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Review

Role of Exosomes in Cardiovascular Disease: A Key Regulator of Intercellular Communication in Cardiomyocytes

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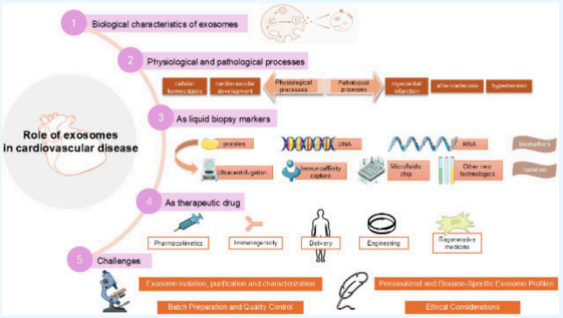
ABSTRACT: In the cardiovascular system, different types of cardiovascular cells can secrete specific exosomes and participate in the maintenance of cardiovascular function and the occurrence and development of diseases. Exosomes carry biologically active substances such as proteins and nucleic acids from cells of origin and can be used as biomarkers for disease diagnosis and prognosis assessment. In addition, exosome-mediated intercellular communication plays a key role in the occurrence and development of cardiovascular diseases and has become a potential therapeutic target. This article emphasizes the importance of understanding the mechanism of exosomes in cardiovascular diseases and systematically details the current understanding of exosomes as regulators of intercellular communication in cardiomyocytes, providing a basis for future research and therapeutic intervention.



1. INTRODUCTION

According to data from the World Health Organization, the number of deaths caused by cardiovascular diseases (CVD) worldwide in 2019 exceeded 17 million, accounting for 32% of the total deaths. Exploring effective prevention and treatment strategies is critical to improving global public health.

The delicate communication between cardiovascular cells plays a key role in maintaining cardiovascular function and responding to various physiological and pathological stimuli. In recent years, small extracellular vesicles have attracted widespread attention as a new intercellular signaling mechanism. Exosomes can transport information across membranes, mediate signal transmission between cells, and play an important role in regulating physiological processes and the occurrence and development of diseases.[1] In the cardiovascular system, different types of cardiovascular cells can secrete specific exosomes and participate in the maintenance of cardiovascular function and the occurrence and development of diseases.[2]

On the one hand, exosomes carry biologically active substances such as proteins and nucleic acids from cells of origin and can be used as biomarkers for disease diagnosis and prognosis assessment. on the other hand, exosome-mediated intercellular communication plays a role in the occurrence and development of CVD.[3] It plays a key role and becomes a potential therapeutic target. In recent years, a large number of studies have confirmed the important role of exosomes in regulating cardiovascular function and participating in the pathogenesis of CVD. For example, exosomes secreted by endothelial cells and smooth muscle cells can regulate vascular function; exosomes released by cardiomyocytes participate in myocardial remodeling; exosomes produced by inflammatory cells mediate inflammatory responses; exosomes released by cells with metabolic disorders influence Energy metabolism, etc. These findings lay the foundation for further elucidating the role of exosomes in cardiovascular physiological and pathological processes, and provide new ideas for the development of new exosome-based diagnosis and treatment strategies. This review uses a more detailed and systematic approach to elucidate our current understanding and research progress of exosomes in CVD.[2]

2. BIOLOGICAL CHARACTERISTICS OF EXOSOMES

2.1. Definition and Classification of Exosomes.

Exosomes are diminutive vesicles ranging in diameter from30 to100 nm, originating from the endosomal system. They are released when the limiting membrane of multivesicular bodies (MVBs) fuses with the plasma membrane or can emerge from the fusion of other internal membranes, such as those of the

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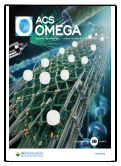

endoplasmic reticulum and nuclear envelope, interacting with the cellular membrane.[4] Exosomes were first identified in1983 in the reticulocytes of sheep, and in1987, Johnstone introduced the term "exosomes" to describe them.[5,6] Various cell types secrete exosomes under both physiological and pathological conditions. Based on their biosynthetic pathways of secretion, extracellular vesicles (EVs) can be classified into three major classes:[7] (1) Exosomes (diameter ≈30−150 nm), small vesicles released via exocytosis when multivesicular bodies (MVBs) fuse with the plasma membrane; (2) Shed microvesicles, vesicles with a diameter of approximately 50−1000 nm that are shed directly from the plasma membrane; and (3) Relatively large apoptotic bodies (diameter 1000−5000 nm) released from dying cells. All cultured cell types are capable of secreting exosomes, which are also naturally present numerous bodily secretions, such as blood, saliva, urine, cerebrospinal fluid, and breast milk, contain these substances.[8,9]

2.2. Mechanisms of Biogenesis and Secretion of Exosomes. The biogenesis of exosomes is regulated by molecules involved in the synthesis and transport of intracellular vesicles,[10,11] for example, Ras-related GTPase Rab proteins, Syntenin-1, tumor susceptibility gene 101 (TSG101), Alix (apoptosis-linked gene 2-interacting protein X), and ESCRT (endosomal sorting complex required for transport) are all involved in the origin and transport of exosomes. Exosomes are generated intracellularly, differing fundamentally from the conventional budding process (Figure 1). Their

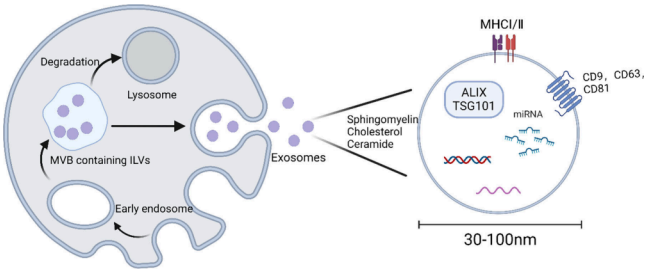

Figure 1. Exosome biogenesis and secretion.

