

REVIEW

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Endothelial extracellular vesicles: their possible function and clinical significance in diabetic vascular complications

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Abstract

Diabetic vascular complications attract increased attention due to their high morbidity, mortality and disability rate. Comprehensive and in-depth exploration of the etiology and pathogenesis of diabetic vascular complications is important for diagnosis and treatment. Endothelial extracellular vesicles (EVs) serve as potential intercellular communicators, transmitting biological information from the donor cell to the recipient cell, exerting both harmful and beneficial effects on vascular function. Endothelial EVs are new diagnostic and therapeutic targets and biomarkers in diabetic vascular complications. This review summarizes the biogenesis and release of endothelial EVs, as well as isolation and characterization methods, and discusses the role of endothelial EVs in the maintenance of vascular homeostasis along with their contributions to vascular dysfunction. Finally, the article illustrates the impact of endothelial EVs on the pathogenesis of diabetic vascular complications and evaluates their potential as therapeutic tools and diagnostic markers in diabetic vascular complications.

Keywords Extracellular vesicles, Endothelial cells, Diabetic vascular complications, Exosome, Microvesicles

Diabetes mellitus (DM) and its complications pose a serious threat to human health and have become a global public health issue [1]. Diabetic complications include macrovascular and microvascular complications, such as retinopathy, neuropathy, atherosclerosis, coronary artery disease, hypertension, and peripheral artery disease, which are the main causes of death and disability in patients with DM [2]. The pathology of diabetic complications has a high degree of commonality at the vascular level, mainly manifesting as endothelial dysfunction and atherosclerosis [3]. In the early 1980s, extracellular vesicles (EVs) were first defined as nanoscale bilayer phospholipid membrane vesicles released by cells, which can be divided into microvesicles, exosomes, and apoptotic bodies. As important mediators of intercellular communication, EVs influence the phenotype and function of target cells by transferring nucleic acids, proteins,

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lipids and other signaling molecules from parental cells to recipient cells, and involved in various physiological and pathological processes [4]. Virtually all cell types are known to release EVs. Endothelial is a rich source of EVs, and endothelial cell-derived EVs participate in maintaining vascular homeostasis and play a key role in diabetic vascular complications. This review outlines the biogenesis, release, characterization, and function of endothelial EVs, summarizes research progress in diabetic vascular complications, and evaluates their potential as disease biomarkers and therapeutic targets.

Endothelial cell-derived EVs

Endothelium has a rich vesicular network and is a major contributor to the EVs pool in the systemic circulation, accounting for approximately 5–15% [5]. The heterogeneity inherent to endothelium is reflected by their EVs released in circulation [6]. Endothelial cell-derived EVs deliver cargos such as lipids, proteins, mRNA, and miRNA in a paracrine manner. These cargoes may affect biological processes such as inflammation, apoptosis, proliferation, migration, and angiogenesis of recipient endothelial cells, thus regulating endothelial homeostasis.

Biogenesis and release of endothelial EVs

Endothelial EVs are released from endothelial cells upon activation or apoptosis. Various factors such as pro-inflammatory cytokines (TNF- α , IL-1, IFN- γ , LPS), thrombin, CRP, PAI-1, ox-LDL, and reactive oxygen species (ROS) can trigger the release of endothelial EVs into the circulation, causing blood vessel damage [7]. According to their different biogenesis and release mechanisms, endothelial EVs can be classified into three main subtypes: microvesicles, exosomes and apoptotic bodies. Microvesicles (about 100–1000 nm in diameter) are formed by direct outward budding from the plasma membrane. Exosomes (about 30–200 nm in diameter) are vesicles formed by inward invagination of the endosomal membrane and fusion of the multivesicular body (MVB) with the plasma membrane. The process of exosome biogenesis comprises four essential stages: cargo sorting, the formation and maturation of MVBs, the transportation of MVBs, and the eventual fusion of MVBs with the plasma membrane [8]. Initially, the cytoplasmic membrane invaginates, encapsulating various extracellular elements alongside cell membrane proteins, thereby forming early endosomes. These plasma membrane invaginations wrap some extracellular components and membrane proteins, leading to the formation of early sorting endosomes (ESEs). These ESEs can transfer their cargo to the Golgi apparatus and to the endoplasmic reticulum, or different ESEs fuse to form late sorting endosomes (LSEs), and eventually generate MVBs, which contain several intraluminal vesicles (ILVs). The MVBs can either fuse with

autophagosomes or lysosomes for degradation, or to fuse with the plasma membrane in order to release the contained ILVs as exosomes [9]. Numerous proteins play a role in exosome biogenesis and consist of Rab GTPases, endosomal sorting complex required for transport proteins (ESCRT), as well as other proteins that additionally serve as markers for exosomes (CD9, CD81, CD63, flotillin, TSG101, ceramide, and Alix). Apoptotic bodies are large clumps (about 1–5 μ m in diameter) formed during apoptosis by outward blebbing of the plasma membrane of cells [10]. (Fig. 1)

Isolation and characterization of endothelial EVs

In order to fully understand the function of endothelial EVs and their role in disease, emerging technologies need to be developed to isolate and characterize it. Commonly used EV isolation techniques mainly include ultracentrifugation-based methods for EV isolation, e.g., differential centrifugation, density gradient centrifugation; size exclusion-based EV isolation, e.g., ultrafiltration, size exclusion chromatography, asymmetrical flow field-flow-fractionation; EV isolation by precipitation, e.g., protein organic solvent (PROSPR) precipitation, polyethylene glycol (PEG) precipitation; as well as immunoaffinity capture-based EV isolation methods and EV isolation based on microfluidic technologies (Table 1) [11]. Each isolation method varying in yield and purity of EVs, labor-intensity, time-consuming, and cost [12]. Different isolation methods greatly impact the downstream analyses of EVs cargo and physicochemical properties.

