Chapter

Part One: Extracellular Vesicles as Valuable Players in Diabetic Cardiovascular Diseases

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Abstract

Extracellular vesicles (EVs) are particles released in the extracellular space from all cell types in physiological and pathological conditions and emerge as a new way of cell-cell communication by transferring their biological contents into target cells. The levels and composition of circulating EVs differ from a normal condition to a pathological one, making them real circulating biomarkers. EVs have a very complex contribution in both health and disease, most likely in relationship between diabetes and cardiovascular disease. The involvement of EVs to the development of cardiovascular complications in diabetes remains an open discussion for therapists. Circulating EVs may offer a continuous access path to circulating information on the disease state and a new perspective in finding a correct diagnosis, in estimating a prognosis and also in applying an effective therapy. Besides their role as biomarkers and targets for therapy, EVs can be exploited as biological tools in influencing the different processes affected in diabetic cardiovascular diseases. This chapter will summarize the current knowledge about EVs as biological vectors modulating diabetic cardiovascular diseases, including atherosclerosis, coronary artery disease, and peripheral arterial disease. Finally, we will point out EVs' considerable value as clinical biomarkers, therapeutic targets, and potential biomedical tools for the discovery of effective therapy in diabetic cardiovascular diseases.

Keywords: extracellular vesicles, microvesicles, exosomes, diabetes, cardiovascular disease

1. Introduction

Lately, research has been increasingly focused on understanding of the biology of extracellular vesicles (EVs). Finding a more accurate name to define and classify EVs remains an open and, at the same time, a real challenge in the scientific world.

There are many reasons why it is difficult to find a very precise name for EVs: they are secreted by near all cell types in living organisms; the mechanisms through which they are released into the biological fluids are different and multiple; moreover, they have different sizes (30–2000 nm in diameter) which make the methods of obtaining and analyzing them to be diverse, but at the same time, some of them are slightly controversial. Once released from the cells, EVs are not inert particles, but they have complex functions in both physiological and pathological processes due to their specific cargo and factors stimulating their secretion. Thus, EVs are now viewed as early noninvasive biomarkers for various disorders in order to establish a correct diagnosis, but they also can be real targets for an effective treatment and, at the same time, valuable tools for treating several diseases such as diabetic cardiovascular diseases.

2. Terminology and biogenesis pathways of extracellular vesicles

EVs are a large term used to define a variety of membrane-limited vesicles involved in the intercellular communication. A nomenclature has been proposed but there are still numerous papers using different terms for EVs [1–3]. The EVs comprise different types of vesicles, and based on the size, morphology, and mechanism of biogenesis, they are largely classified as: **exosomes and ectosomes**, also referred as shedding microvesicles (MVs) or microparticles (MPs) [4].

As for the apoptotic bodies, the researchers' opinions are different; some of them think that they can be included in the EV category and others do not include them. Apoptotic bodies result from cells undergoing programmed cell death (apoptosis) and could be identified in EV probes [5]. The large cellular fragments resulted from apoptosis are phagocyted by neighboring cells and recycled; therefore, they should not be regarded as EVs involved in intercellular communication.

Exosomes (50–100 nm) have been described since 1980s as "exfoliated membrane vesicles," which may serve as a physiologic function occurring in many normal and neoplastic cells [6]. An ultrastructural study [7] showed that about 50 nm small vesicles are exocyted from multivesicular bodies (MVBs) after receptor-mediated endocytosis. For reticulocytes, exosomes' exocytosis determines the loss of transferrin receptors during red cell maturation [8].

MVBs (**Figure 1A–D**) of 0.5–1 μm large vesicles containing 2–50 small intraluminal smaller vesicles belong to the endolysosomal compartment. This pleomorphic cellular compartment comprises early and late endosomes where a highly controlled molecular sorting mechanism drives MVBs to the lysosomes or to the extracellular space. During endosome maturation into late endosomes, inward budding from the limiting membrane of the endosome leads to the formation of intraluminal vesicles in MVBs [9]. Usually, MVBs fuse with lysosomes, the terminal compartment of the endocytic pathway, where they are digested and the final components are recycled. Some MVBs can fuse with the plasma membrane and their intralumenal vesicles are released from cells as exosomes. The process by which the fate of endosomal content is determined is not fully understood [10, 11]. Accumulating evidence suggests that the release of EVs often serves as an alternative disposal pathway to the overloaded lysosomes [12, 13]. This mechanism may be involved in a vascular calcification [14].

It is demonstrated that exosomes are not only cell specific but also they carry RNAs between cells and play major roles in intercellular communication [15]. How

