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Microparticles: Protagonists of a Novel Communication Network for Intercellular Information Exchange [*Circ Res.* 2010;107:1047–1057]

Microparticles in Hemostasis and Thrombosis [*Circ Res.* 2011;108:1284–1297]

Microparticles in Angiogenesis: Therapeutic Potential

Formation, Fate, and Function of Platelet Microparticles

Microparticles in Vascular Function and Atherothrombosis

Leukocyte-Derived Microparticles in Vascular Homeostasis

Christian Weber and Sebastian Mause, Guest Editors

Microparticles in Angiogenesis Therapeutic Potential

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Abstract: Considered during the past decades as cell dust, microparticles are now deemed true biomarkers and vectors of biological information between cells. Depending on their origin, the composition of microparticles varies and the subsequent message transported by them, such as proteins, mRNA, or miRNA, can differ. Recent studies have described microparticles as “cargos” of deleterious information in blood vessel wall under pathological situations such as hypertension, myocardial infarction, and metabolic syndrome. In addition, it has been reported that depending on their origin, microparticles also possess a therapeutic potential regarding angiogenesis. Microparticles can act directly through the interaction ligand/receptor or indirectly on angiogenesis by modulating soluble factor production involved in endothelial cell differentiation, proliferation, migration, and adhesion; by reprogramming endothelial mature cells; and by inducing changes in levels, phenotype, and function of endothelial progenitor cells. This results in an increase in formation of *in vitro* capillary-like tubes and the generation of new vessels *in vivo* under ischemic conditions, for instance. Taking into consideration these properties of microparticles, recent evidence provides new basis to expand the possibility that microparticles might be used as therapeutic tools in pathologies associated with an alteration of angiogenesis. (*Circ Res.* 2011;109:110-119.)

Key Words: biological vectors ■ neovascularization ■ endothelial cells ■ microvesicles ■ progenitor cells

Modulation of neovascularization represents a potential therapeutic tool against a large number of diseases. An increase of neovascularization leading to healing of wounds and reconstruction of hypoxic injury is an attractive approach during ischemia; however, clinical data show that tumor development can be delayed when neovascularization is targeted and blocked. Among the available therapeutic tools, recent studies report that small vesicles, called micropar-

ticles (MPs), released from the plasma membrane possess the capacity to increase or decrease angiogenesis depending on their composition and concentration. This review highlights the notion that circulating MPs and engineered *in vitro* or *ex vivo* MPs can modulate different steps leading to angiogenesis, including cells involved in this process, and in this way affect *in vivo* neovascularization and finally tissue repair.

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Vasculogenesis, Angiogenesis, and Neovascularization: Different or Common Mechanisms?

Formation of new blood vessels depends on 2 processes, vasculogenesis and angiogenesis. Vasculogenesis refers to the de novo formation of blood vessels via the differentiation of endothelial progenitor cells (EPCs; initially identified as bone marrow-derived cells, which express endothelial cell-specific markers) into endothelial cells.¹ One has to take into account that the EPC concept has evolved since their discovery¹ and the phenotype and function of EPCs are still under debate. Despite the lack of consensus on surface expression of antigenic markers that are characteristic of EPCs, the same group currently describes EPCs as cells that: (1) show the ability for endothelial lineage commitment; (2) acquire an endothelial cell equivalent phenotype; (3) initially lack mature endothelial cell markers; (4) are capable of differentiation; and (5) present proangiogenic and vasculogenic properties, with a strong biological activity toward neovascular formation resulting in functional recovery and regeneration of the injured vessel.² Angiogenesis is the formation of new capillaries from the preexisting vasculature,³ is controlled by a number of growth factors and signaling pathways, and is the balance between proangiogenic and antiangiogenic factors.⁴ Thus, it is largely accepted that vasculogenesis may play a key role in embryogenesis, whereas angiogenesis occurs during both prenatal and postnatal life. However, recent evidence suggests that, in the adult, EPCs could be considered to participate in postnatal vasculogenesis. In addition, a third process, arteriogenesis, completes the expansion and growth of the vascular system in adults. Arteriogenesis describes the development and growth of preexisting arterioles into physiological relevant arteries that form collateral vessels.^{5,6} In the literature, the involvement of these mechanisms in the development of the cardiovascular system is frequently confounded.