After isolation, EVs need to be characterized by various methods to distinguish and classify the heterogeneous and intricate populations of EVs into more precisely defined subgroups based on their physicochemical and molecular properties. Characterization techniques for EVs encompass optical methods, such as nanoparticle tracking analyses, dynamic light scattering, and flow cytometry, as well as non-optical methods like resistive pulse sensing, transmission electron microscopy, and Raman spectroscopy [13]. Endothelial EVs can be characterized by a variety of specific markers, such as CD105-endothelin (endoglin), E-selectin (CD62E), S-endo (CD146), CD144-vascular endothelial cell cadherin (VE-cadherin), CD31-platelet cell adhesion molecule (PECAM-1), CD54-intracellular adhesion molecule-1 (ICAM-1), and von Willebrand factor [7]. However, apart from CD62E and CD144, most of these proteins are common to other cell types. CD144 is considered to be the most specific surface marker of endothelial EVs, but its sensitivity is not very high [14]. Hence, several markers need to be combined to assess the endothelial origin of EVs and exclude EVs of different origins. Depending on different types of endothelial cell damage, the surface antigen of endothelial EVs can exhibit different

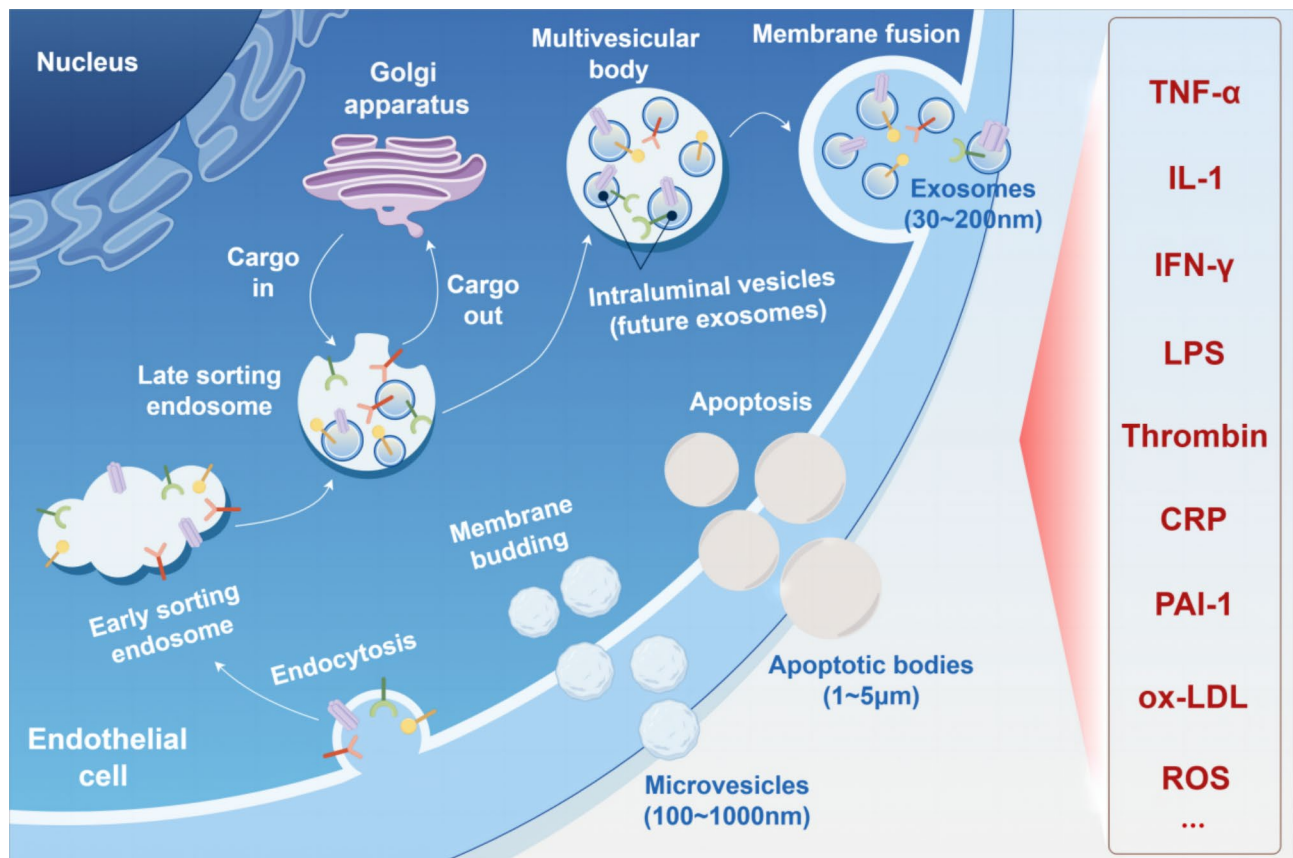


Fig. 1 Schematic representation of EVs biogenesis in endothelial cells (By Figdraw)

characteristics [15]. Higher levels of CD54-ICAM-1, CD62E-E-selectin, and CD106-vascular cell adhesion molecule-1 (VCAM-1) are carried on the surface of EVs released from activated endothelial cells. Additionally, these EVs express low levels of CD31-PECAM-1, CD105-endoglin, and CD144-VE-cadherin on their surfaces. Conversely, EVs released from apoptotic endothelial cells exhibit elevated levels of CD31-PECAM-1, CD105-endoglin, and CD144-VE-cadherin while displaying decreased levels of CD54-ICAM-1, CD62E-E-selectin, and CD106-VCAM-1 on their surfaces [16].

Beneficial effects and detrimental effects of endothelial EVs on vascular function

Detrimental effects of endothelial EVs on vascular function

Endothelial cells secrete a low proportion of EVs in physiological circumstances, but their release significantly escalates in pathological states such as diabetes or other conditions characterized by endothelial dysfunction [17]. Endothelial EVs serve as essential messengers for inflammation, coagulation, and angiogenesis, and their release is one of the main cellular responses to endothelial damage. High levels of endothelial EVs is considered as a key biomarker of vascular injury [18].

