Cellular Membrane Microparticles: Potential Targets of Combinational Therapy for Vascular Disease

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Abstract: Vascular disease constitutes the leading health problem throughout the entire world. Current therapies for vascular disease mainly rely on comprehensive strategies including control of risk factors, vascular interventions and conventional supportive treatments. To improve the preventive and therapeutic efficacies of current approaches, novel combinational therapies are required. Microparticles (MPs) are small membrane vesicles derived from cells undergoing stress, activation or apoptosis. They carry the characteristics of their parent cells, enabling them to serve as potential biomarkers for various diseases. Of note, MPs also have been shown to mediate cell communications through transferring membrane proteins, phospholipids and RNAs from their parent cells to recipient cells. Recent novel approaches have started to reveal the functions of MPs. In this review, we summarize the general concepts and the latest research progress in MPs. And the potential of MPs as novel targets of combinational therapy for vascular disease will be discussed.

Keywords: Combinational therapy, microparticles, vascular disease.

INTRODUCTION
Vascular disease refers to the pathology affecting cardiovascular, cerebrovascular and peripheral blood vessels. Acute heart infarction and stroke are the common vascular diseases which remain the leading causes of morbidity and mortality in the western countries [1]. Atherosclerosis is the basic pathological character underlying most vascular events. The degree of endothelial dysfunction, inflammatory activity, atherosclerotic stenosis, platelet agreeability and neovascularization are important factors affecting the progression of atherosclerosis [2]. Current combinational therapies for vascular disease mainly focus on modification of lifestyle, management of risk factors and interventional treatments [3, 4]. Although these approaches have gained certain achievements, the development of novel avenues should be an endless goal.

Cellular microparticles (MPs) are submicron membrane fragments released from stressed, activated or apoptotic cells, which have been documented as predictive biomarkers and cellular vectors of biological information in recent years [5-7]. Accumulating data have demonstrated that MPs could bind and fuse with their target cells, and deliver genetic materials to the recipient cells [8-12]. Of note, numerous studies have suggested that MPs have different functions which might depend on their cell of origin and agents used for stimulation [13, 14]. A recent study described that circulating MPs are the major transport vehicles for distinct microRNAs (miRNAs) in circulation [11]. We found that endothelial progenitor cell-derived MPs (EPC-MPs) released under serum deprivation stress had protective effects on hypoxia/reoxygenation-induced brain microvascular endothelial cell dysfunction and apoptosis; whereas, EPC-MPs produced under apoptotic stimulation by tumor necrosis factor- had opposite effects [15]. Therefore, a better understanding of the formation, composition, and function of MPs may lead to new therapeutic strategies to treat vascular disease either through removal or inhibition of "detrimental" MPs or through administration or stimulation of "favorable" MPs.

This review summarizes the general concept and recent research advance of MPs. The perspective of MPs as potential pharmacological targets of combinational therapy for vascular disease is discussed.

DEFINITION, FORMATION AND BASIC COMPOSITION OF MPS
Cellular MPs are submicron membranous vesicles that shed from various cells undergoing stress, activation, or apoptosis. Many terminologies have been used, like "exosomes", "exosome-like vesicles", "microvesicles"
(MVs)” and “apoptotic MPs”’. To clarify these terminologies, the key determinants are the biogenesis and secretary mechanisms of these subcellular fragments [16]. In general, apoptotic MPs are released in the final stage of programmed cell death and may contain fragmented DNA [9, 17, 18]. MVs and exosomes are two well characterized categories. They are surrounded by a phospholipid bilayer with exposure of phosphatidylserine (PS), which allows Annexin V to bind [10, 19]. MVs are released from the outward budding plasma membranes of their parental cells during cell activation or apoptosis [8]. Exosomes are released via the endocytic recycling pathway. The intraluminal vesicles (ILVs) are formed by membrane budding to the inside of the late endosome. The ILV-containing endosomes are called multivesicular bodies (MVBs). The MVBs could fuse with either lysosomes for cargo degradation or with the plasma membrane to secrete the ILVs as exosomes [20]. Exosomes display the surface markers of their maternal cells [21, 22], nevertheless, the sorting process of membrane proteins during ILV formation is an active process but a simple representation of surface markers from the cell of origin [23]. Exosomes are known to contain heat shock proteins (HSP70, HSP90), tetraspanin family molecules (CD9, CD63, CD81), and components of the endosomal sorting complex required for transport machinery (Alix, TSG101) [24, 25]. Exosome-like vesicles have similar characteristics of exosomes except do not contain lipid rafts [26]. In addition, these subcellular fragments can also be distinguished by size. According to previous studies, the size of exosomes ranges from 30 to 100 nm [27], and the size of exosome-like vesicles range from 30-90 nm [26]. MVs have a size of 100 to 1000 nm [16], while apoptotic MPs are much larger (1000-4000 nm) [28]. The comparisons between MVs and exosomes are summarized in Table 1.