Tissue homeostasis is dependent on a sufficient supply of oxygen and nutrients as well as the removal of metabolite products via blood vessels.⁷ Deregulation of vasculogenesis and angiogenesis have been indirectly involved in a number of pathological situations, such as ischemic diseases associated with a reduction in blood flow supply, which in some cases are secondary to the dysfunction of existing blood vessels resulting from atherosclerotic lesion formation and an increase in the requirement of oxygen and nutrient needs during tumor development. In both situations, tissue hypoxia is considered to be essential for the paracrine mechanism to induce the formation of new vessels. This ischemic effect has been shown to be mediated by a marked increase in paracrine signals, such as vascular endothelial growth factor (VEGF) and stromal cell-derived factor-1.^{8–10} The release of soluble factors initiates a cascade that degrades the extracellular matrix, disrupts cell–cell contacts, allowing for the migration and proliferation and capillary tube formation of endothelial cells.¹¹ The new capillary channel forms an anastomosis with a preexisting capillary, creating a new patent capillary. A growing body of evidence indicates that after mobilization of EPCs from the bone marrow into circulation, they are recruited to ischemic sites and tumor vasculature, where they

Non-standard Abbreviations and Acronyms

CD40L	CD40 ligand
CXCL12	chemokine (C-X-C motif) ligand
CXCR4	chemokine (C-X-C motif) receptor
EPC	endothelial progenitor cell
EGFR	epidermal growth factor receptor
MP	microparticles
MPs^{Shh+}	MPs expressing the morphogen Sonic hedgehog
PI3K	phosphoinositol 3-kinase
Shh	Sonic hedgehog
VEGF	vascular endothelial growth factor

differentiate into mature endothelial cells. This suggests that EPCs may play a critical role in adult postnatal endothelial repair and vasculogenesis.¹² In addition, EPCs can indirectly contribute to vascular regeneration through the paracrine production of proangiogenic cytokines and growth factors that promote proliferation and migration of preexisting endothelial cells and activate angiogenesis.^{13,14}

In most cases, soluble factors, cellular steps leading to angiogenesis, or the different cell types implanted in these steps are the potential targets for novel therapeutic strategies. Thus, during pathologies associated with ischemia, an improvement of both angiogenesis and vasculogenesis may have beneficial effects; in contrast, antiangiogenic strategies could represent new approaches for treatment of tumor development.

Failed Angiogenesis in Metabolic Diseases

Among the metabolic diseases, diabetes mellitus is one with alterations in angiogenesis that represent a major problem. Diabetes-associated vascular complications are the most common major clinical problem that patients with diabetes exhibit. In addition to an elevated incidence of macrovascular complications (such as atherosclerosis), which increase the risk for myocardial infarction and stroke, patients with diabetes can have peripheral artery disease (that may lead to limb amputation)¹⁵ and microvascular complications (such as retinopathy and nephropathy) develop, which can cause blindness and renal failure.¹⁶

Dysfunctional angiogenesis associated with diabetes can have several origins. Impairment of endothelial function can reduce tissue vascularization, leading to delayed wound healing in diabetic patients.¹⁷ Notably, expression of growth factors related to angiogenesis, such as VEGF and fibroblast growth factor, is reduced in *db/db* mice,^{18,19} probably as a consequence of an increased oxidative stress.²⁰

Also, patients with diabetes display impairment of re-endothelialization after vascular injury, associated not only with the reduction of number of EPCs but also with alterations of their functions (mobilization, homing, and endothelium repair).^{21,22} It has been shown that EPC levels in patients with diabetes are reduced compared to those of control subjects.²³ Concomitantly, dysfunction in the recruitment of EPCs in the affected zones has been reported.²⁴ In addition, EPCs from

patients with diabetes produce excessive superoxide anions, which results from the uncoupling of endothelial nitric oxide synthase.²⁵ Another mechanism potentially implicated in the impaired function of EPCs is the downregulation of the production of soluble factors involved in neovascularization such as VEGF and insulin-like growth factor-1. Thus, a reduced release of these factors has been shown under in vitro high glucose conditions, as well as in diabetic animal models and patients with diabetes mellitus.^{26,27} In addition, hypoxia-inducible factor-1 transactivation is reduced, leading to decreased hypoxia-inducible factor-1 α activity under exposure to high glucose²⁸ and reduced expression of hypoxia-inducible factor-1 α in skin wounds of *db/db* mice.²⁹ Recently, it has been shown that long-term diabetes not only impairs repopulation of hematopoietic progenitor cells but also dysregulates the cytokine expression in the bone marrow microenvironment in mice, suggesting that diabetes alters the stem cell niche.³⁰ Consequently, the improvement of failed functions of endothelial mature cells and EPCs during diabetes represents the goal of future therapeutic approaches.

Exacerbated Angiogenesis in Cancer

Cancer progression is dependent on abnormal angiogenesis, in particular, exacerbated neovascularization forms new blood vessels that supply adequate nutrients, oxygen, and growth factors to facilitate the growth of the tumor and metastasis development.³¹ Tumor angiogenesis results from an imbalance of proangiogenic and antiangiogenic factors released from tumor cells, in turn leading to erratic and irregular angiogenesis, which is characterized by poorly formed vasculature.³² Elevated levels of proangiogenic factors have been detected in cancer patients and a negative correlation has been reported between the levels of proangiogenic factors and their poor outcome.^{33,34} For instance, circulating levels of soluble VEGF receptor-1 are increased in hepatocellular carcinoma and are associated with poor survival in these patients.³⁵ Also, patients with breast cancer have higher plasma VEGF and angiogenin levels than control subjects,³⁶ and in metastatic gastric patients, VEGF and regulated-on-activation normal T-cell-expressed and secreted levels correlate with the severity of the metastasis.³⁷ Endothelial cells that are implicated in tumor angiogenesis divide more rapidly and express higher levels of immature phenotype markers such as the tyrosine kinase receptors Flk-1 and Tie-2 compared to normal endothelial cells, which is indicative of aberrant endothelial function in cancer.^{38,39}