Certain stimuli or pathological conditions affect EVs released by endothelial cells in vivo and in vitro, impairing vascular function. By contrast, endothelial EVs derived from healthy subjects did not affect endothelial function. Multiple in vivo and in vitro studies showed that endothelial EVs contribute to coagulation, vascular inflammation, disruption of angiogenesis, in addition to other vascular effects. Exposed membrane phosphatidylserine (PS) and tissue factor (TF), along with a plethora of other surface molecules, conferring a procoagulant potential to endothelial EVs [19, 20]. However, recent studies have demonstrated that endothelial EVs can also expose endothelial protein C receptor (EPCR), express the urokinase-type plasminogen activator (uPA) and its receptor (uPAR), and exhibit anticoagulant properties [21]. Therefore, endothelial EVs play a role in regulating the balance between procoagulant and anticoagulant. EVs derived from different cell types are involved in vascular inflammation by activating endothelial cells, promoting monocyte adhesion and migration [22]. EVs released from oxidatively altered endothelial cells carry oxidized phospholipids that trigger inflammatory reactions in endothelial cells, resulting in monocyte adhesion [23]. Oxidized low-density lipoprotein (ox-LDL) may augment the release of endothelial EVs harboring elevated levels

Table 1 Comparison of different EV isolation techniques

Technique	Advantage	Disadvantage
Differential centrifugation	Simple to operate; Suitable for large batches of samples.	Many variations in g-forces and centrifugation times; Co-isolation of contaminants; Destroy EVs during high-speed centrifugation.
Density gradient centrifugation	Higher purity and yield.	Time-consuming and labor-intensive; Not suitable for high-throughput applications.
Ultrafiltration	Fast and cheap; Separate vesicles of different sizes; Greatly reduces the likelihood of EVs rupture.	Impure and often contaminated with molecules of a diameter similar to that of EVs; High pressure breaks larger EVs into smaller ones.
Size exclusion chromatography / Asymmetrical flow field-flow-fractionation	Fast; No co-isolation of protein and vesicle aggregates; Preserve biophysical and functional properties of the isolated vesicles.	Not suitable for large volumes.
PEG/PROSPR precipitation	Fast and cheap; Produce high-purity vesicle preparations suitable for downstream proteomic analysis.	High toxicity and reduced activity of PEG/PROSPR-derived EV preparations.
Immunoaffinity technology	High specificity and purity.	Higher cost; Low-yield; Limited by the antibody preparation technology.
Microfluidic technology	High sensitivity and specificity.	Some high-viscosity biological samples may block the microfluidic channels; Use small sample volumes.

of ICAM-1 that can be transferred to endothelial cells elsewhere, leading to increased monocyte recruitment in other regions and inducing endothelial inflammation [24]. Experiments conducted on cultured endothelial cells demonstrated a correlation between the release of endothelial EVs and the release of interleukin-6, implying a strong link between endothelial vesiculation and traditional inflammatory pathways involved in cytokine production [25]. Furthermore, endothelial EVs stimulate the secretion of chemokines, specifically monocyte chemoattractant protein 1 (MCP1) and IL-8, in a manner that depends on IL-1 α but is independent of IL-1 β ,

thereby contributing to inflammation [26]. Endothelial EVs can serve as paracrine mediators, inducing an increased release of soluble intercellular adhesion molecule-1 (sICAM-1) in immature endothelial cells and triggering inflammatory responses. Endothelial EVs have been shown to impair vasodilation and induce endothelial dysfunction by inhibiting nitric oxide (NO) production in target endothelial cells. This effect is accompanied by decreased endothelial nitric oxide synthase (eNOS) activity and increased local oxidative stress levels and superoxide production [27, 28]. Notably, the involvement of endothelial EVs in angiogenic response remains controversial. Previous studies have confirmed that EVs derived from endothelial progenitor cells (EPCs) trigger an angiogenic program in endothelial cells through the horizontal transfer of mRNA [29]. Endothelial cells participate in the regulation of angiogenesis by shedding vesicles containing matrix metalloproteinase-2, matrix metalloproteinase-9 and membrane type 1-matrix metalloproteinase [30]. The low concentrations of endothelial EVs promote angiogenesis in vitro, the high levels of endothelial EVs suppress angiogenesis [31].

In addition, endothelial EVs play an important role in the pathogenesis of atherosclerosis by transferring cell contents including miRNA, proteins, and other biomolecules [32]. Generation and abnormal miRNAs sorting of endothelial EVs induced by ox-LDL are considered to be associated with atherosclerotic events. Research indicated that paracrine cellular crosstalk between human coronary artery vascular endothelial cells (HCAECs) and human monocyte-derived macrophages (HMDMs) likely plays a role in promoting atherosclerosis in the presence of ox-LDL stress. It has been observed that EVs released by ox-LDL-stimulated HMDMs can impede the formation of capillary tubules in HCAECs. Additionally, the miR-4306 contained within EVs derived from ox-LDL-stimulated endothelial cells has been found to promote lipid accumulation in HMDMs by activating the Akt/NF- κ B signaling pathway [33]. Exosome-encapsulated miR-505 from ox-LDL-treated vascular endothelial cells exacerbates atherosclerosis through the promotion of neutrophil extracellular traps formation [34]. In vitro, ox-LDL and IL-6 stimulation upregulates the expression level of miR-92a-3p in endothelial EVs. The transfer of functional miR-92a-3p from endothelial EVs to recipient endothelial cells regulates angiogenesis through a mechanism that relies on thrombospondin 1 [35]. Besides, other bioactive molecules with an atherogenic role have also been detected in endothelial EVs. For example, exosomal LINC01005 from ox-LDL-treated endothelial cells promotes vascular smooth muscle cell phenotype switch, proliferation, and migration and regulates atherosclerosis by regulating the miR-128-3p/KLF4 axis [36]. Exosomal MALAT1 derived from ox-LDL-treated endothelial cells

initiated neutrophil extracellular traps formation, which in turn deteriorated atherosclerosis [37].

