Published in final edited form as:

Curr Opin Endocrinol Diabetes Obes. 2012 April ; 19(2): 121–127. doi:10.1097/MED.0b013e32835057e9.

Microvesicles: potential markers and mediators of endothelial dysfunction

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Abstract

Purpose of review—Microvesicles (MVs, also known as microparticles) are small membranous structures that are released from platelets and cells upon activation or during apoptosis.

Microvesicles have been found in blood, urine, synovial fluid, extracellular spaces of solid organs, atherosclerotic plaques, tumors, and elsewhere. Here, we focus on new clinical and basic work that implicates MVs as markers and mediators of endothelial dysfunction and hence novel contributors to cardiovascular and other diseases.

Recent findings—Advances in the detection of MVs and the use of cell type-specific markers to determine their origin have allowed studies that associated plasma concentrations of specific MVs with major types of endothelial dysfunction – namely, inappropriate or maladaptive vascular tone, leukocyte recruitment, and thrombosis. Recent investigations have highlighted microvesicular transport of key biologically active molecules besides tissue factor, such as ligands for pattern-recognition receptors, elements of the inflammasome, and morphogens. Microvesicles generated from human cells under different pathologic circumstances, e.g., during cholesterol loading or exposure to endotoxin, carry different subsets of these molecules and thereby alter endothelial function through several distinct, well-characterized molecular pathways.

Summary—Clinical and basic studies indicate that MVs may be novel markers and mediators of endothelial dysfunction. This work has advanced our understanding of the development of cardiovascular and other diseases. Opportunities and obstacles to clinical applications are discussed.

Keywords

Microvesicles; microparticles; endothelial dysfunction

Introduction/Background

Microvesicles (MVs, also known as microparticles) are small membranous structures that are released from platelets and cells upon activation or during apoptosis [1–6]. The budding process is characterized by sorting of protein cargo, blebbing then abscission of a section of the plasma membrane [7], and a loss of membrane asymmetry that results in the exteriorization of phosphatidylserine (PS) to the outer leaflet. Thus, MVs harbor a subset of cell-membrane lipids and membrane-associated proteins derived from their parental cells, including cell type-specific markers that definitively distinguish their origin. Microvesicles were first described as “platelet dust” more than four decades ago in studies of the coagulant activity of platelet-rich plasma during storage [8]. A major conceptual advance occurred with the realization that MVs in plasma transport biologically significant amounts of tissue factor (TF), a transmembrane molecule that functions as a potent initiator of coagulation [9]. Now, MVs are understood to comprise a complex of molecules that facilitate TF action, such as exteriorized PS, which enhances clot propagation [3], and P-selectin glycoprotein ligand-1 (PSGL1), which targets TF-positive MVs to exposed P-selectin on activated platelets [10,11].

Nevertheless, studies in several settings from our group [4,5] and others [12] indicate that only a minority of MVs are TF-positive [4,5,12]. Thus, MVs might arise from, or mediate, novel physiologic and pathologic effects that are independent from coagulation. During the past dozen years, investigations of MVs have spread from the field of coagulation to atherosclerosis and vascular biology [2,4,5,13,14], diabetes [15,16], infectious diseases [17–20], rheumatology and autoimmunity [21–23], and cancer [24–29]. MVs have been found in blood, urine, synovial fluid, extracellular spaces of solid organs, atheromata, tumors, and elsewhere [30].

The formation, composition, catabolism, measurement, and general functions of MVs have been the subject of recent studies and review articles [30–35]. Here, we focus on new work implicating MVs as markers and mediators of endothelial dysfunction.

Vascular endothelium plays a crucial role in regulating vascular tone and structure. In healthy states, the endothelium also inhibits leukocyte and platelet adhesion and maintains a balance of profibrinolytic over prothrombotic activities [36–38]. Endothelial dysfunction is characterized by a shift towards inappropriate or maladaptive vascular tone, leukocyte recruitment, and thrombosis [37]. Examples of clinically important vascular dysregulation include chronic hypertension and impaired endothelial-dependent vasodilation in response to key physiologic stimuli, such as hypoxia, flow, and insulin. Healthy responses to hypoxia and flow increase local blood flow and hence oxygen delivery [39]. Insulin-mediated vasodilation increases the flow of blood through striated muscle, to facilitate the normal postprandial uptake of glucose from plasma [40]. In each of these situations, MVs originating from endothelium and other sources may indicate or contribute to vascular dysregulation.