formation involves the parent cell contributing particulate matter to vesicles that are formed within the endosomal cavity due to membrane invagination. These endosomes, which encapsulate the vesicles, are subsequently referred to as secondary endosomes or MVBs. After exiting the lysosomal pathway, the multivesicular bodies fuse with specific regions of the outer cellular boundary, or plasma membrane. Consequently, it follows that small vesicles within the endosome bud inward toward the endosomal lumen, leading the parent cell to extrude these budding vesicles into the extracellular space via an exocytic mechanism.[12] The formation of MVBs is mainly dependent on the endosome-sorting transport complex (ESCRT) pathway. ESCRT includes four different protein complexes, ESCRT-0, ESCRT-I., ESCRT-II., and ESCRT-III., among which ESCRT-0, I., and II. are involved in the identification and targeted sorting of external biomolecules into MVBs to form endoluminal vesicles, and ESCRT-III. is involved in the process of intraluminal vesicles forming extracellular vesicles (EVs) outside the cell through budding.[13]

Exosomes arise from the membrane undergoes inward protrusion, specifically at MVBs, resulting in the formation of intraluminal vesicles (ILVs). MVBs subsequently undergo fusion with lysosomal compartments or the plasma membrane, leading either undergo degradation of MVBs or release exosomes into the extracellular milieu. Exosomes can interact with recipient cells by fusing their lipid membranes with the recipient cell membrane or via endocytic pathways, enabling the exocytosis of exosome cargo into the cytosolic compartment of recipient cells. The lipid bilayer of exosomes is composed of sphingomyelin, cholesterol, ceramide, and various other components. Notable constituents of exosomes include miRNA, mRNA (mRNA), DNA, and four integral membrane proteins associated with exosome biogenesis (CD9, CD63, and CD81). Additionally, proteins from endosomal-related pathways, such as ALIX and TSG101, including proteins that mediate signal transmission and facilitate antigen display, including MHC I and II, are frequently detected in the interior of exosomes as well.

2.3. Molecular Composition of Exosomes. Previously, exosomes were primarily regarded as mere cellular debris. However, recent investigations have illuminated the fact that exosomes encapsulate cell-specific proteins, lipids, and nucleic acids, which can function as signaling molecules to neighboring cells, thus modulating their cellular activities.[14−16] Owing to their distinctive secretion mechanisms, the protein composition of exosomes diverges from that of other extracellular vesicles; they are devoid of proteins derived from the endoplasmic reticulum and instead exhibit elevated levels of proteins associated with the major histocompatibility complex (MHC) and integrins, alongside a variety of other protein families involved in cellular interactions. Notable among these are tetraspanins like CD9, CD81, and CD63, In addition, thermal stress proteins are also involved, such as HSP60, HSP70, and HSP90. Additionally, certain intracellular proteins such as Alix and TSG101, as well as low levels of phosphatidylserine, are present.[17] Lipids are integral to the physiological functions of exosomes, although research exploring this aspect remains limited. The lipid bilayer predominantly comprises phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine, phosphatidylinositol, phosphatidic acid, cholesterol, ceramides, sphingolipids, glycosphingolipids, and other lipids found in trace amounts.[18,19] Exosomes derived from various cell types exhibit subtle variations in their lipid composition. Exosomes have high concentrations of miRNAs and play a key role in cell development, differentiation, and proliferation. miRNAs are a class of noncoding RNAs that are involved in various cell developments and affect gene expression.[20] Moreover, a range of genetic materials has been identified within exosomes. In rare cases, exosomes may contain DNA, including single-stranded and double-stranded DNA, mitochondrial DNA, genomic DNA, and even reverse transcription complementary DNA.[21] However, it is noteworthy that exosomes are predominantly enriched in noncoding RNA, chiefly consisting of microRNAs (miRNAs), small nuclear RNAs (snRNAs), rRNAs (rRNAs), tRNAs (tRNAs), small nucleolar RNAs (snoRNAs), Piwi-interacting RNAs (piRNAs), and various RNA fragments.[22,23]

2.4. Exosome-Mediated Intercellular Communication Methods. Under physiological and pathological conditions, extracellular vesicles transport biological information to nearby target cells or are taken up by distant tissue cells through blood circulation and body fluids. This transport can lead to the reorganization of the genetic makeup of target cells, enabling them to acquire new functions, lose certain abilities, or even undergo cell death.[24] There are three primary mechanisms

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involved in intercellular information transfer:[25] first, the specific surface molecule ligands carried by the vesicles bind to corresponding receptor sites on target cells; second, the vesicles fuse directly with the target cell membrane, mediating intercellular communication through exosomes and releasing their contents into the target cell's cytoplasm; last, information can be directly transmitted to target cells through a mechanism similar to endocytosis. Studies have shown that exosomal miR-34a participates in the control of processes such as apoptosis, autophagy, inflammation, senescence, fibrosis, and remodeling through signaling pathways including Smad4/TGF-β1, Foxo3/PUMA, Notch1/ETBR, PTEN/PI3K/SIRT1, and FOXM1/NRF2/HO-1.[26] The miRNAs in mesenchymal stem cell exosomes (MSC-Exos) can also be taken up by recipient cells and affect their gene expression, a process called cell-to-cell RNA transfer. This mode of communication plays a key role in regulating immune responses, promoting tissue repair, and inhibiting inflammation.[27]

2.5. Role of Exosomes in Cardiovascular Diseases. EVs, carrying substances such as proteins and nucleic acids with specific surface markers, exhibit high stability under various conditions. This characteristic makes EVs well-suited for biomarker identification and detection.[28] At present, studies have found that EVs that can be used for the treatment of cardiomyopathy are mainly derived from mesenchymal stem cells and cardiac progenitor cells. Mesenchymal stem cell-derived EVs mainly improve the inflammatory microenvironment of myocardium by regulating lncRNA/miRNA, and play a role in postinjury repair and cardioprotection.[29] The miRNAs and lncRNAs in MSC-Exos, such as miR-21, miR-146a, miR-181b and miR-126, regulate cardiomyocyte function and angiogenesis through specific signaling pathways.[30] In a mouse model of myocardial infarction, an injection of MSC-Exos enriched with miR-22 into the ischemic heart blocked ischemic cardiomyocyte apoptosis and reduced the area of cardiac fibrosis. Experiments have shown that MSCs target and inhibit miR-155, blocking upstream signaling of cardiac fibrosis, thereby preventing cardiac fibrosis and restoring cardiac function.[31,32] Research has found that exosomal miR-192, miR-194, and miR-34a, as p53-responsive miRNAs, can inhibit the activity of hypoxia-inducible factor-1 and antiangiogenesis. Their elevated levels after acute myocardial infarction may indicate the progression of heart failure (HF).[33] In addition, the epicardial adipose tissue contains miRNAs that can regulate resting membrane potential. The miR-1-3p and miR-133a-3p carried by extracellular vesicles are potential mediators of arrhythmias associated with epicardial adipose tissue and may serve as therapeutic targets for antiarrhythmia treatment.[34]