The detailed mechanisms of MP biogenesis are largely unknown. However, numerous studies have reported that MPs exert different effects under various stimuli and express distinct surface membrane antigens [29-34]. For example, apoptosis-induced EMPs express CD31 and CD105, whereas activation-induced EMPs express CD62E and CD54 [29, 31]. MPs released from platelets (PMPs) during physiological condition may play an important role in the normal homeostatic response since these PMPs exhibit prothrombinase activity [32]. Whereas, PMP generated under high shear stress may be crucial for the occurrence of thrombogenesis in various pathological states [33, 34]. Generally, the function of MPs relates to the asymmetric phospholipid distribution in the cell membrane, with phosphatidylycerol and sphingomyelin located on their external leaflet, phosphatidylethanolamine and PS on their inner side [37]. MPs exhibit negatively charged phospholipids (mainly PS) which account for their procoagulant and proinflammatory properties, thereby alteration of vascular function [38].

### ISOLATION AND DETECTION OF MPS

There are several experimental protocols describing isolation of MPs from blood or cell culture medium [28, 39, 40]. Although a standardized centrifugation protocol has not been established, the first and slower centrifugation is used to eliminate cells and debris, whereas the second and third centrifugation speed are adjustable depending on the MPs of interest. To pellet circulating MPs, a blood (anticoagulation with sodium citrate or heparin) sample is centrifugate at low speed (160 × g for 20 min at room temperature) to get rid of red blood cells and obtain platelet-rich plasma (PRP). The PRP is then centrifugate at 1,500 × g for 20 min at room temperature to remove cell debris and obtain platelet-poor plasma (PPP). The PPP is further centrifugate at 2,000 × g for 20 min to obtain platelet-free plasma (PFP). The PFP is finally centrifuged by a rapid short centrifugation (13,000 × g for 2 min) to obtain a MP pellet. The MP pellet can be resuspended in 1 x Tris-buffered saline with gentle vortexing for analysis by flow cytometry [40, 41]. In our previous studies [42], we isolated MPs from circulation by several centrifugation steps. Firstly, a peripheral blood sample was taken from the left ventricle of mouse heart (anticoagulation with 1% heparin). Blood was diluted in phosphate-buffered saline (PBS) and then gently layered over gradient medium for centrifugation (800 × g for 30 min at 4°C) [43]. After gradient density centrifuge for isolation of mononuclear cells

### Table 1. Overview of the characteristics of main cellular microparticles

<table>
<thead>
<tr>
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<th>Microvesicles</th>
<th>Exosomes</th>
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<tr>
<td>Size range</td>
<td>100 – 1000 nm</td>
<td>30 – 100 nm</td>
</tr>
<tr>
<td>Mechanism of generation</td>
<td>By directly budding from plasma membrane</td>
<td>By endocytic recycling pathway</td>
</tr>
<tr>
<td>Release type</td>
<td>Regulated process</td>
<td>Constitutive and regulated process</td>
</tr>
<tr>
<td>Compositions</td>
<td>Proteins, lipids, mRNA, and microRNA</td>
<td>Proteins, lipids, mRNA, and microRNA</td>
</tr>
<tr>
<td>Surface markers</td>
<td>Annexin V, tissue factor and cell-specific markers</td>
<td>Annexin V, HSP70, HSP90, CD9, CD63, CD81, Alix and TSG101</td>
</tr>
<tr>
<td>Detection</td>
<td>Flow cytometry, electron microscopy, dynamic light scattering, nanoparticle tracking analysis</td>
<td>Electron microscopy, western blot, mass spectrometry, nanoparticle tracking analysis</td>
</tr>
<tr>
<td>Key functions</td>
<td>Coagulation, inflammation, angiogenesis, varies on different stimuli</td>
<td>Antigen presentation and immune response</td>
</tr>
<tr>
<td>References</td>
<td>[8, 10, 16, 19, 42, 49, 50, 52]</td>
<td>[20-25, 27, 39, 46, 49, 50]</td>
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(MNCs), the upper layer was collected and centrifuged (3,000 × g for 15 min at 10°C) to obtain PPP. The PPP was centrifuged (20,000 × g for 45 min at 10°C) to pellet MPs [39, 44]. The MP pellet was resuspended in PBS and fixed with 1% paraformaldehyde, and then stored at 4°C for flow cytometric analysis within 24 h. To pellet MPs from cultured cell medium, medium was collected and centrifuged (300 x g for 10 min) to remove cell debris. The supernatant was removed and the pellet was kept after ultracentrifugation at 10,000 x g for 60 min [45].