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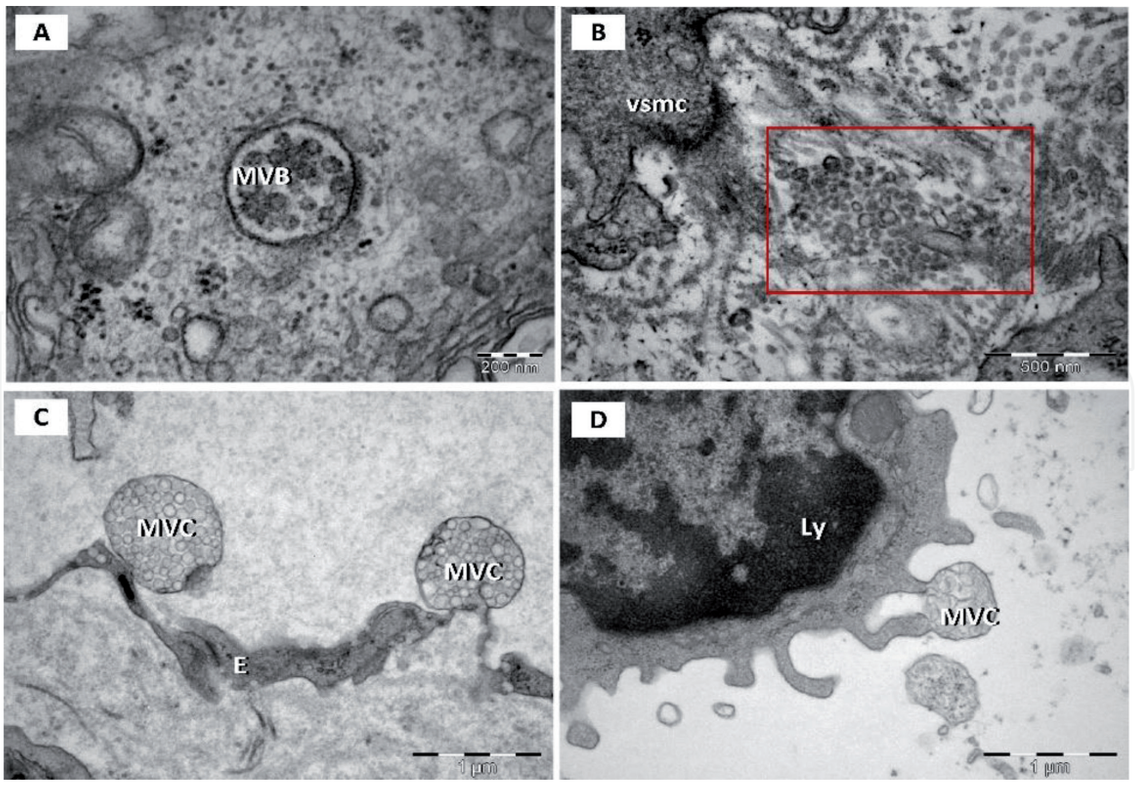

Figure 1.
Transmission electron microscopy of the extracellular vesicles in diabetic kidney. (A) Multivesicular body (MVB) with intraluminal vesicles in the cytoplasm of endothelial cell. (B) Numerous extracellular vesicles (square area) present between vascular smooth muscles cells (VSMCs) in vascular media. (C) Multivesicular cargos (MVCs) released by an endothelial cell (E) into the lumen of a peritubular capillary. (D) Multivesicular cargos (MVCs) released by a circulating lymphocyte (Ly).

RNAs reach the MVB vesicles is not clear, but it is supposed that cytosolic RNAs are taken up into intraluminal vesicles undergoing inward budding from the limiting membrane of the MVBs [9, 16].

Ectosomes (MVs or MPs) are slightly larger vesicles (100–500 nm) compared with exosomes and are also cell specific as they are released from plasma membrane by budding. Ectosomes do not require exocytosis as they are generated by outward budding of a plasma membrane domain, which enclose a cargo gathered at the cytosolic face. The detachment of the ectosomes from the donor cells involves contraction of cortical actin beneath the plasma membrane [17]. These plasma membrane-derived vesicles are also reported to carry RNAs and proteins as an effective mechanism for intercellular communication.

Multivesicular cargos (**Figure 1A–D**) have also been described as EVs with a particular appearance: clustered vesicles (80–200 nm) shielded by plasma membrane [18]. This type of EVs has been described as mediating bone mineralization [19], vascular calcifications [20], or intercellular communication between telocytes [18], which often surround the vessels [21]. In our experience, endothelial cells (ECs) from diabetic kidney often release multivesicular cargos in the vascular lumen (**Figure 1C**). Possible mechanism of multivesicular cargo biogenesis based on electron microscopy images [18, 19] involves an initial aggregation of vesicles in the cortical cytoplasm which further will bulge a segment of the plasma membrane. Finally, gathered vesicles are released into the extracellular space as a cargo shielded by plasma membrane. The dissolution of the shielding membrane of the multivesicular cargo will release individual or grouped cytoplasmic-derived vesicles into the extracellular space.

3. Function of extracellular vesicles in physiology

3.1 Physiological role of extracellular vesicles

EVs are connected to different physiological and pathological processes, such as tumor growth modulation, cytokine production, or cardiovascular disorders [22–24].

EVs contain lipids, and pools of proteins, some specific for the cell type generating them—MHC class I and II, and some which are present in most EVs—proteins from the plasma membrane, cytosol, and endosome. This latest feature suggests the shared biogenesis pathway for these EVs. On the surface of EVs, proteins similar with the ones from the originating cells can be found [25–28]. Different types of nucleic acids such as DNA, small RNA, ribosomal RNA (rRNA), microRNA (miRNA), long noncoding RNA (lncRNA), and mRNA are enclosed within the EVs, which transfer their content into recipient cells, inducing transient or persistent phenotypic changes, which will modify their cellular functions.

According to Barros et al. [25], there are at least four mechanisms by which the EVs can influence the target cells: (1) direct contact between the proteins from the target cell and EV membrane, which changes the intracellular signaling of the recipient cells; (2) cleavage of proteins on the EVs' surface and further interaction between the protein fragments and receptor-proteins on the recipient cell; (3) fusion between EVs and target cell membrane, followed by EV content release within the recipient cell; and (4) internalization of EVs by phagocytosis or endocytosis.

3.2 Role of extracellular vesicles in immunological response

The immune response involves participation of innate and adaptive immune system to regulation of body homeostasis, defense, and surveillance, thus maintaining the equilibrium between health and disease.