Beneficial effects of endothelial EVs on vascular function

Although endothelial EVs are contributors to pathology associated with endothelial dysfunction, increasing evidence highlights their beneficial effects on vascular function, including promoting angiogenesis and endothelial repair and inhibiting vascular inflammation. The release of EVs might be a way cells use to remove oxidized and potentially harmful molecules. For example, EVs release could protect endothelial cell apoptosis by diminishing levels of caspase-3 in endothelial cells [38]. Endothelial cell protection and repair are important processes in maintaining endothelial integrity and function. Endothelial EVs carrying the EPCR-activated protein C (APC) complex can induce protease activated receptor-1 (PAR1)-dependent cytoprotective and anti-inflammatory effects on endothelial cells, protecting the vascular endothelial barrier [39]. Quiescent endothelial cells suppress monocyte activation through secretion of EVs containing antiinflammatory microRNAs. In particular, miR-10a was transferred to monocytic cells from endothelial EVs

and inhibited the activation of the proinflammatory nuclear factor κ B pathway, thereby repressing inflammatory signaling [40].

In summary, endothelial EVs express a large repertoire of endothelial molecules and biological functions, significantly contributing to inflammation, thrombosis, and angiogenesis [41]. These paradoxical beneficial and deleterious effects can be influenced by factors such as the cellular origin, the functional status of the releasing cells, the intravesicular content, and the recipient cells [42]. The involvement of endothelial EVs in regulating vascular homeostasis might be much more complicated than initially thought, and their relevant biological effects still need to be further explored. (Fig. 2)

Endothelial EVs in diabetic vascular complications

Utilizing endothelial EVs as biomarkers for diabetic endothelial damage with prognostic values

Endothelin-1 (ET-1) is a potent vasoconstrictor peptide produced and released primarily by endothelial cells [43]. Overexpression of ET-1 has been implicated in the etiology of atherosclerotic vascular disease. Previous research showed that ET-1 induce an increase

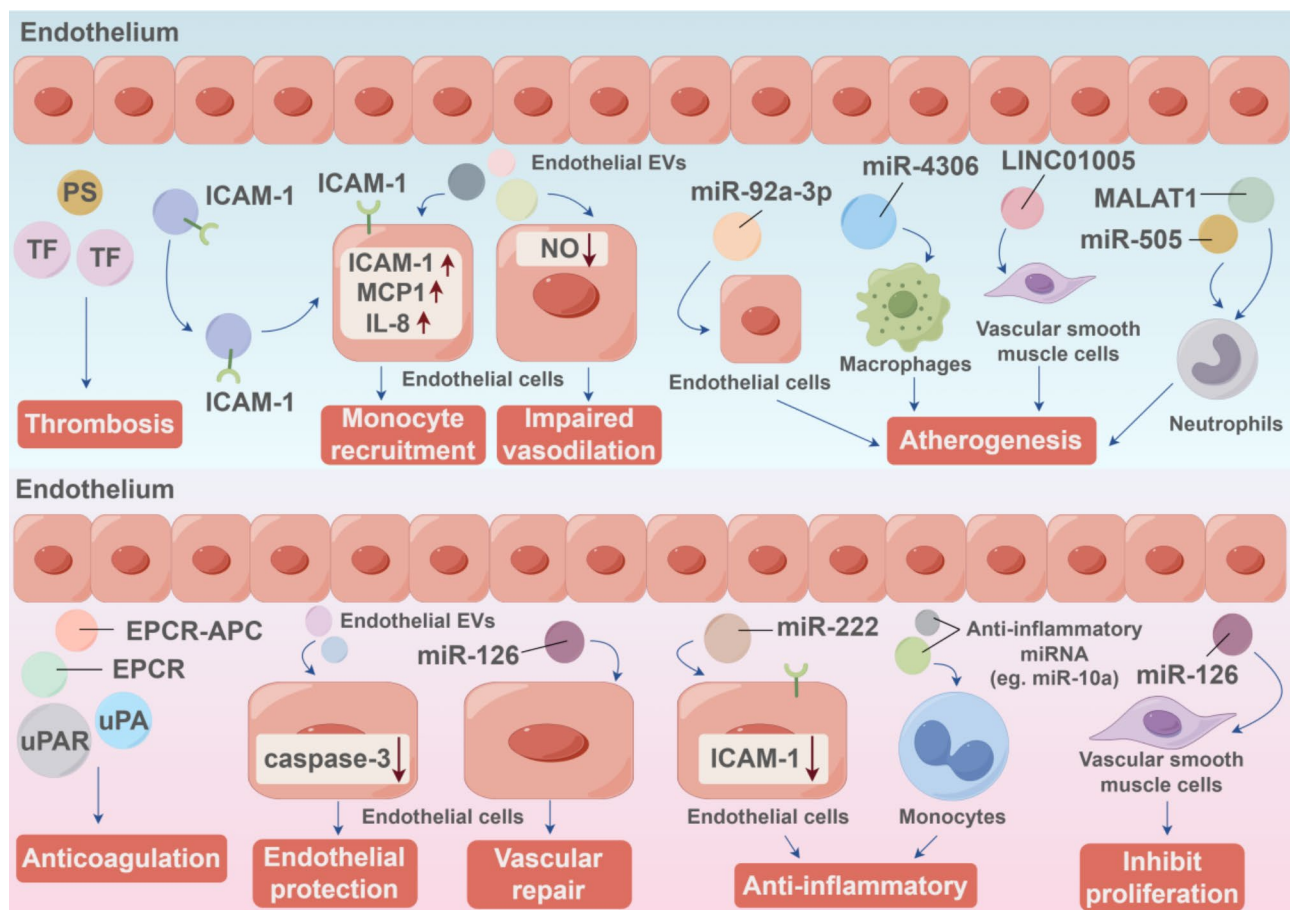


Fig. 2 Beneficial effects and detrimental effects of endothelial EVs on vascular function (By Figdraw)