3. ROLE OF EXOSOMES IN CARDIOVASCULAR PHYSIOLOGICAL AND PATHOLOGICAL PROCESSES

A large amount of research has investigated the role of exosomes in various aspects of cardiovascular health which associate with cardiovascular development and homeostasis within the cardiovascular system. The involvement of exosomes has been investigated in the progression of cardiovascular conditions, including atherosclerosis, myocardial infarction, heart failure, hypertension, and pulmonary hypertension. A detailed analysis of these topics will be presented in the following sections.

3.1. Role of Exosomes in Revascularization and Maintenance of Cellular Homeostasis. Vascular remodeling plays a vital role in the regenerative microenvironment after cardiovascular system and peripheral nerves injury. More and more research has explored exosomes and vascular reconstruction have a strong link. It is to be expected exosomes are key to maintaining cellular equilibrium.

3.1.1. Exosomes and Revascularization. Exosomes promote both revascularization and cellular homeostasis. The electron micrographs have revealed the presence of numerous MVBs in the cytoplasm of CD34+ cells which contain vesicles similar to exosomes, characterized by their bilayer membrane structure.[35] The $CD34^+$ cells are particularly promising candidates for therapies aimed at enhancing revascularization. Because they can mobilize swiftly from the bone marrow into peripheral blood when tissue is injured.[36−38] These cells in circulation migrate to the damaged tissue sites and participate in vascular repair and angiogenesis.[39−41] Sahoo et al. demonstrated that $CD34^+$-derived exosomes simulate the angiogenic effects of CD34+ cells by enhancing the viability, proliferation, and tube formation of endothelial cells on Matrigel.[42] In some vivo studies, both $CD34^+$ cells and their exosomal counterparts were found to promote the development of endothelial structures that mimic blood vessels. And there was a notable elevation in the endothelial cell content within the Matrigel plug. Pellets containing $CD34^+$-derived exosomes were associated with significant enhancements in vascular growth during experiments centered on corneal neovascularization. These results underscore that $CD34^+$-exosomes are essential paracrine factors that can facilitate the vascular growth driven by $CD34^+$ cells.[42] Sahoo et al. provided compelling evidence that these $CD34^+$-exosomes are notably enriched with more pro-angiogenic miRNAs, particularly miR-126 and miR-130a, when compared to exosomes derived from $CD34^-$ cells.[42] Despite these findings, there remains a considerable unknown mechanism. Further investigation is necessary to fully elucidate these mechanisms.

Exosomes from hypoxia-cultured Schwann cells (H-SCs exosomes) can induce the metabolic alterations in endothelial cells (ECs) which promotes the reorganization of intraneural blood vessels after injury through the delivery of miR-21-5p. This mechanism targets the von Hippel-Lindau protein, which leads to an increase in hypoxia-inducible factor-1α (HIF-1α) levels and influences glycolytic processes via the suppression of the pyruvate dehydrogenase-E1α subunit while inhibiting mitochondrial oxidative phosphorylation. Moreover, preconditioning under hypoxic conditions boosts the remodeling of endoneural vasculature via SCs-Exos.[43] The metabolic state of ECs is largely dependent on glycolysis. Because after an injury, glycolysis influence modulating endothelial functions, stimulating angiogenesis, and facilitating the restoration and development of both vascular and tissue architectures.[44−46] In addition, Tβ4-exosomes are capable of persistently releasing functional exosomes postmyocardial infarction, thereby augmenting the angiogenic capacity of coronary artery endothelial cells via the miR-175p/PHD3/HIF-1α signaling pathway. This measure promotes the development of alternative blood flow pathways. It may become a new approach in revascularization therapy in the future.[47] Exosomal hsa_circ_0007047 enhances angiogenesis through the miR-1178-3p/PDPK1 pathway to alleviate remodeling after myocardial infarction.[48] In general, exploring more clinical

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mechanisms of exosomes will help the development of future clinical revascularization treatments.

3.1.2. Exosomes and Cellular Homeostasis. Studies have found that extracellular vesicles(EVs) are essential for cells to operate correctly. Suppressing EV secretion may result in a rise of nuclear DNA levels in the cytoplasm, which can eventually lead to apoptosis.[9] There are many investigations have revealed that exosomes have the capacity to impact cellular wellbeing and pathology through diverse mechanisms.[49] Exosomes maintain cellular balance by removing the damaged or harmful substances, such as proteins, lipids, and nucleic acids.[50] Decreased secretion of exosome secretion triggers a reactive oxygen species (ROS)-dependent DNA damage response (DDR). STING, as a cytoplasmic DNA sensor, detects the existence of nuclear DNA in the cytoplasm and activates the ROS-DDR. There are fragments of chromosomal DNA that were detected in exosomes. The presence of fragments of chromosomal DNA indicates that exosome plays a crucial role in preserving cellular equilibrium by removing harmful cytoplasmic DNA.[51] Because the buildup of nuclear DNA fragments in the cytoplasm can initiate a ROS-dependent DDR in both young and aged cells.[52−55] When the secretion of exosomes is suppressed, there is a marked increase in the levels of proteins encoded by viruses, which indicates that exosome secretion may also eliminate viral DNA from infected cells. It suggests that the secretion of exosomes plays a crucial role in preventing viral entry into cells, although there may be other mechanisms at work.[51] In summary, exosomes are integral to cardiovascular development and homeostasis, particularly in upholding cellular balance.

3.2. Role of Exosomes in the Development of Atherosclerosis. Vascular endothelial cells (VECs) have been found to generate a substantial number of exosomes. These exosomes are important mediators of crosstalk not only among VECs themselves but also between VECs and vascular smooth muscle cells (VSMCs), as well as various immune cells.[56] Exosomes influence the physiological and pathological processes associated with atherosclerosis through these exosomes. A detailed explanation of this mechanism will be provided in the subsequent sections.