3.2.1 Activation of the helper T cells (CD4+)

Molecules of MHC class II complex are specific to antigen-presenting cells (APCs), such as dendritic cells (DC), macrophages, and B lymphocytes, which present internalized exogenous peptides for the activation of CD4+ T cells. B cells release functional EVs with increased amounts of MHC class II molecules coupled with peptides, which are able to generate T helper cell response. T cells are strong stimulators of the EVs' synthesis by B cells due to activation of CD40, and IL-4 receptors [29–31], and the B cell-derived EVs also contain molecules of MHC class I, components of B cell receptor (BCR)—CD19, immunoglobulins, and tetraspanins [30, 31]. Content of EVs derived from DC, with MHC class II—peptide complexes, contributes to amplification of adaptive immune response [32–34].

3.2.2 Activation of the cytotoxic T cells (CD8+)

Because all nucleated cells express MHC class I molecules, the nucleated cells-derived EVs contain the MHC class I—endogenous/exogenous antigens complexes, thus giving the potential to activate the cytotoxic T cells [35]. These findings were confirmed for the first time by Admyre et al., who demonstrated that monocyte-derived DC released exosomes capable of inducing antigen-specific immune response from peripheral blood-isolated CD8+ T cells [36].

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3.2.3 Immunomodulation induced by EVs

The production and release of EVs presenting on the surface factors which are capable of triggering apoptotic pathways, such as Fas ligand or galectin 9, can induce immunosuppression. On the other hand, platelet-secreted EVs can induce secretion of pro-inflammatory cytokines, such as IL-8, IL-1β, and IL-6, thus triggering an inflammatory immune response [37].

4. Extracellular vesicles as biological vectors modulating diabetic cardiovascular diseases

4.1 Role of extracellular vesicles in coronary artery disease

Individuals with type 2 diabetes mellitus develop cardiovascular disorders, including coronary artery disease, more frequently than healthy controls, mainly through the chronic, damaging exposure of the vascular system to hyperglycemia. Therefore, it is important to understand the exact mechanisms through which diabetes contributes to the development and severity of these complications.

EVs generated in patients with diabetes mellitus promote inflammation and contribute to the development of atherosclerotic lesions, stimulating monocyte adhesion and their infiltration in the subendothelial layer, promoting the migration and proliferation of vascular smooth muscle cells (VSMCs) and also the calcification of the atherosclerotic plaque.

4.1.1 Extracellular vesicles and the coronary atherosclerotic plaque

Recent studies have shown that atherosclerotic lesions of all stages contain MVs. Higher levels of circulating MVs have been discovered in individuals with cardiovascular risk factors, such as smoking [38], dyslipidemia [39], diabetes mellitus [40], and arterial hypertension [41], probably through activation or from apoptosis of different cells being exposed to a damaging stimulus. Data extracted from in vitro studies suggest that MVs can have both pro-inflammatory and anti-inflammatory effects, depending on the stimulus that induces their formation [42]. MVs increase the release of proinflammatory cytokines, mainly interleukin-6 and -8 (IL-6 and IL-8), from ECs and leukocytes, promoting the adhesion of monocytes to the endothelium and their migration to the atherosclerotic plaque [42, 43]. Also, endothelial MVs can activate monocytes by transferring miR-10a and thus interfering with the nuclear factor-κB inflammatory pathway. Another effect of MVs is the decrease of the nitric oxide (NO) production by ECs, consequently impairing endothelial vasodilating properties [44]. Endothelial-derived MVs and platelet-derived MVs increase endothelial permeability by delivering two enzymes (caspase 3 and Rho-kinase) to target cells and inducing apoptosis [45]. MVs promote adhesion of monocytes to the endothelium by increasing endothelial expression of adhesion molecules, such as intercellular adhesion molecule-1 (ICAM-1), or adhesion molecule receptors, such as CD11a, on monocytes [46]. ICAM1 expression can also be regulated by miR-222 in MVs [42, 47].

Various MVs contribute to foam cell formation in the atherosclerotic plaque by stimulating lipid and cholesterol formation in macrophages. Macrophages and foam cells undergo afterward apoptosis, forming a core of extracellular lipids. Increased monocyte and macrophage apoptosis contributes to augmented MV release in the plaque. MVs of monocyte and macrophage origin are the largest population of MVs in human atherosclerotic lesions [48].

Infiltration of LDL particles in the vascular wall during the atherosclerotic process can induce the formation and release of tissue factor-enriched MVs from the VSMCs, which in turn influence VSMC proliferation and migration [49].

EVs of different origins, with different miRNA content, contribute to VSMC proliferation and migration; for example, MVs with miR-223 induce a decrease in atherosclerotic plaque size by inhibiting VSMS proliferation and migration, while endothelial vesicles with miR-143 and miR-145 prevent VSMC dedifferentiation [50].

4.2 Role of extracellular vesicles in peripheral arterial disease

4.2.1 Atherosclerosis and vascular calcification in diabetes mellitus

Adipose tissue-derived EVs were shown to have immunomodulatory effects on macrophages which in turn, after contacting said EVs, secreted factors that interfered with insulin signaling in human adipocytes [51]. Moreover, EVs released from adipocytes from obese individuals act in a paracrine manner on neighboring adipocytes and impair insulin-mediated glucose transport [52]. In turn, the exosomes derived from insulin resistant adipocytes carry sonic hedgehog (SHH) protein that increases the expression levels of TNF-α, IL-1β, IL-6, VEGF-A, ICAM-1, MMP2, and MMP9 in the atheroma plaque and promotes vasa vasorum angiogenesis, leading to greater plaque burden and vulnerability [53]. Thus, EVs provide a link between obesity, low-grade inflammation, insulin resistance, and atherosclerosis progression.