The methods to isolate exosomes and apoptotic MPs are slightly different from those of MPs. To pellet exosomes, the most rigorous protocols are based on ultracentrifugation of the sample after it has been cleared by a series of low speed centrifugations. Exosomes are final pellet at 100,000 x g to 110,000 x g for 60 to 90 min [39, 46]. For purer preparations, some researchers use sucrose gradient ultracentrifugation. Electron microscopy is typically used to characterize exosomes due to their small size [46, 47]. To pellet apoptotic MPs, medium from apoptotic cells is centrifuged at low speed (800 × g for 10 minutes) to remove dead cells and cell debris. The cell-free supernatant is transferred to a new tube and further centrifuged (16,000 × g for 20 minutes) to isolate the apoptotic MPs [28].

Flow cytometry (FC) is the most commonly used method for analysis of MPs. This method enables the analysis of thousands of MPs in one sample, with the simultaneous determination of multiple markers. Standard FC can detect vesicles above approximately 200 nm, therefore exosomes and smaller vesicles cannot be analyzed directly by this method. Despite these limitations, FC is still a widely used method for MP detection and many studies have reported that the counts of MPs are correlated with different physiological conditions and diseases by using FC analysis [19]. Most FC protocols use the binding of fluorescein isothiocyanate (FITC)-labeled Annexin V to PS as the common marker for MPs [48]. MPs are usually characterized by FC based on both their size and specific markers of their parent cells. For examples, EMPs can be characterized as CD144+ particles from 1-2 μm in size, EPC-MPs can be recognized as CD34+ and vascular endothelial growth factor receptor-2 (VEGFR-2)+ particles from 1-2 μm in size [42]. More information on the specific markers for different origins of MPs is included in the following section. In addition to conventional FC, electron microscopy allows to determine the presence of MVs and exosomes in ultracentrifuge pellets of biological fluids (cell culture medium, plasma or urine), but is not quantitative and requires extensive sample preparation. Nanoparticle tracking analysis (NTA) allows to determine the size distribution of MVs and exosomes based on the Brownian motion of vesicles in suspension, which can measure vesicles as small as 50 nm [49, 50].

Several other methods have been reported for analysis of MPs. Enzyme-linked immunosorbent assay (ELISA) has been reported for the identification of MPs, but this method cannot capture all the vesicles present [40, 51]. Dynamic light scattering can measure size by light scattered from vesicles under Brownian motion and can detect vesicles with size between 1 nm and 6 μm. One major disadvantage is that it cannot distinguish mixtures of MVs and exosomes, because it is biased toward the detection of larger particles [49, 52]. Recently, a novel method has been introduced to detect and quantify cell-derived MPs by the electrochemical potential-modulated electrochemical impedance spectroscopy (EIS), which allows for detection as low as several MPs per microliter [53]. EIS detection and quantification of MPs is based on fundamental interfacial electrochemistry at the sensing electrode, which is driven by electrochemical activities of MPs that can be separated by applied electrochemical potential. Thus, interfacial electrochemistry processes can be used to determine the concentration and size of participating species with higher accuracy, specificity and selectivity than traditional analytical methods [53]. Scanning probe microscopy, especially atomic force microscopy (AFM), can detect MPs with sizes below the detection limit of flow cytometer [54, 55]. With AFM, high-resolution topography imaging of MPs can be obtained with a resolution down to the sub-nanometer range. Moreover, the ability of AFM to image biological samples in aqueous fluids enables the preservation of sample properties in their physiological state [55, 56].