EPCs have been detected at increased frequency in the circulation of cancer patients. Also, tumor production of VEGF was found to correlate with EPC mobilization.⁴⁰ A novel approach to block tumor angiogenesis may be through the inhibition of EPC recruitment. Conversely, mesenchymal stem cells, isolated from bone marrow, can be used as therapeutic vehicles that deliver genes to promote antiangiogenic effects because they share the same property as EPCs to home tumor cells.⁴¹ Mesenchymal stem cells may then represent an alternative source of neural progenitor cells for organ regeneration through their differentiation into classical mesenchymal and neuronal lineages.⁴¹ These authors have obtained mesenchymal stem cells by infection with modified

adenoviral vector encoding human interleukin-2. Intratumoral injection of these cells caused an antitumor effect accompanied by delayed tumor growth and prolonged the survival of tumor-bearing rats.⁴¹ These data suggest that engineered stem cells could be used as new therapeutic approaches for refractory tumors.

Microparticles: Composition and Effects on Target Cells

During cell activation by chemical (apoptotic, proinflammatory, prothrombotic) or physical (shear stress) stimuli, intracellular events and plasma membrane changes occur, leading to plasma membrane blebbing and the subsequent MP release. MPs are heterogeneous in size (0.1 μm –1 μm) and their composition depends on both cell origin and stimulation implicated during their generation. Thus, all types of cells can theoretically release MPs at each stage of their lifecycle, but the intracellular mechanisms involved in MP formation are not completely elucidated. It is widely accepted that a sustained increase in intracellular calcium concentration and the disruption of proteins associated with cytoskeleton are essential for MP formation and release; however, the intermediary steps connecting extracellular stimulation with MP release are not entirely known. The aim of this review is not to describe the mechanisms implicated in MP formation, which has been developed by other authors,^{42,43} but rather to summarize the number of intriguing biological properties associated with angiogenesis exhibited by MPs.

MPs are detectable in plasma from healthy subjects and, in general, their circulating levels are enhanced in pathological situations.^{44–47} Based on the differential profiles of proteins, lipids, and nucleic acids (DNA, mRNA, microRNA) that they can convey depending on their origin (cell stimulation and healthy vs disease) and on their ability to create a communication network between cells, it is plausible to use MPs as therapeutic tools. In this context, ligands carried by MPs can directly interact with receptors in target cells and induce signal transduction. In addition, membranes of MPs can fuse with the plasma membrane of target cells, leading to the transfer of membrane components and delivery of MP cytoplasmic content. This results in the activation or inhibition of intracellular pathways of target cells or in the modification of their phenotype. Regarding the MP effects have on angiogenesis, they can induce production of proangiogenic or antiangiogenic factors,^{48,49} modify endothelial cell function concerning adhesion, migration, or proliferation,⁴⁸ the 3 key steps in the formation of new vessels, and induce an increase in marker expressions of EPC differentiation^{50,51} toward endothelial mature cells. In this review, we summarized the therapeutic potential of MPs isolated using speed sedimentation <100 000g and those of exosomes (<0.1 μm) that are secreted from multivesicular compartments by fusing with the plasma membrane, and their isolation requires ultracentrifugation (>100 000 g).⁵²

Effects of MPs on Endothelial Cell Functions Leading to Angiogenesis

MPs can affect angiogenesis by inducing changes in the secretome of endothelial cells, either increasing the produc-

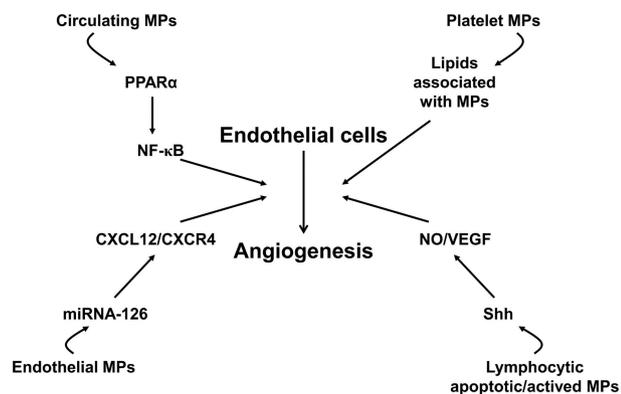


Figure 1. Different pathways activated by MPs on endothelial cells leading to angiogenesis. Circulating MPs from mice are able to activate NF-κB-dependent pathway via peroxisome proliferator-activated receptor-α and induce angiogenesis. In contrast, the effects on angiogenesis evoked by platelet MPs are dependent on lipids associated with MPs. Recently it has been shown that endothelial-derived MPs induce endothelial angiogenesis through the transfer of miRNA-126 and the subsequent activation of the CXCL12/CXCR4 pathway. MPs derived from apoptotic/activated T-lymphocytes through the interaction of Sonic hedgehog (Shh) carried by MPs and their receptors in endothelial cells are able to enhance nitric oxide production and VEGF release and induce angiogenesis.

tion of proangiogenic factors or decreasing the production of antiangiogenic factors.^{48,49} Furthermore, MPs can modify all steps leading to angiogenesis (Figure 1, Table).