in endothelial microvesicles release and significantly increase endothelial cell inflammation and reduce eNOS protein expression and activation, which contribute to the proatherogenic profile [44]. Studies have also shown that circulating endothelial cell-derived microvesicles can affect the production and release of ET-1. Endothelial microvesicles from e-cigarette users enhanced ET-1 production and adversely affected brain microvascular endothelial cells, leading to cerebrovascular dysfunction [45]. ET-1 is recognized as a biomarker of endothelial dysfunction. However, its sensitivity and specificity as prognostic biomarker for diabetic vascular complications are suboptimal.

Endothelial EVs have the potential to serve as excellent biomarkers, as their count, content and source could offer valuable insights into the pathophysiology of endothelial injury. Endothelial EVs help distinguish risk of microvascular and macrovascular complications of diabetes. Research found that the endothelial EVs (CD31⁺/CD42b⁻, CD31⁺/AV⁺) levels were higher in patients with macroangiopathy than in patients with microangiopathy and no complications and altered plasma endothelial EVs levels were independent risk factors for type 2 diabetes complicated by macrovascular complications [46]. CD51/CD41⁺ endothelial EVs levels correlated with albuminuria and microvascular complications (e.g., diabetic nephropathy) [47]. CD144-positive endothelial EVs can be found in human plasma, and these microparticles are linked to coronary endothelial dysfunction and injury in clinical settings. Patients with DM showed higher plasma levels of CD144 endothelial EVs compared to healthy individuals. The increased levels of CD144-positive endothelial EVs were identified as a noteworthy independent predictor for the development of coronary artery disease in diabetic patients [48]. Moreover, certain studies have reported an increased intravitreal concentration of CD144 endothelial EVs in diabetic patients suffered from retinopathy, in comparison to diabetic patients without this complication [49]. lncRNA-MIAT was demonstrated significantly increased in exosomes from high glucose induced human retinal vascular endothelial cell. Mechanistically, the pro-angiogenic effect of lncRNA-MIAT was via the lncRNA-MIAT/miR-133a-3p/MMP-X1 axis [50]. Hu et al. reported an increase in exosomal lncRNA LOC100132249 in the vitreous of patients with proliferative diabetic retinopathy. Human retinal vascular endothelial cells-derived exosomes promotes angiogenesis via an LOC100132249/miRNA-199a-5p/SNAI1/Wnt/ β -catenin axis in a pro-endothelial-mesenchymal transition manner, ultimately leading to diabetic retinopathy [51]. Diabetic nephropathy (DN) and diabetic retinopathy (DR) are the most common diabetic microvascular complications. Yang et al. employed quantitative proteomics and metabolomics to examine

the differential proteins and metabolites produced by exosomes in human glomerular endothelial cells from DR and DN patients to better understand exosome biomarkers for diabetic microvascular endothelial dysfunction. The results showed that fibrinogen overexpression and 1-methylhistidine loss may be linked to the pathogenicity of diabetic endothelial dysfunction in DR/DN [52]. Endothelial EVs, marked by CD62E, serve as potential prognostic markers for type 2 diabetes associated with asymptomatic atherosclerosis, offering an early biomarker for treatment [53]. Additionally, both diabetic and nondiabetic men experiencing erectile dysfunction (ED) were observed to have significantly elevated levels of CD62E endothelial EVs compared to nondiabetic men without ED [54].

Role of endothelial EVs in the pathogenesis of diabetic vascular complications

Endothelial EVs play a crucial role in the development and advancement of vascular complications in individuals with diabetes, such as endothelial injury, inflammation, vascular sclerosis, diabetic cardiomyopathy, diabetic nephropathy and diabetic foot, through the transmission of active biomolecules. High glucose (HG) act as a strong trigger for the creation of endothelial EVs, modifying their molecular composition and increasing their bioactivity. These changes could potentially result in continuous damage to the endothelium and the development of vascular issues linked to diabetes [55]. HG and advanced glycation end products (AGEs) have been shown to induce endothelial EVs generation and alter EV cargo sorting in endothelial cells. These endothelial EVs can promote apoptosis and dysfunction of recipient endothelial cells [56]. miRNAs carried by endothelial EVs play an important role in diabetes and its complications. Jansen F conducted a series of studies to explore the changes in EVs derived from endothelial cells treated with HG after simulating diabetic conditions. Endothelial EVs facilitate vascular repair by transferring functional miR-126 to recipient endothelial cells. Notably, under hyperglycemic conditions, endothelial EVs displayed decreased miR-126 levels and diminished repair capabilities both in vitro and in vivo [57, 58]. Increased vascular smooth muscle cells (VSMCs) migration and proliferation fosters neointimal hyperplasia resulting in arterial narrowing or occlusion [59]. Endothelial EVs reduce the proliferation and migration of VSMCs and inhibit vascular remodeling by delivering functional miR-126 to target VSMCs [60]. Under hyperglycemic conditions, the transfer of antiproliferative miRNA-126-3p from endothelial cells to VSMCs via endothelial EVs was markedly diminished. Consequently, the inhibitory impact of these endothelial EVs on vascular remodeling processes in vitro and in vivo was nullified [58]. Exosomal circHIPK3 derived from