CHARACTERIZATION AND FUNCTION OF MAIN CIRCULATING MPS

MPs can be derived from various cell types and express different surface markers. Elevated levels of MPs have been documented in various vascular diseases and correlated with the severity of disease [42, 57-59]. Numerous studies have shown that MPs have different functions which depend on the cell of origin and stimuli. For example, EMPs released under starvation condition have protective effects on endothelial cells (ECs) against camptothecin-induced apoptosis through inhibition of p38 MAPK activation [60]. On contrary, EMPs generated from high glucose treated ECs have deleterious effects as evidenced by increased macrophage infiltration and adhesion protein expression in an atherosclerotic mouse model [61]. Consistently, our previous study showed that circulating MPs derived from diabetic mice could impair EPC functions such as migration and tube formation in vitro and in vivo [62]. Likewise, circulating PMPs isolated from healthy donors have capacity of increasing post-ischemic capillary density in a myocardial ischemia rat model [62], whereas PMPs from stored blood could lead to thrombosis in recipients during blood transfusion [64]. In the following section, we mainly focus on PMPs, EMPs, and EPC-MPs, which are major categories of circulating MPs.

PMPs

PMPs are the most abundant MPs in the circulating blood. PMPs express glycoprotein IX (CD42a) [65], glycoprotein Ib (CD42b), glycoprotein IIb/IIIa (CD41) [66], platelet endothelium adhesion molecule (PECAM-1; CD31) [67], P-selectin (CD62P) [68], CD63 [69] and CD61 [70]. The elevated PMP level is found in patients with hypertension, atherosclerosis, coronary heart disease and stroke [58, 71-73]. Numerous studies have indicated that MPs are implicated in the pathogenesis of vascular disease due to their highly procoagulant and thrombogenic potentials [19, 65, 74]. For example, increased PMP formation could lead to thrombotic complications in patients who underwent surgical intervention [74]. Another study reported that PMPs have ability to facilitate atherogenesis by enhancing expression of
cellular adhesion molecules, promoting proliferation of smooth muscle cells and stimulating inflammation [65]. PMPs also have beneficial effects on tissue repair. Previous studies showed that a deficiency of PMP generation may contribute to bleeding disorders, because PMPs could bind to subendothelial matrix and act as a substrate for further platelet adhesion at the site of endothelial injury [76, 77]. More interestingly, a recent study suggested that PMPs are able to modify steps involved in angiogenesis, such as proliferation, migration and formation of capillary-like structures in ECs [78].

EMPs

EMPs express CD31, CD54, CD62E (E-selectin), CD105, CD144, and bind von Willebrand factor (vWF) [79-82]. Since CD31 is also expressed by PMP, EMP specificity is ensured by the CD31+/CD41- phenotype (CD41 being the platelet glycoprotein IIb/IIIa). The numbers of circulating EMPs are increased in many vascular diseases including severe hypertension, acute coronary syndromes (ACS) and various forms of vasculitis, and could be used as biomarkers [81, 83-85]. It was believed that EMPs play important roles in coagulation, inflammation, endothelial dysfunction, EC senescence and apoptosis [86, 87]. However, more recent data suggest EMPs could promote cell survival, exert anti-inflammatory and anti-coagulatory effects or induce endothelial regeneration [88, 89]. i.e. EMPs could activate proangiogenic program in ECs by transferring matrix metalloproteinases or mRNAs to target cells [90, 91]. Notably, promotion of angiogenic processes may have dual effects. On one hand, it could promote vascular repair in response to ischemic injury. On the other hand, it could stimulate the growth of cancer and destabilization of atherosclerotic plaque [92, 93]. More knowledge on EMP biological effects could provide new targets for reducing vascular risks.