Few studies have analyzed the effects of total circulating MPs, the physiological or pathophysiological real conditions, on angiogenesis. Recently, we have shown that circulating MPs from peroxisome proliferator-activated receptor-α wild-type mice, but not from knockout mice, induce the formation of capillary-like structures of endothelial cells. This effect, however, was abolished when the NF-κB pathway was

inhibited, indicating the involvement of a mechanism dependent on NF-κB activation.⁵⁰ Thus, peroxisome proliferator-activated receptor-α/NF-κB pathways activated by MPs may play a pivotal stimulatory role for angiogenesis of endothelial cells.

It has been shown that platelet MPs are able to modify steps involved in angiogenesis, such as proliferation, migration, and adhesion of endothelial cells. Kim et al⁵³ were pioneers to demonstrate that platelet MPs increased proliferation, chemotatic migration, and formation of capillary-like tubes of human umbilical vein endothelial cells through a mechanism implicating lipid components of platelet MPs. The effects reported by Kim et al⁵³ were completely abolished by the treatment of platelet MPs with charcoal, a stripper of bioactive lipids, whereas heat treatment only slightly decreased these effects. Although these effects were obtained in vitro, it should be noted that the amount of platelet MPs used can be achieved in plasma from patients with metabolic diseases such as metabolic syndrome⁴⁴ and diabetes.⁴⁶

MPs from leukocytes can induce differential effects on endothelial function depending on the stimulation used for their generation. MPs generated from T-lymphocytes activated with phytohemagglutinin and phorbol ester and undergoing apoptosis with actinomycin D express the morphogen Sonic hedgehog (MPs^{Shh+}) on their surface and promote angiogenesis. In contrast, MPs obtained from T-lymphocytes treated with actinomycin D alone and therefore do not express Sonic hedgehog (MPs^{Shh-}) inhibit angiogenesis. Apparently, the different composition of these MPs may explain the observed differences on target cells.⁵⁴ Regarding the effects of these 2 types of MPs, in vitro treatment of human umbilical vein endothelial cells with MPs^{Shh+} induced proliferation and endothelial adhesion through the increase of

Table. Origins of Microparticles Depending on their Proangiogenic vs Antiangiogenic Properties

Proangiogenic MPs	Antiangiogenic MPs	Involved Pathways	Cellular Mechanisms	Reference
Circulating (total) MPs		PPARα-dependent	Akt and NF-κB activation	50
Platelet MPs		CXCR4 transfer, ERK-dependent and PI3K-dependent	Akt activation, proangiogenic factors, lipid component	51 53
MPs from Activated/apoptotic T cells		Shh-dependent	NO increase, VEGF production	48, 55
	MPs from apoptotic T cells	LDLR-dependent	NO decrease, oxidative stress increase	56, 57
Apoptotic endothelial cells		miRNA-126	CXCR4 pathway	49
TNF-α-stimulated endothelial cells		Plasmin generation	ND	62
MPs from vitreous		ND	VEGF-independent	46
MPs from atherosclerotic plaques		CD40/CD40L interaction	VEGF production	58

ERK indicates extracellular signal-regulated kinase; LDLR, low-density lipoprotein receptor; MP, microparticle; ND, not determined; NF-κB, nuclear factor kappa B; NO, nitric oxide; PPAR, peroxisome proliferator-activated receptor; Shh, sonic hedgehog; TNF, tumor necrosis factor; VEGF, vascular endothelial growth factor.

intercellular adhesion molecule-1 expression and the involvement of Rho kinase and Sonic hedgehog pathways.⁴⁸ These effects were associated with an increase in mRNA and protein expression of several proangiogenic factors (hepatocyte growth factor, VEGF, and interleukin-1 β), indicating that Sonic hedgehog carried by MPs acts on a large number of target genes that regulate angiogenesis at different phases. More interestingly, *in vivo* treatment of hind limb ischemic mice with MPs^{Shh+} enhanced the neovascularization, as reflected by the improvement of both blood flow and number of capillaries in the ischemic leg when compared with the nonischemic leg.⁵⁵ The effects evoked by MPs^{Shh+} were attributable to an interaction ligand (Sonic hedgehog)/receptor (patched/smoothed receptors) and were associated with an increase in nitric oxide production as a consequence of the activation of endothelial nitric oxide synthase and endogenous Sonic hedgehog pathway in ischemic muscles. Moreover, the expression of several proangiogenic factors was increased by MPs^{Shh+} treatment, including fibroblast growth factor-5, fibroblast growth factor-2, and VEGF. Although the mechanisms by which MPs^{Shh+} are able to induce endogenous Sonic hedgehog expression in ischemic leg from mice are not fully elucidated, these studies suggest a relevant potential contribution of MPs^{Shh+} to induce reparative neovascularization after ischemic injury.

