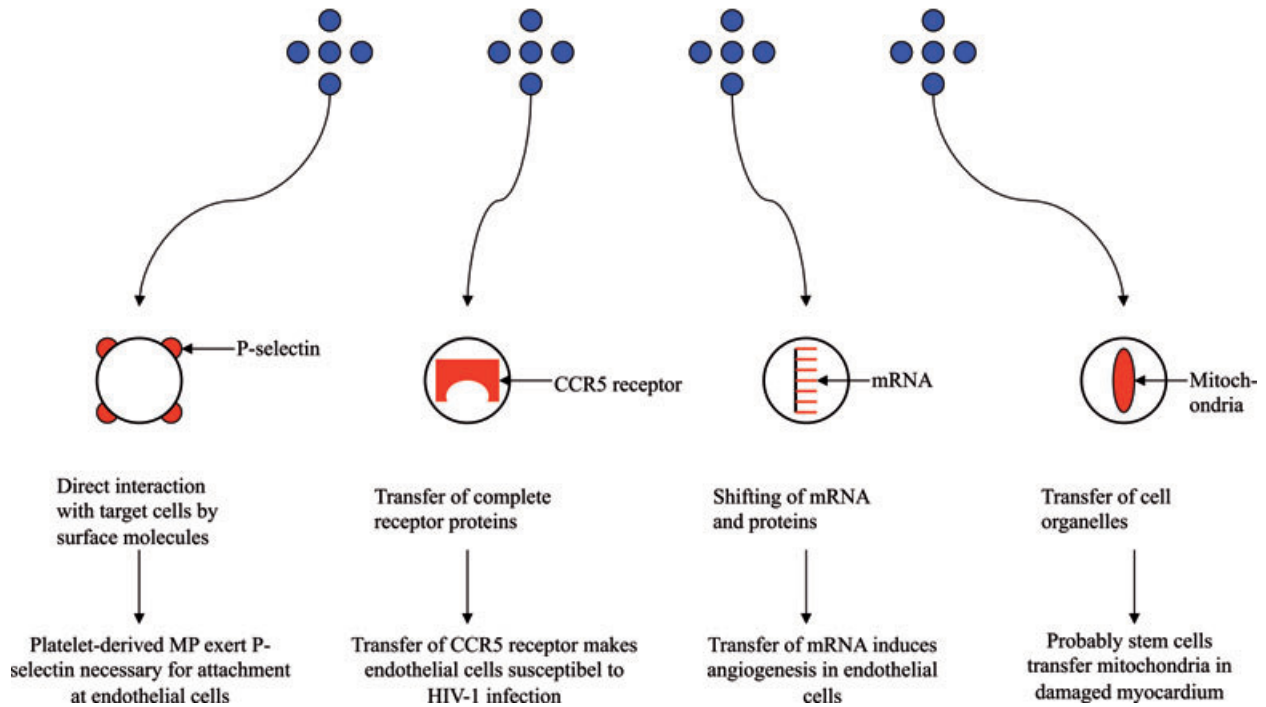
## Understanding microparticles – intercellular communication pathways

There are many known ways in which cells communicate and exchange information. Cytokines, interleukines, growth factors, chemokines and additional small molecules provide one mechanism for cell–cell interaction. Furthermore, *via* specialized adhesion molecules and nanotubules, cells are capable of connecting and exchanging information. Recently, the armamentarium was extended to MP which were identified as a valuable transport and information tool. At present, there is evidence that MP use various mechanisms to transfer information. First, MP function as signalling molecule; second, they are enabled to transfer entire receptors [40]; third, MP shift mRNA and proteins and fourth, they are even capable of transporting whole cell organelles (Fig. 1).