EPC-MPs

EPCs have been shown to play an important role in maintaining vascular integrity and homeostasis. EPC-MPs express CD34 and VEGFR-2 [42], intercellular adhesion molecule (ICAM)-1, integrin α4 (CD49d), CD44 and CD29 [94]. It has been reported that there is a negative association between EPC-MPs and EPCs, suggesting that the level of EPC-MPs could serve as an index for EPC loss and functional incompetence [43]. In addition, EPC-MPs could promote EC survival, proliferation and tube formation ability in vitro, and favor vessel regeneration in a hindlimb ischemia mouse model by transferring functional mRNAs [91]. We recently reported that EPC-MPs could protect cardiomyocytes from Angiotensin II-induced hypertrophy, oxidative stress and apoptosis [95]. Targeting on EPC-MPs may provide a novel therapeutic approach for vascular disease.

Other MPs in the Blood

MPs can also be released from neutrophils, lymphocytes, erythrocytes, etc. Neutrophil-derived MPs express macrophage-1 antigen and CD62L on their surface, and could serve as inflammatory mediators in activating platelets and ECs [96]. They also exert anti-inflammatory effects when carrying Annexin A1 by inhibiting the interaction between neutrophils and ECs [97]. There are two types of lymphocyte-derived MPs: B lymphocyte-derived MPs express CD19, and T lymphocyte-derived MPs (TMPs) express TF, CD4 and CD8 [64, 98]. TMPs generated from apoptotic T cells could impair acetylcholine-induced vessel relaxation in mouse aortic rings by alteration of nitric oxide (NO) and prostacyclin pathways [98]. However, TMPs released from activated T cells carrying Shh could promote endothelium repair through activation of NO pathway and upregulation of proangiogenic factors [13, 99]. Both MPs derived from neutrophils and lymphocytes belong to the family of leukocyte-derived MPs (LMPs). Erythrocyte-derived MPs (ErMPs) are involved in red blood cell elimination and elevated in sickle cell disease [100, 101]. ErMPs represent a “two-edged sword” that can contribute to erythrocyte homeostasis by eliminating sorted oxidized proteins [102], but also induce adverse clinical outcomes in transfused recipients through their immune effects and thrombogenic activities [101, 103-106].

MPS PARTICIPATE IN THE PATHOLOGY OF VASCULAR DISEASE

Vascular disease is one of the major causes of death in developed countries and is increasing in developing countries [107]. Common vascular diseases include hypertension, atherosclerosis, coronary artery disease and stroke [107, 108]. Endothelial dysfunction is a common denominator of vascular disease and has been recognized as an important target for prevention and treatment of vascular disease [109]. Endothelial dysfunction could result in increased vasoconstrictor activity, alterations of local mediators (e.g. cytokines, chemokines and adhesion molecules) [110, 111]. Besides, endothelial dysfunction could lead to an imbalance between tissue-type plasminogen activator and plasminogen activator inhibitor-1, which could predispose the formation of thrombosis [112]. The interaction between MPs and ECs has been demonstrated in several studies, showing that MPs could directly affect the endothelial vasorelaxation via a mechanism that involves diminished production and/or bioavailability of NO [98, 113]. In addition, MP have been shown to promote the adherence of monocytes and leukocytes to ECs, which is one of the key features in the development of atherosclerotic plaques [114-116]. Furthermore, MPs could stimulate cytokine release and TF induction in ECs, which may lead to increased proinflammatory and procoagulant activities [117, 118].

MPs in Hypertension

Vascular dysfunction in hypertension is characterized by impaired endothelial-dependent relaxation and reduced arterial elasticity. Hypertension could cause increased shear stress and direct mechanical damage of arteries. Previous studies have suggested the pathophysiological relationship between MPs and vascular dysfunction/damage in both systemic and pulmonary hypertension [119, 120]. EMPs appear to be very sensitive to hemodynamic changes in hypertension, and their numbers are increase in mild hypertension and rise further in proportion to blood pressure elevation [120]. In addition, artery elasticity is found to closely associate with the EMP generation in healthy subjects [121]. Pulmonary hypertension is associated with the rise of PMPs,
EMPs and LMPs, but only EMPs predict the hemodynamic severity of pulmonary hypertension [57]. More recently, an increased level of circulating small PMPs (300-500 nm in diameter) was identified in patients with several forms of pulmonary hypertension [58].