In contrast, MPs that are generated from apoptotic T-lymphocytes and that do not express Sonic hedgehog⁵⁴ act to inhibit angiogenesis. By using *in vitro* aortic rings and an *in vivo* model of cornea neovascularization in mice, Yang et al⁵⁶ have demonstrated that MPs^{Shh-} suppress angiogenesis and inhibit both endothelial cell survival and proliferation through the increase of reactive oxygen species generation, probably associated with an increase of NADPH activity. Likewise, MPs^{Shh-} from apoptotic lymphocytes act through the downregulation of VEGF receptor-2 and extracellular signal-regulated kinase pathways. More recently, these authors proposed MPs^{Shh-} from apoptotic lymphocytes as promising antiangiogenic agents for the treatment of lung carcinomas. In fact, intratumoral injection of these MPs decreased tumor size and vascularization and VEGF levels in mice.⁵⁷

Endothelial cells undergoing apoptosis are able to release MPs that display vascular protective effects, promote proliferation of endothelial cells, and inhibit endothelial apoptosis.⁴⁹ This elegant study shows that MPs from apoptotic endothelial cells abundantly express miRNA-126, which triggers chemokine (C-X-C motif) ligand (CXCL) 12 production through chemokine (C-X-C motif) receptor (CXCR) 4 pathway; thus, MPs released from apoptotic endothelial cells may act as paracrine elements necessary to protect endothelial cells during atherosclerosis.

MPs from origins other than circulating cells also can participate in regulating angiogenesis. Interestingly, Chahed et al⁴⁶ have shown that MPs from vitreous from patients with diabetic retinopathy, derived from retina and vascular cells, stimulate both *in vitro* endothelial cell proliferation and formation of capillary structures on Matrigel, illustrating the potential involvement of MPs on the progression of this disease. The mechanism implicated is not clear but seems

independent of direct action of VEGF because MPs from vitreous did not contain detectable levels of VEGF.

In addition, MPs from atherosclerotic plaques, primarily from macrophages infiltrated in the plaque, promote endothelial cell proliferation and *in vivo* formation of new vessels on Matrigel plugs. The mechanism involves the interaction between CD40-ligand carried by MPs from atherosclerotic plaques and CD40 expressed in endothelial cells, as deduced by the absence of effects in CD40 knockout mice.⁵⁸ These results suggest that during atherosclerosis, macrophage-derived MPs may trigger neovascularization of atherosclerotic lesions and participate in the plaque vulnerability.

Another interesting aspect is the ability of tumor cells to generate MPs, suggesting that they could play a critical role in angiogenesis facilitating metastasis. In particular, MPs could be involved in the interaction between tumor and stromal cells, which can regulate the mechanisms governing tumor development. This is confirmed by the fact that MPs from prostatic carcinoma cell lines induce activation of fibroblasts (extracellular signal-regulated kinase phosphorylation and matrix metalloproteinase-9 upregulation) and promote MP shedding from activated fibroblasts, which, in turn, increases migration and invasion of highly metastatic cells via the interaction between chemokine (C-X3-C motif) ligand (CX3CL1)/fractalkine ligands and its receptor, CX3CR1.⁵⁹

Also, several antigens identified on breast cancer tissue are expressed in circulating MPs from patients with breast cancer and lymph node metastases, suggesting that MPs could have 2 facets: (1) MPs could be used as prognostic factors and (2) proteins carried by MPs could play an important role in the progression or metastatic spread of cancer by inducing angiogenesis or alternatively by distributing various oncogenes.⁶⁰

MPs and EPC Function

Correction of the EPC dysfunction observed in patients with diabetes is a challenge for cell therapy. *Ex vivo* or *in vivo* expansion of EPCs as well as enhancement of the reparative potential of EPCs constitute real approaches to improve EPC function. Among the *ex vivo* modifications of EPCs, genetic modification of cells or pharmacological treatment have been recently reviewed by Jarajapu and Grant.²¹ *Ex vivo* incubation of EPCs with MPs may represent an alternative tool to increase number and regenerative abilities of EPCs (Figure 2).