3.2.1. Exosomes and Endothelial Dysfunction. ECs act as a barrier, distinguishing circulating cells from adjacent tissues, and are essential for regulating blood flow, modulating vascular inflammation, and stimulating angiogenesis. Their impairment significantly contributes to the onset of atherosclerosis (AS).[57,58] AS induces endothelial dysfunction that manifests as a reduction in vascular function, diminished antithrombotic characteristics, and increased pro-inflammatory activity.[59] More lipids and monocytes accumulate in the subendothelial when endothelial cells suffer detrimental effects on both their structure and function. This mechanism facilitates the development of foam cells and advancement of AS. The presence of endothelial particles within the circulation is associated with the pathogenesis of cardiovascular diseases, especially relevant to the development of AS.

Research indicates that the expression of miRNA-126, miRNA-210, and miRNA-216 can induce the secretion of exosomes from VECs, leading to a reduction in plaque size and inhibiting macrophage infiltration. As a result, this process effectively retards the progression of atherosclerosis.[56,60] Both miRNA-126 and miRNA-214 facilitate neovascularization, whereas miRNA-155 adversely influences the diastolic function of blood vessels.[61] These findings emphasize that miRNA-126 and miRNA-214 are important for the promotion of blood vessel formation and the maintenance of vascular functionality. Conversely, the increased expression of miRNA-155 usually under pathological circumstances and downregulates its target gene, nitric oxide synthase, which subsequently impairs the vasodilatory function of the blood vessels.[62] Exosomes obtained from women suffering from preeclampsia can hinder the proliferation, movement, and tubular structure formation of human umbilical vein endothelial cells in vitro by delivering soluble fms-like tyrosine kinase-1 and soluble endoglin to these endothelial cells.[63] Furthermore, studies show that the lectin-like oxidized low-density lipoprotein receptor-1 is responsible for mediating the endothelial dysfunction triggered by endosomal vesicles derived from preeclampsia placentas' syncytiotrophoblasts.[64] Consequently, this mitigated the endothelial dysfunction induced by angiotensin II in VSMCs caused by these vesicles. Therefore, exosomes are essential in regulating the functionality of endothelial cells.

3.2.2. Exosomes and Phenotypic Transformation of Vascular Smooth Muscle Cells. The development of AS is shaped not only by individual modifications in particular cell types but also by a multitude of pathological alterations among different cell types. The transition in the phenotype of VSMCs plays a crucial role in the advancement of AS. Growing evidence indicates that this shift in phenotype is a key occurrence in the pathophysiology of several cardiovascular diseases, including AS.[65] In normal blood vessels, VSMCs exhibit a mainly stable and contractile phenotype.[66] However, following a vascular injury, these cells shift from their usual contractile state to a synthetic phenotype.[67] During the early phases of injury, VSMCs in their synthetic phenotype proliferate and migrate toward the intima, which holds compensatory importance. Nevertheless, in the later phases, the expansion of the intima is aggravated by the infiltration of medial VSMCs, resulting in thickening of the vascular wall and narrowing of the lumen. At the same time, synthetic VSMCs express a variety of receptors that facilitate the uptake of fatty acids and cholesterol, allowing them to absorb these lipids and accumulate lipid droplets in their cytoplasm, thus enhancing lipid capture and the development of foam cells.[68,69] Additionally, VSMCs have the ability to shift into a phenotype resembling pro-inflammatory and dysfunctional macrophages, which adopt macrophage-like functions that aggravate AS.[70,71] Moreover, VSMCs can undergo a transformation into an osteoblast-like phenotype. Vascular calcification poses a considerable risk for the initiation and mortality linked with AS. This phenomenon closely mimics the process of osteogenesis and is prompted by the alteration of VSMCs into an osteoblast-like form, resulting in the release of bone-related protein biomarkers from these cells. Ultimately, these biomarkers play a role in the calcification of the intima and the development of plaques. Nevertheless, this alteration can also improve plaque stability and assist in reducing the risk of plaque rupture, thus offering beneficial implications for AS management.[72−74] In summary, the phenotypic shift of VSMCs exhibits both harmful and protective effects in atherosclerosis.

Various miRNAs found in exosomes have unique impacts on the progression of AS by affecting the conversion of VSMCs.[75] Specifically, Inhibiting the expression of exosomal miR-92a-3p,[76,77] miR-21-3p,[78] miR-663,[79] and miR-222,[80] while promoting the expression of exosomal miR-133,[81] miR-143/145,[82−84] and miR-155-5p,[85,86] effectively restrains the VSMCs

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proliferation and migration, thereby enhancing vascular remodeling in AS. Moreover, the regulation of exosomal LncRNA H19,[87,88] miR-103-3p,[87] miR-133a,[81] miR-204/miR-211,[71] and miR-34a[89,90] may significantly influence the phenotypic transition of VSMCs into osteoblast-like cells, thereby playing a crucial role in regulating vascular aging, calcification, and the stability of plaques in AS. Exosomal miR-146a is anticipated to serve a protective function by hindering the osteogenic differentiation of VSMCs and mitigating vascular calcification, mainly by focusing on the upregulation of TXNIP within these cells.[91] As a result, different components within exosomes modulate the proliferation, migration, and phenotypic changes of VSMCs variably, thus engaging in several processes related to AS. Investigating this field offers possibilities for adjusting these cellular activities, which could ultimately affect the advancement of AS. Additionally, it can be deduced that employing various exosomes as specific stimulators or suppressors aimed at different phases of AS may assist in alleviating vascular calcification associated with aging or plaque rupture incidents caused by incomplete calcification.