mouse aortic endothelial cell enhances VSMC proliferation triggered by HG through the miR-106a-5p/Foxo1/Vcam1 pathway [61]. According to the study by Sáez and colleagues, exosomes released by endothelial cells and monocytes play a critical role in the activation of these cells under HG conditions, potentially contributing to inflammatory responses in individuals with type 2 diabetes or cardiovascular complications related to diabetes [62]. Endothelial EVs stimulate anti-inflammatory responses in vitro and in vivo by decreasing endothelial ICAM-1 expression through the transmission of functional miR-222 to recipient cells. Under hyperglycaemic pathology, the endothelial EV-mediated anti-inflammatory impact of miR-222 diminishes [63]. Furthermore, endothelial EVs prompt adhesion molecule expression in target endothelial cells and enhance inflammatory cell adhesion and infiltration within the endothelium. Endothelial EVs generated from HG exposure induce an increase in cell adhesion molecules (such as ICAM-1, VCAM-1, PECAM-1 and E-Selectin) on the surface of endothelial cells, heightening atherogenic potential and thrombotic risk [64]. Under pathological HG conditions, endothelial EVs stimulate the expression of adhesion proteins in endothelial cells and promote monocyte adhesion in a NADPH oxidase-ROS-p38-dependent way, promoting vascular inflammation and impairing endothelial function, which leads to atherosclerosis [65]. Endothelial EVs when exposed to HG levels trigger a pro-apoptotic endothelial state marked by heightened levels of intracellular active caspase-3 and lower expression of miR-Let-7a [66]. In vitro experiments showed that hyperglycemia reduced the expression of miR-126 and miR-26a in endothelial EVs. Among them, miR-126 can promote vascular regeneration and miR-26a can inhibit endothelial cell apoptosis [67]. The senescence of endothelial cells is intricately linked to the aging of VSMCs. Notch3 in high glucose-induced endothelial cells might be transported to VSMCs, subsequently triggering the calcification and aging of VSMCs via the mTOR signaling pathway [68]. Exosomes from hyperglycemia-stimulated vascular endothelial cells contain functional versican that is deliverable to VSMCs to promote VSMCs calcification/aging, probably by inducing mitochondrial dysfunction [69]. Under HG conditions, exosomes derived from endothelial cells triggered the senescence of VSMCs by circRNA0077930-miR622-Kras CeRNA network. Exosomes lacking circRNA-0077930 lost their capability to induce senescence in VSMCs [70]. Endothelial EVs were found to carry platelet-derived growth factor-BB (PDGF-BB), which was identified as a crucial factor mediating the resistance of VSMCs to apoptosis in diabetes [71]. Diabetic patients are with the higher risk of generating posterior capsule opacification than non-diabetics after cataract surgery. The miR-1246 was highly expressed in

high-glucose-treated vascular-endothelial-cell-derived EVs, regulated the epithelial-mesenchymal transition via miR-1246/GSK-3 β axis, and played an important role in the development of posterior capsule opacification in diabetes [72]. Diabetic bone marrow EPC-EVs enriched with HDAC1 protein, decrease histone 3 lysine 9 acetylation (H3K9Ac) by enhancing histone deacetylase (HDAC) enzymatic activity. This results in the transcriptional suppression of angiogenic, proliferative, and cell survival genes in the recipient cardiac endothelial cells, thereby impairing the cardioprotective function of EPC-derived EVs [73]. The exosome proteome is epigenetically regulated in type 2 diabetes associated vascular complications. Vasishta et al. demonstrated for the first time in in vivo and in vitro models of type 2 diabetes that DNMT1-mediated epigenetic regulation of endothelial cell-derived exosome proteins contributes to vascular dysfunction in the context of type 2 diabetes [74].

Endothelial cell paracrine effects vary among tissues and promote the development of complications such as diabetic cardiomyopathy, diabetic nephropathy, diabetic foot, and diabetes-related cognitive impairment. Mst1-enriched exosomes released from cardiac microvascular endothelial cells hinder autophagy and promote cardiomyocyte apoptosis in diabetes. Additionally, endothelial exosomal Mst1 disrupts the interaction between Daxx and GLUT4, leading to impaired glucose metabolism in cardiomyocytes [75]. HG-treated glomerular endothelial cells secrete a higher number of exosomes highly enriched in TGF- β 1 mRNA, activate glomerular mesangial cells through TGF- β 1/Smad3 signaling pathway, promote α -smooth muscle actin expression, proliferation and extracellular matrix protein overproduction in glomerular mesangial cells to promote renal fibrosis, and can also trigger podocyte epithelial-mesenchymal transition and podocyte dysfunction through Wnt/ β -catenin signaling [76, 77]. Tsai et al. purified exosome from proximal tubular epithelial cells (PTECs) treated with high glucose and db/db mice and found that PTEC-derived exosomal miR-92a-1-5p modulated the kidney microenvironment in vivo and in vitro models, which altered endoplasmic reticulum stress and myofibroblast transdifferentiation in mesangial cells resulting in DN progression [78]. Recent research has found the pathogenesis of diabetic nephropathy that involves the intercellular transfer of circRNA (such as circRNF169 and circSTRN3) from glomerular endothelial cells to glomerular mesangial cells via exosomes to promote renal fibrosis [79]. Following pretreatment with AGEs, endothelial EVs may exhibit an enrichment of miR-106b-5p. This, in turn, results in a reduction of collagen synthesis and a delay cutaneous wound healing. These effects are believed to be mediated by the induction of autophagy in fibroblasts through the downregulation of ERK1/2

expression, ultimately contributing to the development of diabetic foot ulcers [80]. A recent study indicated that the permeability of the blood-brain barrier and cognitive impairment were correlated with the reduction of tight junctions (TJ) in brain endothelium and pericyte coverage, as well as inflammation in cerebral microvessels and brain tissue paralleling hyperglycemia. EVs derived from diabetic mice were found to exhibit elevated levels of TJ proteins, occludin and claudin-5 [81]. EVs originating from brain endothelial cells of diabetic mice significantly hindered the generation of new neuroblasts by neural stem cells and impaired the cerebral endothelial function. This disruption led to cerebral vascular dysfunction and neurogenesis impairment, ultimately resulting in cognitive decline and an increased susceptibility to dementia [82].