**MPs in Atherosclerosis**

Atherosclerosis is a chronic disease characterized by endothelial dysfunction and local/general inflammation, which is orchestrated by leukocyte infiltration, plaque neovascularization, growth and destabilization [119]. One relatively recent advance in this area is the discovery of MPs and their association with endothelial damage, platelet activation and hypercoagulability, which link the presence of cardiovascular risk factors, atherogenesis and thrombosis [122, 123]. Clinical studies have shown that PMPs are significantly up-regulated in patients with atherosclerosis [119, 124]. A recent study shows that PMPs could induce platelet deposition and thrombus formation via transferring TF to human atherosclerotic arteries [125]. Additionally, a previous study reported that LMPs isolated from human atherosclerotic lesions could augment ICAM-1 expression and subsequently enhance monocyte adhesion [92]. And EMPs may also be responsible for the prothrombotic state through binding platelet and promoting platelet activation in patients with atherosclerosis [119].

**MPs in Coronary Artery Diseases**

Despite significant advances achieved by medical and interventional management during past decades, coronary artery disease (CAD) and its consequences including myocardial infarction, sudden cardiac death and chronic cardiac failure remain the major mortality in western countries [59]. Several reports have highlighted the association of the level of MPs with CAD [59, 126]. A clinical study has reported that elevated number of circulating apoptotic EMPs positively correlated with impairment of coronary endothelial function [127]. The higher level of EMPs was observed in the circulating blood of patients with ACS, contributing to the generation and perpetuation of intracoronary thrombi [128]. Interestingly, the EMP level was suggested to associate with the risk of angiographic lesions in ACS [129]. In addition, PMPs is also important for increased coagulation activation in patients with myocardial infarction [72].

**MPs in Stroke**

In general, ischemic and hemorrhagic stroke are the two major types of stroke. Ischemic stroke constitutes about 87% of all strokes. The pathogenesis of ischemic stroke includes the initial atherosclerotic plaque formation, plaque destabilization and the development of neuronal cell death. A number of studies have reported that MPs are involved in stroke [130-132]. Horstman and colleagues found that the level of MPs is corresponds to the severity, lesion volume and outcome in patients with ischemic stroke [131]. Similarity, another study showed that EMPs were increased and correlated with stroke severity, brain lesion volume and outcome in acute ischemic stroke patients [132]. Additionally, the positive correlation between EMPs and cerebral infarction in subarachnoid hemorrhage (SAH) was also reported [130]. Therefore, a better understanding of MP release and formation could contribute to the treatment and prevention of stroke. Previous studies have suggested that PMPs are prominently increased in patients with cerebral vasooclusion [73], and persisted in the convalescent phase of acute stroke [133]. What's more, the increased level of PMPs positively associated with carotid intima thickness and carotid plaque in convalescent ischemic stroke patients [71].

**MPS AS BIOMARKERS FOR VASCULAR DISEASE**

Circulating MPs reflect activation or damage status of circulating cells. Detectable alterations in MP level have been reported in various vascular diseases [1, 59, 119, 131]. Therefore, measurement and characterization of MPs may provide a new avenue for disease detection and diagnosis, for studying the pathophysiology or progression of disease. For example, elevated levels of circulating MPs were detected in pathological states associated with vascular dysfunction [134] and could serve as potential biomarkers for various cardiovascular diseases, such as ACS, hypertension, and ischemic stroke [131, 135, 136]. Elevated levels of EMPs are found to associate with carotid inward remodeling [119]. We previously reported that increased levels of EMPs and EPC-MPs are positively correlated with blood glucose concentration and infarct volume after ischemic cerebral injury in db/db diabetic mice [42]. In clinical research, the positive correlations between EMP levels and lesion volume or clinical outcome in acute ischemic stroke patients were found [132]. Moreover, Amabile et al. reported that EMP levels could predict the outcome of patients with pulmonary hypertension [137]. Nozaki et al. observed that EMP levels could independently predict future cardiovascular events in patients at high risk for CAD [138]. These findings indicate that MPs could be used as predictive biomarkers for vascular disease.

**THERAPEUTIC POTENCIAL OF MPS FOR VASCULAR DISEASE**

Accumulating evidence suggest that MPs could be either beneficial or deleterious depending on the cellular origin and specific stimuli involved in their generation [29, 30]. Therefore, inhibition of "detrimental" MPs and enhancement of "favorable" MPs could be served as novel approaches for treating vascular disease. The potential of MP-combined therapy for vascular disease is summarized in (Fig. 1).