### MP as signalling molecules

MP may stimulate target cells directly by surface ligands. They are equipped with different signalling proteins and lipids on their

### Information transfer mediated by Microparticles



**Fig. 1** Intercellular communication mediated by MP. Cells communicate and exchange information with each other *via* various systems including cytokines, growth factors, chemokines, small molecules and also adhesion molecules and nanotubules. There is increasing evidence that MP use various mechanisms to transfer biological information and act in cell–cell communication. (1) MP function as direct signalling molecules; (2) MP communicate *via* transfer of entire receptors; (3) MP shift mRNA and proteins and (4) MP are even capable of transporting whole cell organelles.

surface. During morphogenesis of multicellular organisms, secreted MP equipped with signalling complexes like decapentaplegic or wingless proteins on their surface form a gradient necessary for adequate tissue development [41]. MP carrying certain proteins like hedgehog proteins stemming from lymphocytes are also able to direct the differentiation of early haematopoietic cells towards a megakaryocytic differentiation [4]. Platelet-derived MP exert different surface molecules allowing attachment on endothelial cells. Proteins like CD41 ( $\alpha_{IIb}/\beta_{III}$ -integrin) and CD62P (P-selectin) as well as bioactive lipids like arachidonic acid and sphingosine-1-phosphate are involved in these various biological processes mediated by platelet-derived MP [42, 43]. It has been reported that MP activate endothelial cells, polymorphonuclear leucocytes and monocytes [44, 45]. Additionally, they may also induce apoptosis in leucocytes and promote secretion of cytokines and tissue factor expression in endothelial cells [46, 47].

#### Transfer of complete receptor proteins

MP are enabled to transfer complete receptor proteins between cells. This property was observed studying platelet-derived MP

that transfer adhesion molecules like CD41 from platelets to endothelial cells and to tumour cells thereby increasing their adhesiveness [6, 20]. A very impressive finding is the transfer of the CCR5 receptor, making cells susceptible to the infection with human immunodeficiency virus [40]. The human immunodeficiency virus-1 must bind CD4 and one of the several chemokine coreceptors coercively before it can enter and infect the cell. It has been demonstrated that cells that do not typically express these coreceptors such as endothelial cells and cardiomyocytes are made susceptible to the virus by receiving those receptors *via* monocyte-derived MP [40]. The transfer of CXCR4 to AML-derived H60 cells improved migration to SDF-1 and increased homing to the bone marrow of irradiated NOD/severe combined immunodeficient mice emphasizing the importance of the biological vector function of MP [48].

#### MP as exchange vectors of mRNA

MP are also implicated in the exchange of mRNA and proteins between cells. A recent publication suggests that endothelial progenitor cell derived MP activate an angiogenic program in