By incubating bone marrow-derived cells with MPs, Benamer et al⁵⁰ have shown that independently of the increase of the number of EPCs, treatment for 7 days with circulating MPs from mice is able to increase differentiation of these cells toward the endothelial lineage, as reflected by the increase of markers of mature endothelial cells (Flk-1, vascular endothelial-cadherin, platelet endothelial cellular adhesion molecule-1, and intercellular adhesion molecule-1), but not toward the macrophage differentiation. Interestingly, peroxisome proliferator-activated receptor- α carried by circulating MPs seems to be essential for their ability of the proangiogenic reprogramming of EPCs through the Akt and NF- κ B pathway activation, because MPs obtained from knockout mice had no effect on EPC differentiation. In-

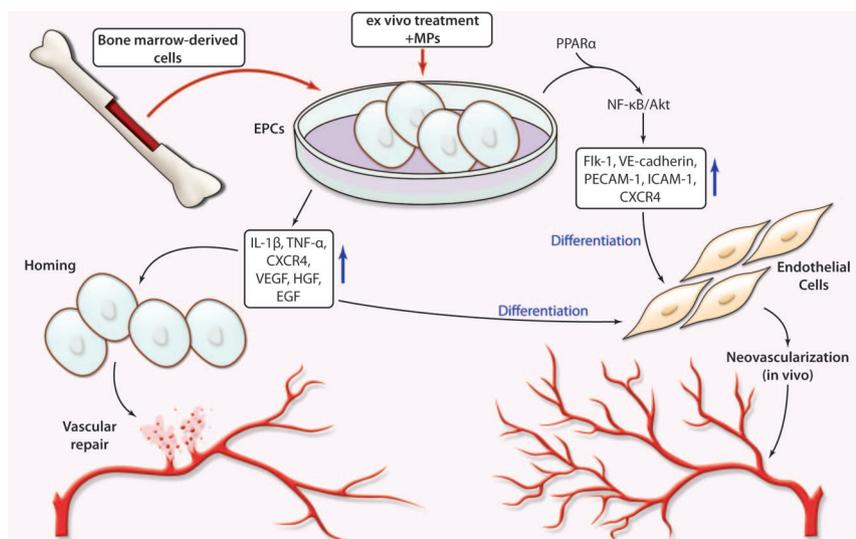


Figure 2. Endothelial progenitor cells and MPs. Ex vivo treatment of bone marrow derived cells with circulating MPs induce cell differentiation toward the endothelial lineage, as reflected by the increase of mature endothelial cell markers via the activation of NF- κ B/Akt pathways. Under these conditions, peroxisome proliferator-activated receptor- α is crucial for EPC differentiation.⁵⁰ Also, treatment with platelet MPs increases EPC homing at the sites of vascular injury and proliferation of mature endothelial cells through a mechanism implicating VEGF, EGF, hepatocyte growth factor, interleukin- β , tumor necrosis factor- α , and CXCR4 secretion.⁵¹ This leads to favor vascular repair and neovascularization. (Illustration credit: Cosmocyte/Ben Smith).

creases in the expression of proangiogenic factors induced by MPs include VEGF, stromal cell-derived factor-1, and angiopoietin-2, whereas antiangiogenic factor expression (thrombospondin-1) was decreased. Because these angiogenic factors are involved in the homing ability of EPCs, the effects induced by MPs harboring peroxisome proliferator-activated receptor- α on EPCs suggest that MPs may be involved in enhancing EPCs proangiogenic abilities. These changes lead to the in vivo formation of new vessels in Matrigel plugs. Together, these data highlight the potential use of MPs carrying peroxisome proliferator-activated receptor- α as ex vivo expansion agents of EPCs.

Interestingly, similar results have been obtained by using platelet MPs. MPs obtained from in vitro activated platelets increase the ability of 7-day cultured angiogenic early outgrowth cells (which exhibit phenotypic features of myeloid and endothelial cells and are known as EPCs) isolated from peripheral blood to regenerate vascular injury.⁵¹ These effects include changes in the phenotype of these cells such as increased expression of the endothelial markers (CD31 and vascular endothelial-cadherin) and CXCR4, indicating their maturation toward endothelial cells. Furthermore, platelet MPs enhance Akt activation in response to CXCL12 on angiogenic early outgrowth cells, suggesting a sensitization of CXCR4 pathway by platelet MPs. Also, treatment of angiogenic early outgrowth cells with platelet MPs modulates secretome composition by increasing levels of proangiogenic factors (VEGF, EGF, hepatocyte growth factor, interleukin- β , and tumor necrosis factor- α), which induce proliferation of mature endothelial cells. Interestingly, in vivo analysis of the effects of platelet MPs on vascular injury showed that re-endothelialization was increased in vessels from mice treated with angiogenic early outgrowth cells incubated with platelet MPs compared to mice treated with angiogenic early outgrowth cells alone. Together, these data and those of Benameur et al⁵⁰ underline the potential effects of circulating or platelet MPs to promote new vessel generation and may constitute an effective therapeutic tool in pathologies associated with endothelium injury by inducing a sufficient recruitment of EPCs in the sites of the vascular lesion.