EVs also play a key role in the cross talk between ECs and macrophages that can either act in the direction of vascular homeostasis or promote atherosclerosis, depending on their composition. It was shown that EVs secreted by Kruppel-like factor 2-treated ECs show anti-inflammatory actions, while oxidized-LDL-treated ECs produce EVs with high levels of miR-155, directing macrophage differentiation toward pro-inflammatory M1 macrophages [54]. In M1, but not in M2 macrophages, the inflammasome is known to be activated [55] and the inflammasome signaling leads to the secretion of pro-inflammatory exosomes [56], further favoring atherosclerosis progression. Furthermore, atherosclerotic patients have high numbers of monocyte/macrophage-derived miR-150-rich EVs that enhance EC migration, a major component of atherosclerosis [57]. Thus, circulating endothelial microparticles (EMPs) were shown to be an independent risk factor for endothelial dysfunction which occurs early in atherosclerosis, and the fact that in type 2 diabetes mellitus their number is increased [58] and their miRNA composition is altered containing miRNAs mainly involved in angiogenesis [59] proves the involvement of EVs in cardiovascular complications of diabetes mellitus.

However, exosomes from other sources can alleviate diabetes mellitus as shown at rats treated with exosomes from human umbilical cord derived multipotent mesenchymal stromal cells (MSCs) that have the ability to reverse peripheral insulin resistance and relieve β-cell destruction [60].

Atherosclerosis, old age, diabetes, and hyperphosphatemia induce VSMC transdifferentiation to osteoblasts [61] characterized as loss of SM22a and SMA markers and gain of Runx2, SP7, osteopontin, osteocalcin, alkaline phosphatase (ALP), transcription factor Sox9, collagen II, and collagen X [62]. These trans-differentiated cells secrete 50–150 nm calcium-phosphorus-rich exosomes that serve as calcification nuclei, in the same manner that osteoblast-secreted matrix vesicles lead to bone mineralization [63]. However, extracellular calcium leads to Fetuin-A uptake in VSMCs mediated by annexins, and high Fetuin-A levels in secreted exosomes prevent further calcification [64]. This control mechanism is affected when Fetuin-A

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levels are low due to chronic dialysis [65] and higher than normal plasma Fetuin-A levels can lead to insulin resistance and diabetes through the direct inhibition of the insulin receptor [66], thus only worsening the cardiovascular diseases (CVDs).

EVs found in intima and media of calcified vascular wall (**Figure 2A–D**) [14] seem to be different of matrix vesicle with role in physiological and pathological calcification [19]. Vascular cell-derived EVs may serve as a continuous source of damaging microcalcifications in atherosclerotic plaques [20]. These vesicles have been described as exosomes from endosomal compartment, plasma membrane-derived ectosomes, and vesicles shielded by a plasma membrane (multivesicular cargos) that are released into extracellular space as a cargo [14]. As the first two types are intense investigated and described, the last type derived from multivesicular cargos is less investigated.

4.3 Role of extracellular vesicles in insulin resistance

The prevalence of type 2 diabetes is rapidly increasing worldwide, in parallel with the current obesity epidemic, posing a major healthcare expenditure burden. Obesity increases the risk for development of diabetes, cancer, and CVDs. In the course of events leading from obesity to type 2 diabetes, several mechanisms are currently outlined. Among them, low-grade systemic inflammation in adipose tissue of obese persons has been proposed as a possible link between insulin resistance and altered functions of vascular cells by increased cytokines production. Furthermore, it has been shown that the molecules that are released by adipose