3.2.3. Exosomes and Foam Cell Formation. Obesity is widely acknowledged as a significant risk factor for AS.[92] Adipose tissue functions as an active endocrine organ, not only producing adipokines but also releasing a considerable number of exosomes. Foam cells, which are macrophages that have become swollen and vacuolated due to lipid accumulation, typically gather along the walls of arteries and signal disruptions in lipid metabolism. These foam cells can be found throughout all phases of AS progression.[68,69] Research has demonstrated that exosomes originating from visceral adipose tissue (VAT) hinder cholesterol efflux through ATP-binding cassette transporters A1 (ABCA1) and ATP-binding cassette transporters G1 (ABCG1), which in turn fosters foam cell development. Additionally, the intravenous administration of VAT-derived exosomes from models on a high-fat diet has been shown to expedite the advancement of AS in hyperlipidemic ApoE$^{-/-}$ mice.[93] These observations imply that exosomes might significantly influence VAT dysfunction and the onset of AS by promoting foam cell creation.

3.3. Role of Exosomes in Myocardial Infarction and Heart Failure. Cardiovascular diseases include myocardial infarction (MI), which is among the most severe and potentially fatal conditions. Exosomes that are released by MI have the function of facilitating communication between receptor cells, similar to those originating from vascular smooth muscle cells.[94,95] The composition and number of circulating exosomes change dramatically during this process of communication, indicating a robust link exists between exosomes and the progression of ST-Elevation Myocardial Infarction (STEMI).[96−98]

3.3.1. Exosomes and Cardiomyocyte Apoptosis and Necrosis. Reperfusion of ischemic myocardium can lead to cell death, including apoptosis and necrosis.[99] Several miRNAs found in exosomes derived from bone marrow stem cells (BMSCs) can lead to apoptosis in cardiomyocytes and cardiac damage. For instance, miR-183-5p,[100] miR-21a-5p,[101] miR-25-3p,[102] and miR-125b[103] within BMSC-derived exosomes can reduce cardiomyocyte apoptosis. This process can also strength myocardial function. MiR-182-5p from exosomes of bone marrow stem cells (BMSCs) can suppress the expression of pro-pyroptosis protein gasdermin D (GSDMD) as well as toll-like receptor 4 (TLR4). This miRNA is also able to simultaneously inhibit the TLR4/nuclear factor kappa B (NF-κB) signaling pathway, generating effects that are both anti-inflammatory and inhibitory of apoptosis.[104,105] There are a variety of miRNAs that have been shown to improve myocardial function through modulating signaling pathways associated with therapeutic response. The regulatory RNAs within exosomes of BMSC can impact the phosphatidylinositol 3-kinase/protein kinase B (PI3K/AKT) pathway, which helps alleviate cardiac fibrosis following a myocardial infarction and myocardial damage from hypoxia.[106,107] The C-Jun N-terminal kinase pathway,[108,109] along with the checkpoint kinase 2/Beclin2 pathway,[110] exerts an inhibitory effect on cardiomyocyte apoptosis. These pathways can be modulated by miR-455-3p[108,109] and miR-143-3p.[110] Exosomes derived from BMSCs can also carry long noncoding RNAs (lncRNAs), which often regulate the expression of genes associated with cardiomyocyte apoptosis and necrosis via their interactions with miRNAs. The lncRNA KLF3-AS1 associates with miR-138-5p, resulting in the upregulation of sirtuin 1 (Sirt1), which impedes pyroptosis and delays the progression of MI.[111] LncA2M-AS1[112] and lncRNA HCP5P[113] found in exosomes have been demonstrated to inhibit the apoptosis of cardiomyocytes.

3.3.2. Role of Exosomes in Myocardial Remodeling and Fibrosis. With the progression of cardiovascular disease (CVD), the heart undergoes irreversible alterations, potentially leading to heart failure (HF) in the end.[114] Processes of cardiac remodeling can have a substantial impact on the morbidity and mortality rates associated with CVD.[115] Exosomal noncoding RNA (ncRNA), as a stable medium for intercellular communication, can influence cell signaling pathways and engage in various cardiovascular pathophysiological mechanisms, such as vascular remodeling, myocardial ischemia, cardiac hypertrophy, and inflammatory immune responses. Several research efforts have clarified how ncRNAs affect cardiac fibrosis.[116,117] This research aims to explore the function of ncRNAs within exosomes during the development of cardiac fibrosis.

Cardiac fibrosis is a key factor that leads to heart failure. Its characteristics are the breakdown of the extracellular matrix and a buildup of collagen due to cardiac fibroblast proliferation. But it has been found that myocardial fibrosis can be inhibited by exosomal ncRNAs. The increased expression of miR-208a has been noted in the exosomes derived from cardiomyocytes throughout the development of cardiac fibrosis, which facilitates the proliferation of fibroblasts and encourages their transformation into myofibroblasts, thus hastening the progress of cardiac fibrosis.[118] Furthermore, exosomes that harbor LINC00636 can inhibit MAPK1 via the overexpression of miR-450a-2-3p found in human pericardial fluid, aiding in the reduction of cardiac fibrosis.[119] Additionally, exosomal miR-29a functions as a communication channel among cardiomyocytes situated in the marginal ischemic zone, fostering antifibrotic actions and averting ventricular dysfunction and heart failure.[120] In addition, miR-294, derived from exosomes of embryonic stem cells, has demonstrated the capability to prevent fibrosis after a myocardial infarction by modulating the p38 MAPK signaling pathway, which in turn helps alleviate heart failure associated with this condition.[121,122] Similarly, exosomal miR-155, present in macrophage-generated exosomes, hinders the proliferation of cardiac fibroblasts in mice postmyocardial infarction by targeting and inhibiting SOS-1, a crucial regulator of RAS activation.[123] Moreover, an increase in the exosomal miR-146a-5p levels in

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Table 1. Exsomal miRNA Identified to Modulate Cardiac Fibrosis