Microvesicles as potential markers of endothelial dysfunction

Studies of human subjects indicate that plasma concentrations of endothelial-derived MVs (i.e., MVs bearing unique endothelial surface markers) increase in obesity [41], physical inactivity [42], type 2 diabetes mellitus [43], end-stage renal disease [44], ischemic left ventricular dysfunction [45], and preeclampsia [46]. Importantly, the circulating levels of endothelial-derived MVs in these studies correlated with the degree of impaired vasodilation in these subjects [41–47]. Likewise, studies of patients with hypertension [48,49] or sickle cell disease [50] have indicated that plasma concentrations of endothelial- [48], platelet- [49], or erythrocyte- [50] derived MVs correlate with circulating endothelial markers of sterile inflammation (soluble VCAM-1, soluble CD40L) and a pro-thrombotic state (von Willebrand factor).

Interventions related to many conventional cardiovascular risk factors and therapies have been reported to affect the generation of MVs from different cell types in vivo and in culture [4,5,35,51]. Consumption of consecutive high-fat meals by type 2 diabetic patients has been associated with impaired flow-mediated vasodilation and elevated plasma concentrations of endothelial-derived MVs [52]. Similarly, brief secondhand smoke exposure also impaired flow-mediated vasodilation and substantially increased plasma levels of endothelial-derived MVs [53]. Separate administration of an LDL-lowering agent (simvastatin), an angiotensin II receptor blocker (losartan), or an antihypertensive calcium channel blocker (nifedipine) to patients with high blood pressure reduced circulating levels of endothelial- and platelet-derived MVs, as well as soluble VCAM-1 [54,55]. Cholesterol-enrichment of cultured human cells, in the absence other stimuli [4], or exposure of human cells to tobacco smoke [5,51] markedly increased the production of pro-coagulant MVs, in part through apoptosis and MAP kinase activation [5,51].

Normal or elevated plasma concentrations of high-density lipoprotein (HDL) have long been associated with lower rates of atherosclerotic cardiovascular events [56–61]. A few clinical studies have reported that circulating levels of MVs from platelets [49] and TF-positive MVs from T cells or neutrophils [16] negatively correlate with plasma HDL levels. In addition to its presumed mediation of ‘reverse’ cholesterol transport from peripheral tissues to the liver [62–64], HDL may also exert a protective role on endothelial function by enhancing eNOS expression and NO release [65–67] and by indirectly inhibiting apoptosis [68] and the generation of MVs [16,49,69]. Each of these actions could affect plasma concentrations of MVs.

Regarding prognosis, several studies indicate that circulating endothelial-derived MVs can predict hemodynamic severity or poor outcomes in pulmonary hypertension [70,71], cardiovascular mortality in end-stage renal disease [72], and subsequent cardiovascular events in patients with heart failure or at high risk of coronary artery disease [73,74].

The clinical work discussed above suggests that plasma concentrations of MVs could serve as a new biomarker of endothelial dysfunction in cardiovascular diseases [73,75]. Nonetheless, merely correlating with a bad clinical state or with future outcomes is not enough for a marker to be useful. It must be relatively easy to measure, consistent, and

reproducible; and it must add significant predictive power over conventional risk factors. Assay methods for MVs include flow cytometry; enzyme-linked immunosorbent assays (ELISA) using plates coated with immobilized annexin V (a protein that preferentially binds exposed PS); brief ultracentrifugation; electron microscopy; and fluorescence confocal microscopy. Flow cytometry remains the most commonly used method for both enumeration and determinations of the cellular origin of MVs, but it is expensive, slow, and cannot currently detect MVs smaller than 200 nm [31,76,77]. In the past few years, new techniques have appeared for MV determination, including atomic force microscopy, dynamic light scattering, enhanced laser microscopy tracking proteomic analysis, and impedance-based flow cytometry [31,76,77]. These new methods are still far from routine practical use for MV assessment. For all of these methods, ease and reproducibility within usual clinical laboratory standards remain unverified, and fresh or promptly fixed samples must be used, which could be inconvenient. Most importantly, no studies of any clinical endpoint to our knowledge have compared the area under the receiver operator characteristic (ROC) curve for conventional risk factors vs. those factors plus measurements of plasma MV concentrations. Further studies will be needed to evaluate the predictive value, discrimination, and reclassification power that are required to demonstrate clinical utility of any proposed biomarker [78].