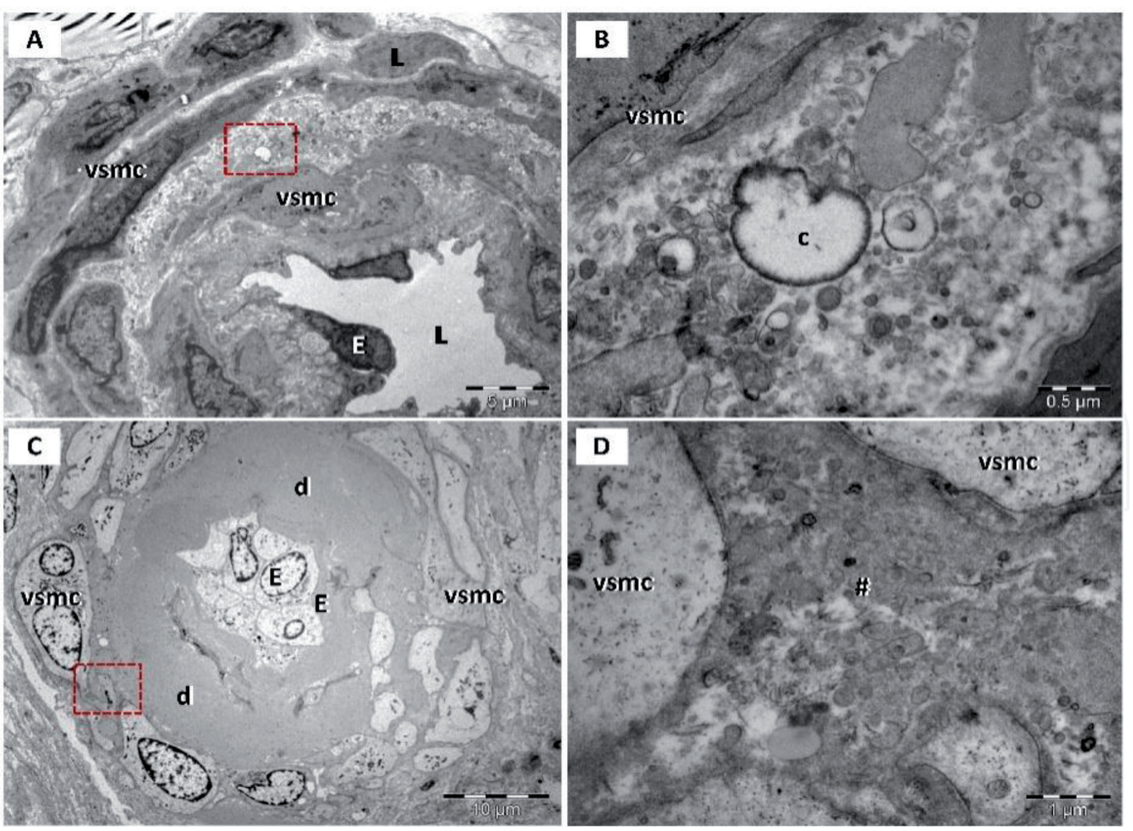

Figure 2.
Transmission electron microscopy of the diabetic arteries in diabetic kidney. (A) Large spaces with vesicular content [square marked area in (A) is magnified in (B)] increase the vascular wall thickness. (B) Numerous extracellular vesicles accumulated between vascular smooth muscles cells (VSMCs) and small calcifications (c) may be seen. (C) Concentric amorphous deposits (d) are located between endothelium (E) and vascular smooth muscle cells (VSMCs). (D) Higher magnification of square marked area in (C) shows also numerous vesicles in the extracellular space (#) between vascular smooth muscle cells (VSMCs).

tissue cells into circulation are enclosed in vesicles. EVs derived from adipose tissue may play a role in the paracrine cross talk between adipocytes and macrophages in adipose tissue in obesity [51], and in endocrine manner for transmission of signals to other cells from cardiovascular system [67]. There are the studies that support the idea that EVs are important mediators for metabolic organ cross talk. Thus, it was hypothesized that insulin-secreting beta (β) cells and insulin-sensitive tissues release exosomes that can be transferred to other metabolic organs, or to immune or endothelial cells. In this way, in an autocrine or paracrine manner, exosomes influence glucose homeostasis and insulin resistance [68].

When circulating miRNA profile of lean and obese individuals was compared, those miRNAs differentially expressed were predicted to regulate inflammatory and fibrotic signaling pathways [69]. Moreover, in obesity, exosomes from adipose tissue-derived MSCs have reduced pro-angiogenic properties due to decreased content in miR-126, VEGF, and MMP-2. A differential EV proteomic profile has also been observed between obese diabetic and obese nondiabetic rats [70]. In a recent study, the lean mice treated with exosomes from obese mice developed glucose intolerance and insulin resistance. In addition, using exosomes transfected with a specific siRNA targeting PPARα, the phenotype induced by obesity-associated miRNAs was recapitulated. Importantly, it was demonstrated that obesity-associated exosomal miRNAs modulate glucose and lipid metabolism in mice [71].

In type 1 diabetes, the imbalances between effector T cells and regulatory T cells, as well as dendritic cell presentation of islet auto-antigens, play an important role in the destruction of islet β cells. It has been shown that MVs derived from endothelial progenitor cells (EPCs) combined with islets can activate angiogenesis, decrease leucocyte-endothelial interaction, and improve pancreatic β cell function [72]. Another study revealed that insulinoma-released exosomes or MPs are immunostimulatory and can activate autoreactive T cells spontaneously developed in non-obese diabetic mice [73]. Exosomes could also serve as triggering factors for specific autoimmunity events leading to diabetes, as shown in another study where in NOD mice exosomes released by islet-derived MSCs trigger autoimmune responses [74]. Thus, specific biological roles of EVs are dependent on functional state and the type of cells from which the EVs are released.

5. Extracellular vesicles as clinical biomarkers, therapeutic targets, and biomedical tools in diabetic cardiovascular diseases

5.1 Extracellular vesicles as clinical biomarkers

Early recognition of prediabetes and diabetes is critical for the prevention or the successful treatment of diabetes-induced cardiovascular complications.

The traditional markers used in clinical practice, such as glycated hemoglobin and glucose determinations, are detected only when diabetes is already established and cannot precisely predict an individual's risk of developing diabetes [75].

Biomarkers for early detection of the disease and identification of individuals at risk of developing complications would greatly improve the care of diabetic patients.

The study of EVs is opening new horizons for their potential application not only as therapeutic tools but also as clinical biomarkers for monitoring disease progression. Even if most clinical data are derived from the studies of tumor patients, increased levels of EVs have been detected in body fluids in a variety of cardiovascular and inflammatory pathologies, obesity, atherosclerosis, diabetes,

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and metabolic syndrome—biomarkers of both incidence and progression diabetic retinopathy in diabetic patients.

Owing to their association with the onset, progression, and pathogenesis of type 2 diabetes, EVs are emerging as a new and attractive class of biomarkers for prognosis, diagnosis, progression/severity, and management of diabetes.

EVs are detectable in most of the body fluids, including blood, and their expression pattern appears to provide valuable information about the functional state of their parental cells [76].

In the study by Sun et al. [77], levels of urinary CD63-positive exosomes were found increased at the early stage of renal injury in diabetic nephropathic subjects.

On the other hand, circulating MPs, in particular platelet-derived microparticles (PMPs) and EMPs, have been found elevated in a wide range of thrombotic disorders, with an interesting relationship between their levels and disease pathophysiology, activity, or progression [78, 79]. EMP plasma levels have been associated with several CVDs and risk factors. Circulating PMPs are also shown to be involved in the progressive formation of atherosclerotic plaque and development of arterial thrombosis [80, 81], especially in diabetic patients [59]. Indeed, diabetes is characterized by an increased procoagulant state and by a hyperreactive platelet phenotype, with enhanced adhesion, aggregation, and activation. Elevated MP levels, such as TF-positive MPs, have been shown to be one of the procoagulant determinants in patients with type 2 diabetes mellitus [82]. Also, it was demonstrated that EVs participate in the transport of cytokines and angiogenic factors in diabetic patients with ocular complications [83]. Moreover, a recent study showed that distribution of pro- and anti-angiogenic miRNAs in patients with type 2 diabetes is in close touch with the upregulation or downregulation of miRNAs in the plasma fraction enriched in ectosomes (MVs or MPs) [84]. This topic has been widely discussed in a paper by Alexandru et al. [84], in which MPs and MPs-associated miRNAs were presented as active players in vascular complications in diabetes.