Exosomes miRNA	Communication	Targets	Functions	Ref
miR-130	Between cardiomyocytes and heart	PPAR-γ	Inhibits cardiac fibrosis and apoptosis	130
miR-208a	Between cardiomyocytes and cardiac fibroblasts	Dyrk2	Increases fibroblast proliferation and differentiation	118
miR-210-3p	Between cardiomyocytes and fibroblasts	GPD1L	promote cardiac fibrosis	132
miR-378	Between cardiomyocytes and fibroblasts	MKK6	Inhibits cardiac fibrosis	122
miR-494-3p	Between cardiomyocytes and fibroblasts	PTEN	Inhibits cardiac fibrosis	133
miR-146a-5p	Between CDCs and heart	ND	Inhibits cardiac fibrosis	124
miR-132	Between CPC and heart	RasGAP-p120	Inhibits cardiac fibrosis	94
miR-294	Between ESCs and heart	AKT/nucleostemin/LIN28	Inhibits cardiac fibrosis	121
miR-29	Between fibroblast and heart	collagen mRNA and fibrotic genes	Inhibits cardiac fibrosis	126
miR-34a	Between fibroblast and heart	Smad4	Inhibits cardiac fibrosis	129
miR-15	Between fibroblast and heart	TGFBR1	Inhibits cardiac fibrosis	127
miR-21	Between fibroblast and heart	TGF-β	Promotes cardiac fibroblast differentiation and collagen production, promote cardiac fibrosis	128
miR-155	Between Macrophages and cardiac fibroblasts	SOS-1	Promotes cardiac fibroblast proliferation and collagen production	123
miR-22	Between MSCs and heart	Mecp2	Inhibits cardiac fibrosis and necrosis	125
miR-24-3p	Between MSCs and heart	ND	Inhibits cardiac fibroblasts differentiation, promotes cardiomyocytes proliferation	134
miR-29a	Between neonatal rat ventricular myocytes (NRVM) and heart	Apaf-19/Hmox-1/Cycs/ Col-I/Col-III	Inhibits cardiac fibrosis	135
miR-425	Between plasma and heart	TGFβ1	Inhibits cardiac fibrosis	136
miR-744	Between plasma and heart	TGFβ1	Inhibits cardiac fibrosis	136
miR-324-3p	Between vascular smooth muscle cells and fibroblasts	TGFβ1	Inhibits cardiac fibroblast proliferation	137
miR-26a	By limiting insulin resistance	FoxO1	Inhibits cardiac fibrosis and improves heart function	138

cardiosphere-derived cells (CDCs) is associated with a decrease in myocardial fibrosis through the suppression of pro-inflammatory cytokines and their transcripts.[124] Exosomes containing miR-22, derived from MSCs, have been indicated to reduce fibrotic development in cardiomyocytes as a result of acute myocardial infarction (AMI), alongside diminishing the severity of myocardial necrosis and fibrosis by interacting with methylated CpG-binding protein 2.[125] Furthermore, downregulating miR-29 is anticipated to release the expression of collagen mRNAs, thereby intensifying the fibrotic response.[126] When miR-15b is inhibited in vivo[127] during prolonged pressure overload on the heart, it leads to heightened cardiac hypertrophy and fibrosis. The downregulation of miR-21,[128] miR-34,[129] miR-130,[130] and miR-378[122] found in exosomes from bone marrow mesenchymal stem cells correlates with the suppression of myocardial fibrosis in mice. Conversely, exosomal miR-208a has been linked to the onset and worsening of cardiac fibrosis.[118] Additionally, the exosomal lncRNA ZFAS1 is known to foster cardiac fibrosis through the Wnt4/β-catenin signaling pathway.[131] Together, these observations highlight that exosomal noncoding RNAs originating from diverse cellular sources can either aggravate or alleviate cardiac fibrosis.

A comprehensive summary of the roles of miRNAs in myocardial fibrosis is presented in Table 1, specifically highlighting the diverse roles of intercellular interactions.

3.3.3. Role of Exosomes in Myocardial Regeneration and Repair. Historically, research into cell therapy has become a significant focus for repairing damage caused by myocardial infarction; nevertheless, its effectiveness in treatment has frequently fallen short of expectations.[121,139,140] Recent investigations indicate that the paracrine signaling of bioactive molecules released from cardiomyocytes can successfully promote the repair and regeneration of endogenous tissues.[141,142] Evidence suggests that exosomes originating from cardiomyocytes can significantly improve cardiac function after myocardial infarction. These exosomes have attributes that reduce pathological inflammation, prevent apoptosis and necrosis in myocardial cells, avert remodeling, and encourage cardiovascular regeneration.[140,143] For example, extracellular vesicles containing Growth Differentiation Factor-15 have been observed to decrease the infarct size, improve cardiac function, inhibit the infiltration of inflammatory cells, prevent cell death, and foster cardiac angiogenesis. The upregulation of telomerase reverse transcriptase expression and the activation of the AMPK signaling pathway can facilitate the protective mechanism against myocardial damage occurs.[144] Exosomes derived from stem cells are crucial for the regeneration and repair processes of cardiomyocytes. Exosomes from embryonic stem cells can stimulate angiogenesis, which increase the survival and proliferation rates of cardiac progenitor cells (CPCs) and assist in cardiac repair postmyocardial infarction. Exhibiting anti-inflammatory traits, these exosomes also improve heart function and reduce fibrotic conditions.[121] Exosomes derived from induced pluripotent stem cells (iPSCs) and MSCs promote the regeneration and repair of cardiomyocytes.[145,146] Considering the exosomal role in the therapy and regeneration of cardiomyocytes, we are convinced that it holds unprecedented potential for the treatment of myocardial injury.

3.4. Role of Exosomes in Hypertension and Pulmonary Hypertension. Pulmonary hypertension (PH) is a diverse disorder characterized by a rise in the average pulmonary arterial pressure.[147] Many researchers are increasingly recognizing the significance of exosomal miRNA in the progression of PH.[148] The intravenous administration of