Microvesicles as potential mediators of endothelial dysfunction

Recent studies indicate that MVs are significant mediators of intercellular communication and may serve as shuttles promoting cellular cross-talk in physiologic and pathologic settings [31,33,79]. Their actions include transmittal of biologically active ligands, receptors, intracellular proteins, RNA including microRNA (miRNA), and even organelles [33,80,81]. Because MV release above baseline levels is stimulated by cellular activation or apoptosis, these vesicles are particularly active in pathology [31,33,79]. Each activated or apoptotic cell can release many MVs, and owing to their small size, the MVs survive longer and diffuse more widely [17] than their parental cells, which are usually cleared quickly by phagocytosis [82]. Thus, the effects of MVs can linger long after their parental cells have disappeared, particularly in the context of disease.

Microvesicles from several abnormal clinical states have been shown to adversely affect endothelial function. Circulating MVs isolated from patients with myocardial infarction [83], metabolic syndrome [84], or obstructive sleep apnea [85] impair endothelium-dependent vasodilation ex vivo [83]. Moreover, MVs isolated from human atherosclerotic plaques, discussed in more detail below, provoke cultured endothelial cells to recruit leukocytes [86]. Basic laboratory studies have shown that MVs obtained from rats with pulmonary hypertension [87] or MVs generated in vitro using different stimuli on smooth muscle cells [88], endothelial cells [89,90] or, in some cases, T lymphocytes [91,92], can inhibit nitric oxide production by cultured endothelial cells [90,92] and by aortic explants ex vivo [88–91]. Moreover, these MVs also induce endothelial dysfunction in vivo [88,91,92]. In addition, MVs produced by stimulation of several cell types are able to induce endothelial cells to recruit monocytes in vitro. Microvesicles with this property come from stimulated platelets in vitro [13,93], from endothelial cells after inhibition of anchorage-dependent cell spreading [94] or exposure to angiotensin II [95], and from human monocytes after

enrichment with cholesterol to mimic the environment within an atherosclerotic plaque [96] or by stimulation with LPS to mimic sepsis [97].

For several reasons, the pathways involved in the production of these biologically active MVs and then their recognition by endothelium appear to be active, regulated, and specific. **First**, MVs generated from calcium ionophore-treated endothelial cells [94] or isolated from the circulation of the patients with carotid artery atherosclerosis [86] cannot induce endothelium to recruit monocytes [86,94], indicating selectivity in MV composition. These observations presumably account for normally functioning endothelium in healthy individuals, despite abundant but quiescent MVs in their circulation. **Second**, the generation of MVs requires active cellular processes, including plasma membrane blebbing via actomyosin-based abscission [3,7,98]. **Third**, the process of plasma membrane blebbing preserves the boundary between intracellular and extracellular compartments and thereby avoids indiscriminant release of intracellular contents. Thus, activation signals have to be transported or created on the external surface.

In three clinically relevant circumstances, mechanistic details for the production then action of MVs that affect endothelial function have been reported. The first of these is MVs produced by human monocytes or macrophages upon enrichment with unesterified cholesterol in the absence of other stimuli (UCMV) [4,96]. Cholesterol enrichment occurs in vivo when monocyte/macrophages ingest retained and aggregated lipoproteins in the vessel wall [99,100], damaged or senescent cells, and related debris. Because macrophages cannot catabolize the steroid nucleus, they must use other processes to accommodate exogenous unesterified cholesterol, including its transport into mitochondria for processing by CYP27A1, also known as sterol 27-hydroxylase [101]. In our experimental system, similar to atherosclerotic plaques in vivo [102,103], the pathways to accommodate unesterified cholesterol become overwhelmed, leading to mitochondrial dysfunction, apoptosis [4], and the release of MVs with biologically active danger signals that activate endothelium [96]. Consistent with this model, our previous work showed that removal of unesterified cholesterol in vivo from the tissues of genetically hypercholesterolemic animals can normalize leukocyte adherence to microvascular endothelium [104]. Strikingly, pretreatment of human microvascular endothelial cells (hMVECs) with a blocking mAb against LOX1, a pattern-recognition receptor, significantly decreased UCMV-induced endothelial activation and monocyte recruitment [96]. As noted above, MVs isolated from human atherosclerotic plaques are also able to induce endothelial cells to recruit leukocytes [86]. By promoting maladaptive immunologic [96] and thrombotic [4] responses, these vesicles may contribute to atherothrombosis and other pathologic conditions in vivo.