Application of endothelial EVs in the treatment of diabetic vascular complications

While numerous endothelial EVs have been recognized as contributing to the development of diabetic vascular complications, there are also endothelial EVs that offer beneficial effects and can function as therapeutic agents for diabetes and its vascular complications [83]. For instance, EVs released by AGEs-stimulated human umbilical vein endothelial cells contain high levels of miR-126-5p, which inhibits vascular calcification linked to diabetes by suppressing the smad1/5/9 signaling pathway [84]. Underexpression of miR-146a upregulates cellular prion protein (PrP^c) production in type 2 diabetic mice. Delivering mouse brain endothelial cell-derived exosomes loaded with miR-146a into the ventricle of type 2 diabetic mice can down regulate PrP^c levels and restore short term memory function [85]. The MRI data indicated that administering exosomes, which are sourced from cerebral endothelial cells, to type 2 diabetic rats significantly reduced neurovascular dysfunction in gray and white matter as well as the hippocampus, and also enhanced cognitive functions compromised by type 2 diabetes [86]. Exosomes derived from human umbilical vein endothelial cells have exhibited encouraging outcomes in enhancing wound healing by modulating immune responses, reducing inflammation, promoting angiogenesis, accelerating skin proliferation and re-epithelialization, and regulating collagen remodeling to inhibit scar proliferation [87]. Xiang et al. designed a multifunctional acellular dermal matrix composite hydrogel containing endothelial cell exosomes for diabetic wound healing [88]. EVs derived from plasma endothelial cells have been found to inhibit fibroblast aging and expedite the healing of skin wounds in diabetic mice through mechanisms that involve reduced YAP phosphorylation and activation of the PI3K/Akt/mTOR pathway [89]. Exosomes derived from hypoxic

endothelial cells have shown promise in improving diabetic wound healing by enhancing endothelial cell function and supporting M2 macrophage activation via the lncHAR1B/BHLHE23/KLF4 axis [90]. EVs derived from brain endothelial cells contain high levels of miR-126 and promote neurorestorative effect in type 2 diabetic stroke mice [91]. miR-16-2-3p is upregulated in microvascular endothelial cell exosomes in patients with diabetic coronary microvascular complications and could alleviate coronary microvascular dysfunction in diabetes through regulating the fatty acid degradation of endothelial cells [92].

EPCs originate from bone marrow and possess the ability to transform into endothelial cells. In instances where endothelial cells incur damage, EPCs can potentially take their place to facilitate the restoration of endothelial function. Various recent research efforts have highlighted the significant role of exosomes derived from EPCs in relation to diabetes and its vascular complications. EPCs released EVs to transport miR-199a-3p for the purpose of suppressing specificity protein 1, thus preventing endothelial cell ferroptosis and retarding vascular endothelial damage, leading to the mitigation of atherosclerosis [93]. The EVs derived from EPCs contained the proangiogenic miR-126 and miR-296 and stimulated the PI3K-AKT and eNOS signaling pathways, enhancing insulin secretion, cell survival, and promoting islet angiogenesis [94]. The exosomes from EPCs derived from human umbilical cord blood have the ability to enhance the angiogenic activities of endothelial cells by activating the Erk1/2 signaling pathway. This ultimately improves the repair of diabetic wounds [95]. EPC-derived exosomes mediated miR-182-5p delivery accelerate diabetic wound healing by suppressing PPARG expression [96]. miRNA-221-3p, a highly expressed miRNA in exosomes derived from EPCs, enhances skin wound healing in diabetic mice by upregulating the protein levels of angiogenesis-related factors like VEGF and CD31, as well as the cell proliferation marker Ki67 [97]. Exosomes secreted by EPCs enhanced the expression of pro-angiogenesis molecules in vascular endothelial cells, such as FGF-1, VEGFA, VEGFR-2, ANG-1, E-selectin, CXCL-16, eNOS and IL-8, thereby promoting skin wound healing in diabetic rats [98]. Exosomes originating from EPCs contain various miRNAs linked to atherosclerosis. These miRNAs notably diminished diabetic atherosclerotic plaque, lowered the levels of ICAM-1, IL-8, and CRP inflammatory markers, reduced oxidative stress indicators such as MDA and SOD, and ameliorated thoracic aorta vasodilation and constriction dysfunction in a mouse model of diabetic atherosclerotic [99]. (Fig. 3)