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ACS Omega 2025, 10, 18145−18169

exosomes derived from MSCs to mice suffering from hypoxia-induced pulmonary hypertension resulted in the suppression of hypoxia-related STAT3 signaling and the miR-17 family, while also promoting the expression of the miR-204 family. This treatment interfered with the STAT3-miR-204-STAT3 feedback loop, redirected the equilibrium toward an antiproliferative condition, and contributed to improvements in pulmonary hypertension, right ventricular hypertrophy, and remodeling of pulmonary vessels.[149] Exosomes obtained from mice injured with monocrotaline (MCT) and from patients suffering from idiopathic pulmonary hypertension (PH) showed higher levels of miRs-19b, -20a, -20b, and -145. Conversely, the miRNAs recovered from exosomes originating from mesenchymal stem cells, such as miRs-34a, -122, -124, and -127, revealed properties that were anti-inflammatory and antiproliferative. Importantly, an increase in miR-124 expression was noted in the exosomes derived from MSCs, whereas a decrease in miR-124 has been linked to pulmonary vascular remodeling related to PH. It is hypothesized that the reversal of pulmonary vascular remodeling observed in mice treated with MSC-derived exosomes might be due to the increased expression of miR-124.[150] MiR-195, originating from endothelial cells, is transmitted from ECs to SMCs, where it suppresses the growth and movement of SMCs by decreasing the levels of the serotonin transporter. As a result, miR-195 could play a protective role in PH.[151] Studies indicate that miR-191 plays a vital role in PH by regulating bone morphogenetic protein receptor 2 (BMPR2) within adipose-derived stem cells (ASCs) and the exosomes derived from ASCs. The inhibition of miR-191 has the potential to decelerate the advancement of MCT-induced PH by averting the degradation of BMPR2. Overall, these results suggest that miR-191 could serve as a possible risk factor for pulmonary arterial hypertension.[152] Chen et al.[153] showed that both MSCs derived from bone marrow and EVs from MSCs significantly reduced MCT-induced PH and right ventricular hypertrophy. Furthermore, adipose tissue-derived exosomes were discovered to deliver miR-125a to ECs, enhancing angiogenesis by downregulating the angiogenic inhibitor Dll4, which supports tissue repair.[154] As a result, exosomes derived from MSCs may positively influence the pathophysiology of pulmonary hypertension and improve mitochondrial function.

4. EXOSOMES AS LIQUID BIOPSY MARKERS FOR CARDIOVASCULAR DISEASE

By scrutinizing the sources and constituents of exosomes, as well as their specificity in cellular targeting, we can ascertain their potential utility as biomarkers for both diagnosis and prognosis. Exosomes are pivotal in maintaining vascular health, facilitating intercellular communication via the transfer of biomolecules to intended cellular targets. The amounts and constituents of exosomes can fluctuate depending on various physiological states and can be identified within bodily fluids.[1,155] Consequently, circulating exosomes and their molecular content, comprising proteins and nucleic acids,which could contribute to the diagnosis of CVD and may serve as biomarkers for these condition.[1,156]

4.1. Isolation and Characterization of Circulating Exosomes. Isolating high-purity exosomes is imperative for elucidating their roles in both physiological and pathological contexts, as well as for exploring their probable clinical applications across a range of diseases, particularly those impacting the cardiovascular system. For example, in a myocardial infarction model, high-purity exosomes can clearly distinguish that their effects on myocardial repair are derived from the exosomes themselves, rather than from confounding cytokines or apoptotic bodies.[157] In addition, the use of high-purity exosomes enhances experimental reproducibility, minimizes batch-to-batch variability, and offers a robust data foundation for mechanistic studies. In Tables 2 and 3, we have summarized the most frequently employed techniques for isolating and characterizing exosomes, along with their respective benefits and drawbacks.

However, the lack of a reliable protocol for accurately distinguishing exosomes from other EVs within biological samples remains a significant challenge in the field. This challenge is especially pronounced When analyzing biological fluids, it is often necessary to enrich or purify exosome samples to improve the signal-to-noise ratio for detecting exosomes amidst nonexosomal contaminants. Each prevailing exosome purification technique introduces specific biases, potentially influenced by the nature of the sample and the contaminants that are copurified along with the exosomes.[2,156,158] Furthermore, the choice of method for isolating exosomes has a notable impact on the quality of the resulting exosomes, which in turn affects downstream analyses. Therefore, establishing a robust protocol for exosome isolation is an essential prerequisite for obtaining reliable data in exosome research.[156]

4.1.1. Ultracentrifugation Techniques. Ultracentrifugation techniques (UC) is the predominant isolation technology used at this stage and is widely considered the benchmark for the extraction and isolation of exosomes. UC is primarily divided into two distinct phases: initially, a sequence of continuous medium-to-low-speed centrifugation steps are conducted to eliminate dead cells, cellular debris, and larger EVs. Subsequently, a centrifugal force of 100,000*g* is applied to isolate exosomes at a higher velocity, followed by washing the exosomes with phosphate-buffered saline (PBS) for the removal of impurities, including contaminating proteins.[158] This technique does not necessitate the labeling of exosomes, thereby minimizing the risk of cross-contamination. For instance, Paul and colleagues have isolated EVs using UC to investigate their role in epicardial signaling in response to heart injury. This research aims to understand how these vesicles contribute to heart repair and regeneration, potentially leading to optimized treatment strategies for heart-related conditions.[159]

Moreover, UC may not always be applicable to clinical samples and is ineffective in separating exosomes from other coisolated biomolecules. The repeated washing steps inherent in ultracentrifugation can diminish the quantity of coisolated contaminants but may also decrease the integrity of the vesicles, leading to a decrease in yield.[156] Density gradient centrifugation is specifically tailored for the purification of exosomes and is frequently used alongside ultracentrifugation for improving exosome purity. In this method, exosomes are initially concentrated using a 60% iodixanol buffer, which maximizes recovery while better maintaining their biological and physical attributes. The concentrated exosomes are subsequently separated using density gradient ultracentrifugation, effectively discarding nonexosomal nanoparticles and protein contaminants. Although this approach demands considerable time, it facilitates the attainment of high-purity exosomes, and the bioinert nature of iodixanol renders it an appropriate choice for subsequent functional assays.[156,158]

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ACS Omega 2025, 10, 18145−18169

Table 2. Commonly Used Purification Techniques

Method	Principle	Pros	Cons
Ultracentrifugation	Exosomes are purified on the basis of their size. Sequential centrifuge steps with increasing force (last step typically ∼100,000*g*)	large sample capacity	expensive equipment, time-consuming, not suitable for small volume, potential mechanical damage
Density gradient ultracentrifugation	Exosomes are separated according to their density in a discontinuous density gradient medium	reduction of coisolated contaminants compared to ultracentrifugation alone	Time consuming, labor intensive, not suitable for small volume
Ultrafiltration	Isolation of exosomes is based on size through filter membrane with defined size-exclusion limit or	low equipment cost, fast procedure	coisolation of contaminants, potential deterioration induced by shear stress
Size exclusion chromatography	Isolation method, based on size, that uses columns with stationary phase consisting of resin particles with known porous size	good purity, shorter processing time compared to differential ultracentrifugation	low vesicle yield, dilution of the sample
Immunoaffinity capture	Exosomes isolation is based on interaction between exosomal antigens and specific antibodies	isolation of specific exosome isolation of specific exosome	low vesicle yield, dilution of the sample
Polymer-based Precipitation	Exosome solubility is altered by water-excluding polymers	easy to use, no specialized equipment, high recovery rate, suitable with small volumes	Co-purification of contaminants, polymer may interfere with the downstream analyses
Microfluidics-Based Techniques	Microscale isolation based on different principles	Highly efficient, low cost	not suitable for large volume, lack of method validation