The second circumstance is MVs produced by human monocytes upon stimulation with endotoxin, to mimic sepsis (LPS-MVs) [97]. In contrast to UCMVs, which are generated by apoptotic blebbing [4], LPS-MVs result solely from cellular activation, with no induction of cell death [4]. These LPS-MVs stimulate cultured endothelial cells to express ICAM1, VCAM1, and E-selectin. Importantly, LPS-MVs contain IL-1 β and components of the inflammasome, including the NACHT, leucine-rich repeat-containing, pyrin domain-containing protein 3 (NLRP3). Knockdown of NLRP3 in the parental monocytic cells reduced the activity of the LPS-MVs, and blockade of the IL-1 receptor on endothelial cells

decreased MV-dependent induction of cell adhesion molecules [97]. These processes appear to be distinct from the sterile inflammation provoked by UCMVs or plaque MVs [4].

Finally, there is at least one circumstance in which MVs produced by stimulated cells can preserve normal endothelial function. The human lymphoid CEM T cell line was treated with phytohemagglutinin, phorbol-12-myristate-13, and actinomycin D to induce activation, apoptosis, and MV production (CEMT-MVs). These CEMT-MVs contained the morphogen sonic hedgehog (Shh) on their surface. Importantly, T cell-derived MVs isolated from human plasma also contain Shh on their surface [80]. The CEMT-MVs preserve NO release by endothelium in vitro and in vivo through an interaction of Shh on their surface with the Shh receptor, Patched, on endothelium, acting mostly through PI3K [81].

These studies indicate that MVs from different cell sources under different conditions can alter endothelial function through a several distinct pathways.

Conclusions

Clinical and basic studies indicated that plasma concentrations of MVs might serve as a marker of endothelial dysfunction and of underlying processes leading to endothelial dysfunction, such as monocyte or macrophage apoptosis. In addition, MVs may contribute to endothelial dysfunction through several pathways, depending on the cellular source and the activation signals that lead to the production of MVs. For MVs to become a clinical biomarker, easy assays with high reproducibility need to be developed, and the predictive power of circulating MVs over conventional risk factors needs to be clarified. As a mediator of endothelial dysfunction, the extent of their contribution in vivo remains to be clarified, owing to the lack of animal models that have selective defects of MV generation or uptake, in which atherosclerosis, sepsis, and other conditions can be induced.

Activation or danger signals on MVs may participate in healthy inflammation. Nevertheless, by promoting inappropriate or maladaptive vascular tone, leukocyte recruitment, and thrombosis, MVs may be novel contributors to the development of cardiovascular diseases.

Acknowledgments

Sources of Funding: This work was supported by American Heart Association (AHA)-Great Rivers Affiliate Beginning Grant-In-Aid (to MLL), Temple University Department of Medicine Career Development Research Award (to MLL), and NIH-HL73898 (to KJW).

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Key points

- Microvesicles (MVs, also known as microparticles) are small membranous structures that have been found in blood and in atherosclerotic plaques.
- Plasma concentrations of MVs have been associated with major types of endothelial dysfunction – namely, inappropriate or maladaptive vascular tone, leukocyte recruitment, and thrombosis. The usefulness of MVs as novel biomarkers remains to be systematically evaluated.
- Microvesicles generated from human cells under different pathologic circumstances transport different sets of biologically active molecules and that alter endothelial function through distinct molecular pathways.