More than that, since urinary EVs (UEVs) have been described in diabetic nephropathy (DN), they immediately became to be proposed and a biomarker in kidney complication [85, 86]. Patients with DN have exceptionally high rates of cardiovascular morbidity and mortality; thus, there is an emerging need to find the link between the risk of DN and CVD progression.

Owing that urine is an easily accessible fluid sample, UEVs can be obtained in bulk, which make them emerging as a valuable source for disease stage-specific molecular signatures potentially useful in diagnostics. Therefore, UEVs has been proposed to be a novel biomarker in diagnostics, prognosis, and disease progression in diabetic kidney complications [87, 88].

Similar to CVDs, in DN, the profile and concentration of proteases and proteases inhibitors is changing in UEVs. For example, Musante et al. [89] have found that cathepsins (A, C, D, L, and XZP) are significantly increased in UEVs isolated from DN patients. Cathepsins are included to the class of lysosomal proteases and their proteolytic activity is related to ECM remodeling [90]. The proteomic study confirmed that in diabetic UEVs, serine proteases and their inhibitors, including SERPINA1 and SERPINA3, are present [90].

Besides protein cargo, also miRNA UEVs content have some specific features, strongly related to CVD pathomechanism. Barutta et al. showed a differential expression of 22 exosomal miRNAs between micro- and normoalbuminuric patients with DN [91]. Among them, miR-130a has been found to play a critical role in cardiac fibrosis by directly targeting peroxisome proliferator-activated receptor-γ (PPAR-γ) [91]. Interestingly, miR-155 was significantly reduced in UEVs from DN patients. This miR is significantly expressed and secreted in Krüppel-like factor 5

(KLF5)-overexpressing VSMCs and it is considered as a potent regulator of endothelium barrier function through regulating endothelial targeting tight junction protein expression. In murine model of atherosclerosis, VSMCs-derived exosomes mediated the transfer of miR-155 from VSMCs to ECs, which led to an increased endothelial permeability and enhanced atherosclerotic progression [92]. These data suggest the possible role of UEVs in kidney remodeling, which can bring the new insight into vascular complications and vascular risk in diabetes.

5.1.1 Therapeutic potential of extracellular vesicles

According to results from studies from the last 5 to 10 years, EVs could play an important role in different cardiac regenerative therapies and could also be used as therapeutic targets and vectors in cardiovascular medicine.

Platelet-derived vesicles induce vascular endothelial growth factor (VEGF)-dependent angiogenesis and stimulate postischemic revascularization after chronic ischemia [93]. Also, plasma-derived exosomes activate Toll-like receptor 4 on cardiomyocytes and thus protect the myocardium from ischemia-reperfusion injury [94]. MSCs-derived EVs could be an alternative to stem cell transplantation after myocardial ischemia by transfer of specific miRNAs through embryonic stem cell EVs [95].

Different cardiovascular medications can influence the level of circulating MVs. Antiplatelet agents (ticlopidine and abciximab) inhibit platelet activation and also the release of platelet-derived MVs [96–98]. Antihypertensive agents (such as angiotensin II receptor inhibitors, beta blockers, and calcium channel blockers) lower the circulating levels of platelet- and monocyte-derived MVs [99]. The effects of statin treatment on circulating MVs of platelet and endothelial origin are still unclear [100, 101].

Statins and antihypertensive medication are able to modify the properties of in vivo-generated endothelial MVs and their effect on the expression of endothelial adhesion molecules, inhibiting the adhesion of monocytes to ECs and improving endothelial function [102].

In other words, MVs are now regarded as novel therapeutic targets to monitor the therapeutic response to treatments in diabetic macrovascular complications. The beneficial effects of several drugs, such as statins, antiplatelet agents, antioxidants, angiotensin II receptor blockers, and calcium-channel blockers, have been reported to be partially due to their effects on reduction of both MV numbers and/or procoagulant factors [103]. Moreover, the cardiovascular benefits of antihyperglycemic drugs used to treat type 2 diabetic patients, such as, glibenclamide [104], acarbose [105], miglitol [106], and gliclazide [107], might be at least partially attributed to the anti-atherothrombotic effects of medication, through the decrease of procoagulant MV levels and platelet-activating factors. Pioglitazone treatment reduced the level of circulating endothelial-derived-MVs and increased the level of EPCs and the endothelial-derived MVs/EPCs ratio, improving the imbalance between endothelial damage and repair capacity [108]. Moreover, in our studies on atherosclerotic animal model and patients with hypertension and dyslipidemia, we showed that administration of irbesartan, an AT1 receptor antagonist, decreases the levels of circulating MVs, and also of specific MVs (endothelial-, platelet-, and leukocyte-derived MVs), and increases EPC levels, preventing the appearance of vascular endothelial dysfunction [78]. The mechanisms underlying this response include the reduction/increase of a number of specific membrane receptors exposed by MPs (TF, P-selectin, E-selectin, PSGL-1, Rantes), respectively, by EPCs (β2-Integrins and α4β1-integrin), the augmentation of endothelium-mediated

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vasodilation and the reduction of protein expression of VEGF/stromal cell-derived factor-1α (SDF-1α) [109].