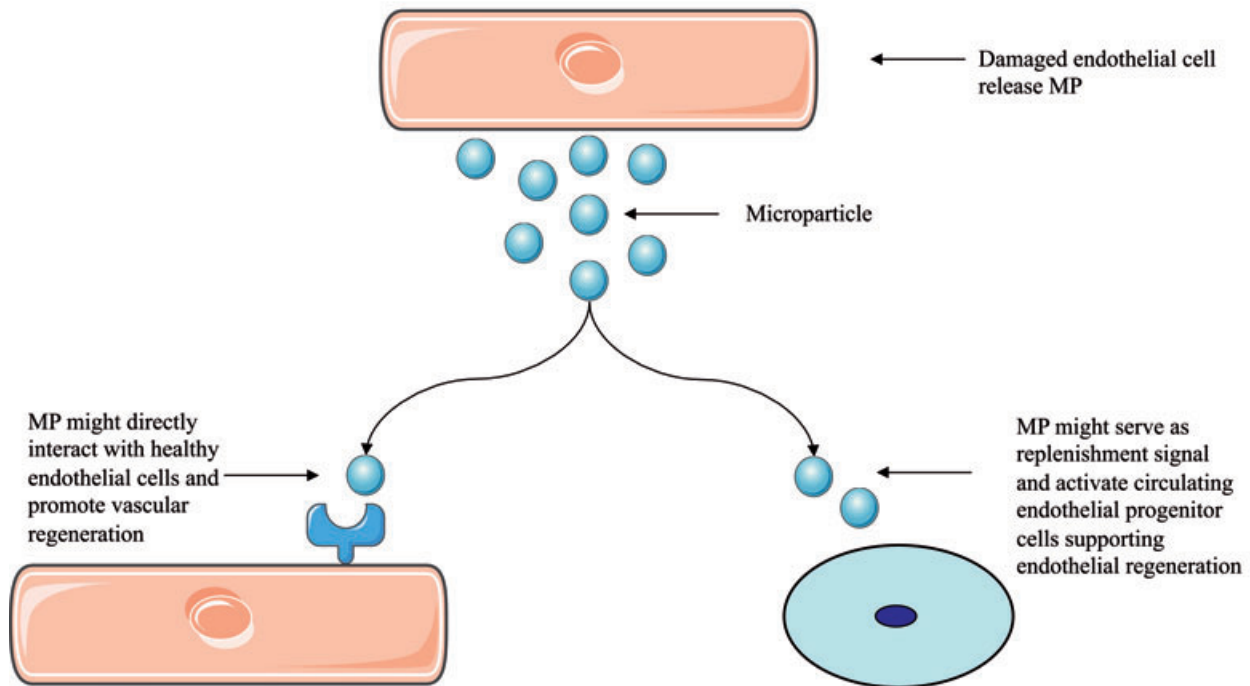
endothelial cells by a horizontal transfer of mRNA [49]. *In vitro*, MP promoted endothelial cell survival, proliferation and organization in capillary-like structures. *In vivo*, stimulation of human endothelial cells by MP led to the organization of patent vessels in severe combined immunodeficient mice. The authors showed that endothelial progenitors cell derived MP were incorporated into endothelial cells by interaction with  $\alpha_4$  and  $\beta_1$  integrins expressed on the MP surface. When incubated with RNase, despite their internalization into endothelial cells, MP failed to induce *in vitro* and *in vivo* angiogenic effects. mRNA transfer was shown by transduction of GFP in endothelial cells by MP containing GFP-mRNA and the biologic relevance by the angiogenic effect of MP-mRNA extract delivered by lipofectamine. This observation finds support in the phenomenon that HSPC cocultured with extracts of damaged liver cells induced the expression of genes specific for hepatocytes [50]. Furthermore, and in contrast to other publications, Agouni and coworkers showed in a very elegant way that MP carry sonic hedgehog proteins and are able to induce nitric oxide release from endothelial cells [51]. Proteins and mRNA delivered by MP may influence the target cell and even influence epigenetic reprogramming [52]. But there are further substances that might use MP as vector systems. Evidence increases that

infectious proteins like prions are mainly stored in plasma and platelets in human blood and that these particles are probably associated with platelet-derived MP [53, 54]. Finally, MP might even be involved in the transfer of cell organelles. This observation was obtained in cells defective in aerobic respiration, in which transfer of mitochondria and mitochondrial DNA took place. Most likely this transfer was mediated by MP, nevertheless, additional investigative effort must be spent on this issue [55]. Probably the beneficial effects in hypoxia-injured myocardial tissue seen after infusion of stem cells might be because of a transfer of healthy mitochondria mediated by MP [56].

## Conclusions and perspectives

MP represent an important biomarker in cardiovascular disease. Considerable new insights concerning MP as an integrative element in cell–cell communication have added new aspects to the MP story and might open fascinating windows in terms of therapeutic modulation of target cells (Fig. 2). Although this aim

### Perspectives: Conceivable mechanisms by which MP might influence the endothelial regeneration



**Fig. 2** Conceivable mechanisms by which MP might influence angiogenesis and endothelial regeneration. MP might directly interact with healthy endothelial cells and promote vascular regeneration *via* autocrine and paracrine action. In addition, MP might serve as replenishment signals and activate differentiation in endothelial and endothelial regenerating cells, which set the stage for neoangiogenesis, *e.g.* in ischemic tissue. SHH and CD40 have been shown to play an important role in the described pathway.



is lying far ahead and numerous obstacles have to be overcome, it is conceivable that therapeutic strategies using MP in cardiovascular medicine may include influencing the apoptotic mother (endothelial) cell to release MP with special (vasculo-protective) properties or the use of engineered MP with the intention of enhancing vasculoprotection. Although considerable challenges clearly exist, deepening our knowledge concerning mechanisms and molecular pathways in short- and

long-range signalling of MP, will bring us substantially closer to these emerging concepts.

## Conflict of interest

The authors confirm that there are no conflicts of interest.

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