Several reports show that MPs from endothelial cells also can affect EPC function and angiogenesis. MPs from apoptotic endothelial cells are able to activate a proangiogenic program in 7-day cultured human EPCs isolated from peripheral blood, increasing their number and inducing differentiation toward mature endothelial cells. The mechanism involved is related to the phagocytosis of endothelial MPs by EPCs.⁶¹ Similarly, endothelial-derived MPs expressing urokinase-type plasminogen activator and its receptor served as a surface for the generation of plasmin and favored or inhibited tube formation by cord blood EPCs, depending on MP amounts; low amounts of endothelial-derived MPs increased tube formation and were associated with matrix metalloproteinase activation, whereas higher concentrations inhibited it.⁶²

Another in vitro study shows that MPs generated from murine apoptotic endothelial cells induce a strong differentiation of bone marrow-derived mononuclear cells from endothelial phenotype.⁶³ These authors also have evaluated the effects of MPs isolated from mouse ischemic muscles. This type of MP promotes in vitro bone marrow-derived mononuclear cell differentiation and in vivo postnatal vasculogenesis through a mechanism implicating elevated reactive oxygen species production.⁶³ These results suggest that MPs from ischemic tissues could act as endogenous survival signals responsible for vascular repair.

Finally, it has been recently described that stem cells can also generate MPs that may play both autocrine and paracrine roles. Interestingly, Chen et al⁶⁴ have detected RNA and miRNA in the supernatant of mesenchymal stem cells after a 10 000g centrifugation step, suggesting that RNA was associated with MPs. Although the biological function of secreted miRNA is not completely determined, the fact that they are carried by MPs suggest that MP-associated membrane can protect miRNA against degradation by forming an adequate environment, and MPs may act as vectors of miRNA-dependent messages between cells.

Effects of Exosome-like Microvesicles on Angiogenesis

As described, cells can release another type of microvesicles named exosomes. The results published in recent years have

shown that exosomes modify the angiogenic program of endothelial cells or EPCs through the release of angiogenic factors and by acting at the different steps of angiogenesis in a similar manner to MPs. Although the aim of this review is not related to exosome effects, they represent a potential therapeutic tool; therefore, we briefly explain (by showing recent data) their potential use under several pathological conditions by acting on angiogenesis. It should be noted that the experimental protocols used to obtain exosomes do not allow the isolation of a pure population because they may contain MPs. Under this context, it is difficult to conclude whether the observed effects are induced by MPs or by exosomes.

Circulating exosomes obtained from plasma of glioma patients were positive for the mutant/variant mRNA of epidermal growth factor receptor (EGFRvIII), which defines a clinical subtype of glioma. Interestingly, these exosomes display proangiogenic properties, indicating that glioma-derived exosomes play a role in initiating angiogenesis.⁶⁵

Exosomes generated from platelets present a dual effect in angiogenesis. Whereas an interesting beneficial proangiogenic potential, by delivering a cocktail of proangiogenic proteins, such as VEGF, basic fibroblast growth factor, and PDGF, has been demonstrated in a model of myocardial ischemia,⁶⁶ Janowska-Wieczorek et al⁶⁷ have shown that exosomes derived from platelets can induce angiogenesis in lung cancer, suggesting an implication in metastasis. These differences can be attributed to the different method of platelet activation to obtain exosomes and their different concentration used.

Recently, it has been shown that exosomes released by Delta-like 4–treated endothelial cells bear this protein, indicating that Notch signaling is regulated in these cells. Under these conditions, Delta-like 4–containing exosome increases capillary-like structure formation *in vitro* and *in vivo* by a mechanism that implicates the transfer of Delta-like-4 into the endothelium. This suggests that the Delta like/Notch pathway does not require direct cell–cell contact to expand its signaling potential on angiogenesis.⁶⁸ Other proteins also can be carried by endothelial-derived exosomes and can be

implicated in their proangiogenic potential. Tarabozetti et al⁶⁹ have shown that matrix metalloproteinases harbored by exosomes from endothelial cells are functionally active and lead to endothelial cell invasion and capillary-like formation. Microvesicles obtained by ultracentrifugation from EPCs isolated from peripheral blood mononuclear cells carry various mRNA associated with PI3K/Akt pathway and can exert proliferative effects and proangiogenic activity on endothelial cells, such as their organization in capillary-like structures.⁷⁰

Finally, exosomes represent a delivery system that confers stability to mRNA and miRNA packaged by them and protects from external RNases by the surrounding membrane. Thus, miRNA-150 is actively secreted from monocytes into exosomes and evokes *in vitro* endothelial cell migration, whereas injecting mice with exosomes increases miRNA-150 in mouse blood vessels.⁷¹ Exosomes enriched in proteins⁷² and cell cycle-related mRNAs⁷³ with the potential to facilitate angiogenesis and metastasis also can be released by squamous carcinoma and colorectal cancer cells, respectively. Taken together, generation of exosomes may represent an alternative approach to favor angiogenesis in pathological conditions.