In addition to their role as drug targets, EVs are an attractive drug delivery vehicle. The use of EVs as therapeutic vectors could be done through bioengineering, either by modifying the cytosolic content of the vesicles which could be transferred to the target cells in order to influence cell metabolism, or by loading of EVs with molecules to be delivered to target cells. Studies regarding the use of EVs as therapeutic vectors in CVDs are few and are only on animal models.

EVs present some individual features, which make them promising therapeutic tools, and emphasize EV-based therapies as a promising alternative to cell therapy in cardiovascular medicine. Using EV-based therapeutics avoids biological issues associated with cell-based strategies, such as stress-induced necrosis or aberrant differentiation [110].

Thus, EVs have a particular stability over time conferred by their membranous structures that make them real "off-the-shelf" tools allowing careful maintenance of stability, integrity, and biological activity during their manufacture, storage, and subsequent administration [111]. Moreover, EV lipid bilayer coat protects their bioactive cargo from degradation when they circulate from one cell to another. The small size of EVs, compared to whole cells, also offers therapeutic benefits, such as decreased macrophage phagocytosis and vascular occlusion, and easier injection [110]. Additionally, EVs have innate biocompatibility, low toxicity and immunogenicity, and selective uptake that make them an excellent delivery vehicle for therapeutics [112].

With all these features, at this time, EVs represent attractive nanocarriers for drugs as well as therapeutic small molecules, nucleic acids, and proteins.

In order to enhance the EVs' therapeutic capabilities and applicability, methodologies have been developed for loading them with non-native cargo and also, several targeting strategies for systemically delivery. The two main categories of current strategies are: (i) approaches focused on cellular modification and (ii) methods focused on direct EV alteration [113].

The most common therapeutic approaches that have used EVs are: (i) to deliver small RNAs attempting to reverse pathological miRNA-based communication with anti-miRNA oligonucleotides or (ii) to stimulate protective communication with synthetic miRNA mimics [114, 115]. More specific delivery of anti-miRNAs or miRNA mimics to target cells is realized by engineering vesicles with cell-selective surface proteins [116], which should reduce off-target effects. The ability to load EVs with miRNAs suggests the possibility of using EVs to deliver miRNA-based therapeutics in CVDs. The field of miRNA-based therapies is advancing rapidly, and research focused on circulating EVs and their miRNA content has revealed diverse and important roles [112].

However, not many studies have focused their objective in the use of EVs as therapeutic tools against CVDs. In this regard, in a mouse model of type 1 diabetes, it was shown that MSCs-derived EVs delayed the onset of type 1 diabetes through modulation of IL-1β-mediated pancreatic B-cell damage [117]. Moreover, EVs secreted by induced pluripotent stem cells deliver cardioprotective miR-21 and miR-210, preventing cardiomyocyte apoptosis in the ischemic myocardium [118].

More information exists in the literature concerning the individual subsets of EVs: exosomes and MVs as therapeutic targets and biomedical tools. For instance, it was reported that cardiomyocytes exert an anti-angiogenic function in type 2 diabetic rats through exosomal transfer of miR-320 into ECs [119]. Further research showed that exosomes derived from cardiomyocytes overexpressing

heat shock protein 20 (Hsp20) protect against in vitro high glucose-triggered cell death as well as in vivo diabetes mellitus-induced cardiac adverse remodeling, suggesting that Hsp20-engineered exosomes might be a novel promising therapy [120]. Exosomes from human fibrocytes stimulated with platelet-derived growth factor-BB for 7 days and transforming growth factor-β for the following 3 days displayed both, in vitro and in vivo, wound healing properties in diabetic *db/db* mice [121]. Although it has been shown that this pharmacological treatment of human fibrocytes increased expressions of miR-126, miR-130a, miR-132, miR124a, miR-125b, and miR-21 into exosomes, the exact mechanism implicated in these effects is still unknown. In addition, administration of mouse brain endothelial cell-derived exosomes, loaded with miR-146a by chemical transfection method, into the brain's ventricle attenuates dementia-like pathology in diabetic *db/db* mice [122].

Several experimental data and preclinical models have demonstrated the excellent potential of stem cell-derived exosomes to be used as therapeutic tools in CVDs [111]. Thus, exosomes enriched with miR-22 secreted by MSCs following ischemic preconditioning was reported to have a significant benefit in cardiac recovery after myocardial infarction, by targeting the methyl CpG binding protein 2 [123]. Exosomes derived from human MSCs, carrying miR-21-5p, mediates effects on cardiac contractility and calcium handling, likely via PI3K signaling, opening new research ways in optimizing future stem cell-based cardiotherapies [124]. Furthermore, it was shown that exosomes secreted by human cardiosphere-derived cells enriched in miR146a inhibited apoptosis and promoted proliferation of cardiomyocytes, improving in this way angiogenesis. In another study, it has been showed that in cardiomyocytes cultured in a hypoxic environment, GATA-4 overexpressing MSCs-derived exosomes contribute to increased cardiomyocyte survival, reduced cardiomyocyte apoptosis, and preserved mitochondrial membrane potential [125]. Importantly, the use of exosomes isolated from MSCs for the reduction of inflammatory state during type 1 diabetes mellitus is mentioned into an Egyptian clinical trial (phase II-III, NCT02138331) [126].