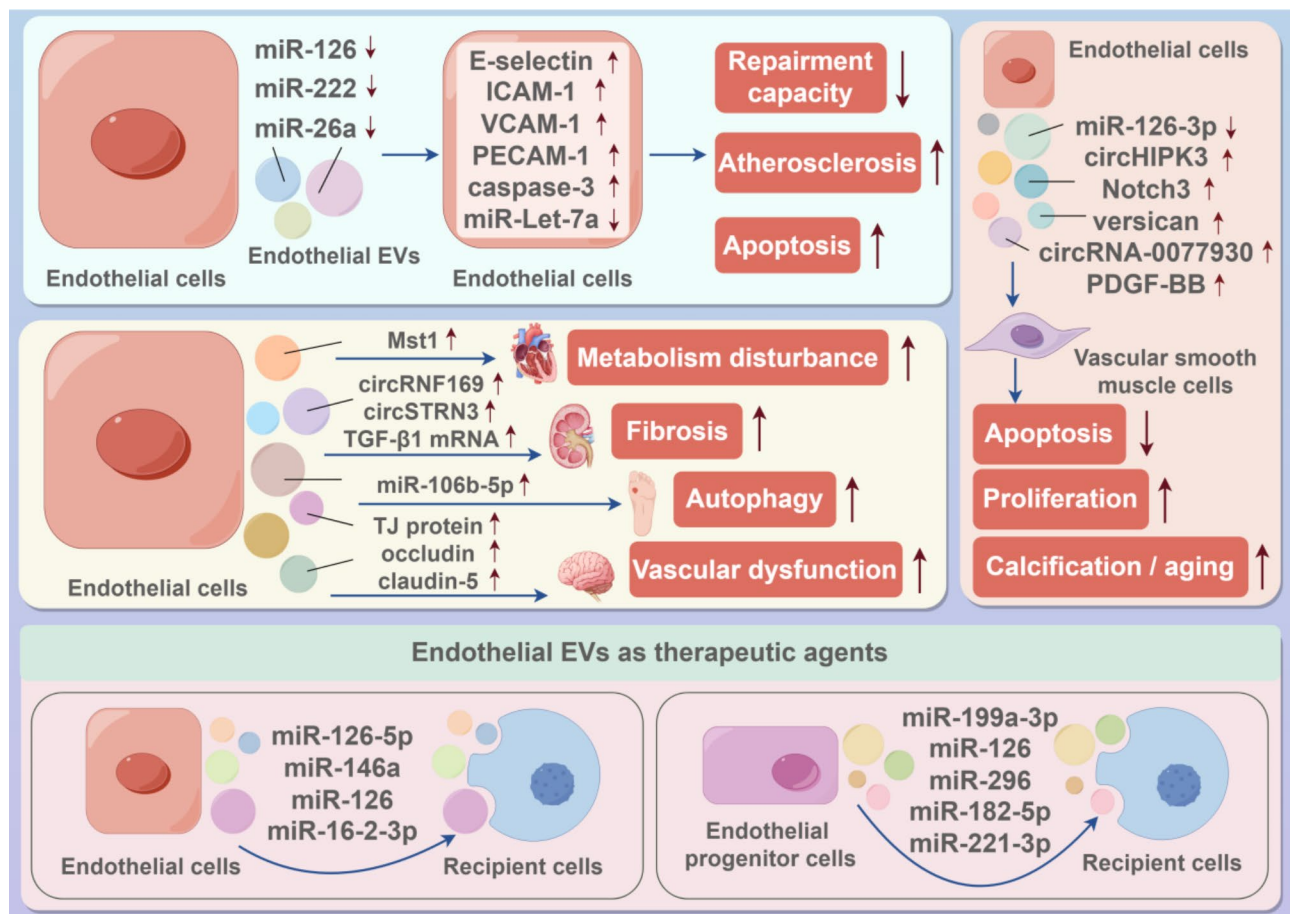


Fig. 3 Role of endothelial EVs in the pathogenesis and treatment of diabetic vascular complications (By Figdraw)

Summary and outlook

EVs, as an important means of intercellular communication, play a key role in both physiological and pathological conditions. Endothelium has a rich vesicular network, and intercellular signal transduction mediated by endothelial EVs exerts beneficial or harmful effects on the regulation of vascular health and disease. Endothelial EVs have the ability to induce endothelial dysfunction, vascular calcification, atherogenesis, plaque instability, and thrombosis, as well as exert favorable effects on endothelial and vascular health, atherosclerotic progression, and the stabilization of plaques.

Diabetic vascular complications impose a huge social and economic burden on patients with diabetes. Immediate action is required to prevent diabetic complications and reduce the risks of major organ damage, known as secondary prevention of DM. In recent years, increasing evidence has confirmed that endothelial EVs are key regulators of diabetic vascular complications and have the potential to become biomarkers and therapeutic targets. The production pathways and mechanisms of endothelial EVs differ significantly across various disease conditions, indicating that unique endothelial EV epitopes can aid in

identifying diabetic vascular complications. Additionally, lipids, proteins, miRNAs, and other non-coding RNAs, along with metabolites present in endothelial EVs, play a role in modulating the biological functions of recipient cells and contribute to the progression of diabetic vascular complications through multiple pathways. Notably, miRNAs have been implicated in the epigenetic regulation of essential metabolic homeostasis in diabetes and related vascular complications. A variety of miRNAs, including miR-126, miR-222, miR-26a, and miR-1246, are involved in regulating vascular homeostasis.

The pathophysiological role of endothelial cell-derived EVs and their cargo in diabetic vascular complications is increasingly recognized, elucidating their clinical potential in new treatment options. Endothelial EVs has raised great interest in the drug delivery field as carriers of functional RNA and proteins and can efficiently deliver cargo to recipient cells. Endothelial EVs have certain advantages as natural drug delivery carriers compared with synthetic carriers. For example, endothelial EVs have the intrinsic ability to cross tissue and cellular barriers, have strong targeting and tropism to specific cells or tissues, protects its biological cargo from degrading enzymes,

and is stable in circulation. However, several challenges remain in using endothelial EVs to treat diabetic vascular complications. First, clinical treatments require high purity of endothelial EVs. However, current isolation and purification technologies have many limitations, and new technical methods need to be developed to improve the purity of each vesicle subpopulation and further isolate endothelial EVs that contain only the intended cargo or target specific cells. Additionally, it is essential to standardize the classification, isolation, and purification of endothelial EVs in order to guarantee that EV analyses are reproducible and can be compared across different international research groups. Secondly, although studies have shown the therapeutic potential of endothelial EV as health regulator of endothelial dysfunction, clinical translation application is still lacking. The use of endothelial EV as drug delivery vehicle to treat diabetic vascular complications still requires further research and verification. Finally, the mechanisms responsible for the uptake of endothelial EVs and their vesicle contents by target cells are currently unclear, and further understanding of the complex roles of these vesicles is needed.

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Author contributions

Jiaxing TIAN developed the review question. The initial literature review was performed by Xinyi FANG, Yuxin ZHANG, and Yanjiao ZHANG. The first draft of the manuscript was written by Xinyi FANG, Yuxin ZHANG, and Yanjiao ZHANG with all authors commenting on subsequent versions of the manuscript. All authors read and approved the final manuscript.

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Competing interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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