Future Directions

Accumulating evidence described here suggests that MPs are important mediators of cell communication, especially between circulating cells, endothelial cells, and EPCs. Based on the effects induced by MPs on these cells, MPs represent a true therapeutic potential. An interesting aspect is the use of MPs as therapeutic tools based on the generation of *in vitro*-engineered MPs.⁷⁴ Engineered MPs can be produced on request to modify their molecular composition and properties. Namely, it has been shown that engineered MPs can overexpress several proteins by inducing the cells from which they originate to increase protein synthesis. Two types of approaches could be considered. First, specific stimulation by pharmacological treatment of MP-producing cells evokes inclusive or exclusive protein sorting, leading to MPs with a particular composition. Thus, we have engineered MPs from activated/apoptotic T-cell lines bearing Sonic hedgehog,⁵⁴

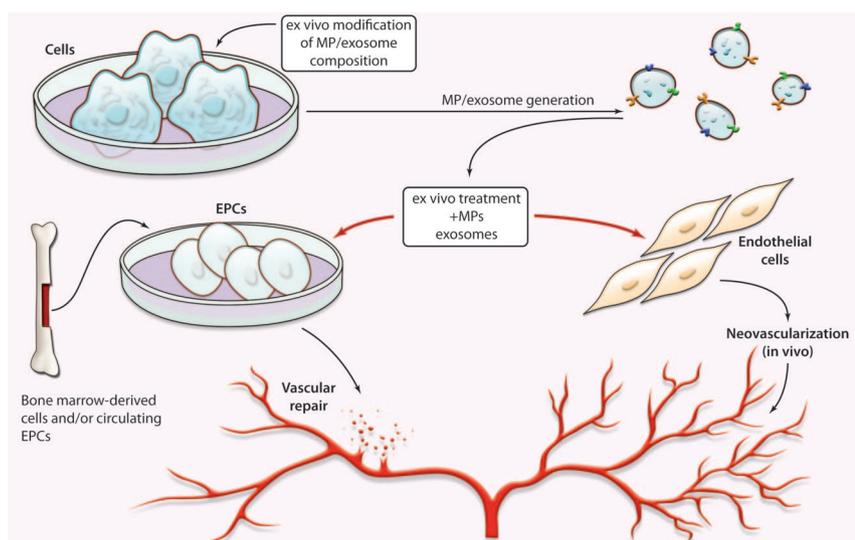


Figure 3. Future directions for the use of MPs or exosomes as therapeutic tools. Ex vivo modifications of composition of MPs^{48,55,75} and exosomes^{76,77} can be performed by inducing changes in the isolated cells through the overexpression of several desired proteins that may be transferred to EPCs or endothelial cells and, consequently, rescue the failed angiogenesis. (Illustration credit: Cosmo-cyte/Ben Smith).

which evoke nitric oxide production on endothelial cells, restore endothelium-dependent relaxation after ischemia-reperfusion injury, and favor in vitro angiogenesis and new vessel formation in an ischemic hind limb model.^{48,55,75} These findings suggest that MPs^{Shh+} may represent a potent tool in stimulating neovascularization in disease states associated with impaired angiogenesis. In contrast, MPs from apoptotic T-cell line that do not express Sonic hedgehog⁴⁸ inhibit angiogenesis, suggesting a potential therapy to reduce tumorigenesis via inhibition of tumor vascularization and possibly tumor development.⁵⁶ Second, and because of the ability of MPs to transfer and incorporate mRNA into target cells and modify their phenotype, transfection of MP-producing cells with “new” proteins or mRNAs and their subsequent delivery to target cells may represent a new opportunity to transfer a “desired” biological message into target cells and modify their phenotype, for instance, recovering the function of mutated/failed protein (Figure 3). For instance, it has been reported that MPs from lung cells contain mRNA that can be released into bone marrow cells and induce their epigenetic reprogramming by transfer of genetic material, thereby modulating their phenotype.⁷⁶ This promising approach has been validated, under pathological conditions, with other types of microvesicles. Akao et al⁷⁷ have recently shown that transfected miRNA molecules in human monocytes are released from these cells as contents in microvesicles. In this form, microvesicle-entrapped miRNA remains undegraded and are recruited into tumors. Likely, they could exhibit an antitumor effect in tumor-bearing mice. However, further experiments are needed to evaluate the therapeutic potential for patients.

Overall, MPs are complex entities that display a large number of activities affecting cells involved in angiogenesis. Although the mechanisms involved in these effects are not completely understood, considerable efforts are performed to attempt to use MPs as autologous therapeutic tools in diseases associated with altered angiogenesis. Future investigations should be addressed to combine beneficial effects of MPs and exosomes, because both are complementary in inducing angiogenesis regulation. Another important aspect is related to the complex composition of MPs and exosomes. Proteomic analyses are needed to identify all components of these microvesicles to provide extensive evidence about their side effects.

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Disclosure

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