In addition, it has been demonstrated that abnormal miRNA expression in MVs is involved in neoangiogenesis: (i) diminished expression of miRNA-200b reduces VEGF levels [127] and (ii) augmented expression of miR-29b regulates certain apoptotic genes and increases VEGF levels [128]. These data suggested that acting on these miRNA levels in MVs may control cell proliferation in diabetic retinopathy. Likewise, MVs cargo with miR-126 play an important role in angiogenesis and vascular integrity [129], while administration of the miR-126-enriched MVs to ApoE−/− mice could reduce the development of aortic plaques of atherosclerosis [130]. Importantly, it has been shown that MVs derived from EPCs, carrying specific miRNAs, activate angiogenesis through phosphatidylinositol 3 kinase/protein kinase B signaling pathway [129]. MVs derived from human acute monocytic leukemia cell line (THP-1 cells) treated by inflammatory factors contain miR-150 which may be involved in EC migration [226]. In a recent study, we showed that MVs of healthy origins promote EPC proliferation, adhesion, and migration, supporting reestablishment of EPC ability to incorporate in damaged endothelium and working in concert with existing ECs to form blood vessels [131]. These beneficial effects of MVs on late EPC dysfunctionality in atherosclerosis could be explained by the ability of MVs to transfer specific miRNA (miR-10a, miR21, miR-126, miR-146a, and miR-223) into recipient cells and by insulin-like growth factor- 1 expression activation [228].

Data summary concerning exosome and MV charge and their therapeutic effects are presented in the **Table 1**.

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Exosome charge	Exosome source	Recipient	Therapeutic effects	Reference
miR-320	Rat cardiomyocytes	Cardiac endothelial cells	Decreases angiogenesis in type 2 diabetes	[119]
Hsp20	Mouse cardiomyocytes	Endothelial cells	Improves cardiac function and angiogenesis in diabetes	[120]
miR-126, miR-130a, miR-132, miR124a, miR-125b, miR-21	Human fibrocytes	Dermal fibroblasts, keratinocytes	Accelerate diabetic wound healing	[121]
miR-146a	Mouse brain endothelial cell	Brain's ventricles	Attenuates dementia-like pathology in diabetes	[122]
miR-21, miR-210	iPSCs	Cardiomyocytes	Rescue ischemic cardiomyocytes	[118]
miR-22	hMSCs	Cardiomyocytes	Enhances cardiac function	[123]
miR-19a	hMSCs	Cardiomyocytes	Restores cardiac contractile function and reduces infarct size	[125]
miR-21-5p	hMSCs	iPSCs-derived cardiomyocytes and iPSCs-derived fibroblasts	Increases engineered cardiac tissue contractility via PI3K signaling	[124]
MV charge	**MV source**	**Recipient**	**Therapeutic effects**	**Reference**
miR-126	ECs	Vascular cells from ApoE^{-}/$^{-}$ mice	Reduces the development of aortic plaques of atherosclerosis	[130]
mRNAs	EPCs	hMECs	Activates angiogenesis through phosphatidylinositol 3 kinase/protein kinase B signaling pathway	[129]
miR-150	THP-1 cells	hMECs	Modulates endothelial cell migration	[129]
miR-10a, miR21, miR-126, miR-146a, miR-223	Plasma from healthy hamsters	Late EPCs	Promote EPC proliferation, adhesion and migration in atherosclerosis	[131]

iPSCs, induced pluripotent stem cells; hMSCs, human mesenchymal stem cells; ECs, endothelial cells; EPCs, endothelial progenitor cells; hMECs, human microvascular endothelial cells; and THP-1 cells, human acute monocytic leukemia cell line.

Table 1.
Exosome and MV charge components and their therapeutic effects in diabetes and CVDs.

6. Progress and challenges in extracellular vesicle field

Although research into EV field is gaining ground, some challenges need to be overcome before using them in the clinic, such as: (i) optimization of EV isolation procedures, especially the time of protocols, decrease of amount of samples, and the selective isolation of distinct EV subtypes; (ii) the large-scale production in good manufacturing conditions; and (iii) increase of the specificity of engineered EVs vis-à-vis target cells to avoid the possible side effects [126].

Additionally, much still remains to be revealed regarding the role of EVs in cell-cell communication both in health and diabetic cardiovascular disorders. Specifically, understanding the effects of the chronic inflammatory environment in diabetes on the packaging and release of endothelial-EVs and their following interactions with cardiomyocytes could be useful [112]. Advancing the knowledge regarding the cellular source and destination of EVs in CVDs will allow exploration of the specific cellular interactions, while understanding EV organ-tropism will help to target specific tissues, improving the efficiency of miRNA-based therapies.

Even so, with many problems remaining to be resolved, as we mentioned above, prior EV-based therapeutics might be clinically used to treat CVDs. Anywise, the many studies underline their potential as successful therapeutic targets in combatting the heavy millstone of metabolic disease [112].

7. Conclusions

Overall, our chapter strongly suggests that EVs may function as significant regulators of both physiological and pathological conditions and demonstrates their universal role in the relationship between diabetes and cardiovascular disease. Their unique properties as biological vectors modulating diabetic cardiovascular diseases, including atherosclerosis, coronary artery disease, and peripheral arterial disease, are also highlighted.

Undoubtedly, elucidation of terminology, biogenesis, biological content, and function of EVs contributes to better understanding of the complexity of their role in influencing the different processes affected in diabetic cardiovascular diseases. Consequently, we envisage that for EVs used as clinical biomarkers, therapeutic targets, and biomedical tools in diabetes and associated complications, there is a need for developing a molecular system of EVs based on their lipidomic, metabolomic, and miRnomic signature. Once these issues are clarified, preventative and therapeutic strategies can be implemented and further developed.

Despite the fact that existing literature discussed in this chapter describes the EV importance in diabetic cardiovascular diseases, it also leaves some significant questions unanswered. Thus, it becomes increasingly complicated to establish an EV structure either beneficial or harmful, to clarify their role either good or bad, in both health and disease. Incontestably, more research evaluating such properties is necessary to establish EVs' value as clinical biomarkers, therapeutic targets, and biomedical tools based on concrete scientific results for diabetic cardiovascular disease treatment.

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Conflict of interest

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

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