**Pharmacological Modulation of Circulating MPs**

There is an emerging interest in evaluating the change of circulating MPs in response to pharmacological treatments. A previous study showed that decreased PMP level was associated with reduced vascular damage in peripheral circulatory disorder patients who treated with ticlopidine [139]. The calcium channel blockers could decrease PMP level in patients suffering from transient ischemic attacks, resulting in decreased ischemic thrombogenic risks [140]. More interestingly, a recent study found that polyphenols could decrease the levels of PMPs, EMP and ErMPs in aldosterone-salt induced hypertensive rat, which is associated with reduced vascular inflammation and endothelial dysfunction [141]. All of these studies have shown the association between MP
level and the effects of pharmaceutical agents, suggesting that pharmacological modulation of MPs could be a novel approach for correcting vascular pathologies. However, the underlying mechanism of MP biogenesis and release needs further investigation.

**Administration of Therapeutic MPs**

Recent studies suggested that EMPs, PMPs, EPC-MPs, and mesenchymal stromal cell-derived MPs (MSC-MPs) possess therapeutic potentials through modulating cellular processes and subsequently inducing tissue repair [62, 88, 142, 143]. Local administration of PMPs could increase the amount of capillaries in a myocardial ischemia rat model [62]. Intravenous application of apoptotic EMPs could increase mobilization and incorporation of progenitors into plaques and limit the progression of atherosclerosis in an atherosclerosis mouse model [88]. Additionally, intravenous administration of EPC-MPs could protect rat kidney from ischemia/reperfusion injury through transferring pro-angiogenic miRNA-126 and miRNA-296 to target cells [142]. Systemic administration of MSC-MPs could promote neurovascular remodeling, neurogenesis and angiogenesis in a stroke rat model [143]. Furthermore, it is further illustrated that the therapeutic effect of MSC-MPs for stroke is mediated by transferring miRNA-133b to adjacent astrocytes and neurons, which evokes neurite remodeling and functional recovery [144].

In order to enhance the specific therapeutic effects of MPs, engineered MPs with modified compositions were investigated in several studies. For example, TMPs engineered with shh could improve endothelial function via increasing NO release in animal models [99, 145]. Engineered embryonic stem cell-derived MPs could delivery exogenous mRNAs/proteins to recipient cells and mediate cell signaling [146]. Recently, another study demonstrated that over-expression of transcription factor peroxisome proliferator-activated receptor-γ (PPARγ) in MPs could increase transcription of the target gene, suggesting the capacity of MPs for transcellular communication [35]. Interestingly, a very recent study has shown that local administration of engineered MPs by over-expressing the suicide gene mRNAs/proteins could induce tumor cell apoptosis and inhibit tumor growth [36]. Taken together, administration of MPs or engineered MPs may provide a novel therapeutic approach for treating vascular disease.

**Combinational Therapy**

The promising application of MPs is to combine MPs/engineered MPs with cell-based therapy for vascular disease. It has been shown that PMPs could boost the ability of EPCs to restore endothelial integrity after vascular injury *in vitro* and *in vivo* [63]. Another study demonstrated that EPC-MPs could promote EC survival, proliferation, organization in capillary-like structures and stimulate angiogenesis in patent vessels in a mouse model, probably through shut-
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ting cellular mRNAs associated with the PI3K/AKT signaling pathway [91]. In addition, it has been illustrated that MPs generated locally after ischemia are endogenous triggers for bone marrow progenitor cell differentiation and subsequently revascularization in response to ischemic injury [75]. All these studies indicate that MPs could offer a promising combined therapy for vascular diseases.

CONCLUSION

In conclusion, accumulating evidence supports the perspective of MPs as biomarkers and effectors for vascular disease. Of note, MPs could be beneficial or deleterious depending on the cellular origin and specific stimuli involved in their generation. The deleterious effects of MPs could be pro-inflammatory, pro-coagulant and pro-thrombotic, while their beneficial effects could be angiogenic, anti-inflammatory, homeostatic, etc. Nevertheless, more specific strategies to maximize the application of MPs wait for future exploration. The development of MPs as targets, in combination with existing therapies, is of great pharmacological significance for vascular disease.

CONFLICT OF INTEREST

No conflicts of interest, financial or otherwise, are declared by the authors.

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