Table 3. Commonly Used Characterization Techniques

Method	Parameter analyzed	Pros	Cons
Nanoparticle Tracking Analysis	Dimension and concentration	Easy sample preparation, fast analysis/measurements, sample acquisition performed in a liquid phase, high resolution, vesicles are directly observed	Possible overlaying effect of larger vesicles, fail to distinguish exosomes from other nanocontaminants, determination of the proper dilution of the sample
Dynamic Light Scattering	size	high resolution	Reliable data only when working with a monodispersed suspension, fail to distinguish exosomes from other nanocontaminants
Tunable resistive pulse sensing	Size, concentration, and surface charge	information about surface charge of vesicles	Pores may be easily blocked by particles, particles may be too small to generate a signal higher than the background noise of the system
Transmission Electron Microscopy	Morphology and size	High resolution, discrimination between exosomes and other similar-size contaminants, immunostaining	change in the morphology of exosomes due to sample preparation,potential damage of sample by electron beam
Atomic Force Microscopy	structure, biomechanics, and biomolecular content of individual exosomes	Minimal sample preparation and without any destructive mode of operation	analysis may be influenced by temperature, state of the tip, scan speed
Flow Cytometry	detection of exosomal marker	fast analysis	Exosomes fall below the resolution of flow cytometry
Exoview platform	cargo, size, concentration	small volume, no vesicles isolation required, low purification biases	expensive instrumentation, time-consuming

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ACS Omega 2025, 10, 18145−18169

In comparison to differential centrifugation, density gradient ultracentrifugation yields exosome products of superior purity but at the expense of lower yield and throughput. Additionally, this approach is labor-intensive and costly due to the necessity of constructing layers of polymer density. Consequently, density gradient ultracentrifugation is typically not advised for processing exosomes on a large scale.[160,161] Numerous emerging alternatives have been developed to enhance exosome isolation, including the use of density gradients and sucrose-based centrifugation steps. This method facilitates the upward flotation of vesicles into the overlaid sucrose gradient, effectively allowing for the precipitation of proteins and impurities at the bottom of the tube. Consequently, this approach enables the straightforward removal of contaminants, resulting in aggregate-free separation of exosomes.[16]

4.1.2. Immunoaffinity Capture. Immunoaffinity capture selectively isolates specific exosomes from heterogeneous populations by targeting distinct surface markers. Typically, this methodology uses magnetic beads covalently coated with streptavidin, which enables high-affinity binding to any biotinylated capture antibody. This method yields promising outcomes in isolating subpopulations of exosomes originating from specific cell types.[156] Furthermore, it is a gentle method that could preserve the biological activity of exosomes after isolation and is often utilized in combination with size-exclusion chromatography (SEC) and other preparation processes.[160] Recent advances in the field of exosome isolation have demonstrated that antibody-coated magnetic beads can effectively isolate exosomes from antigen-presenting cells. The selection of appropriate exosome membrane markers, such as CD81, CD9, and CD63, facilitates efficient immune capture. Compared with traditional exosome separation techniques, antibody-coated magnetic beads enable the direct separation of exosomes from bodily fluids, thereby minimizing time-consuming centrifugation steps. For example, Zarovni et al. utilized antibody-functionalized magnetic beads to isolate exosomes from cell cultures and plasma samples.[162] Recently, another immunological approach has been reported involving a lipid nanoprobe system designed to isolate exosomes from serum-free cell culture supernatants and plasma. In this method, exosomes are first labeled with biotin-labeled 1,2-distearoyl-SN-glycero-3-phosphate ethanolamine-poly-(ethylene glycol) to target the lipid bilayer. The labeled vesicles are then collected using magnetic submicron particles coated with NeutrAvidin, allowing for subsequent extraction and analysis of the exosomes.[16] A method for the direct isolation of exosomal media from complex samples has also been described. This technique employs Gold-Loaded Nanoporous Ferric Oxide Nanozymes, which are initially functionalized with CD63 and dispersed within the sample fluid. These nanozymes serve as "dispersible nanocarriers", enabling the efficient capture of a significant quantity of exosomes. After magnetic collection and purification, the exosomes bound to AU-NPFe_2O_3NC are transferred to screen-printed electrodes modified with tissue-specific antibodies.[163] Also, a novel and direct method for exosome separation utilizing a new class of carboxyl-functionalized magnetic iron oxide nanoparticles (C-IONPs) has also been reported.[164] These nanoparticles were synthesized using a starch-assisted method, followed by surface functionalization and characterization. C-IONPs were initially modified with the universal antibody CD9, serving as a "dispersible nanocore" for the direct capture of exosomes from cell culture. The captured exosomes were then immobilized on screen-printed carbon electrodes (SPCE) that had been prefunctionalized with ovarian cancer-specific CA125 antibodies. Subsequently, chronoamperometric assays were conducted using a hydrogen peroxide/hydroquinone (HO/HQ) system, leveraging the peroxidase-like activity of C-IONPs.

4.1.3. Microfluidic Chip Technology. A microfluidic device, or lab-on-a-chip, harnesses the principles of fluid dynamics to enhance conventional isolation methods. This technique not only reduces costs and enhances time efficiency but also address the discontinuous separation processes inherent in other commonly used methods.[165] This technology also holds significant promise for advancing biomarker detection by providing sensitive measurements over a broad range, all within a compact format.[166]

For instance, the ExoChip designed by Kanwar et al. features a microfluidic platform where several round capture chambers are interconnected through narrow channels.[167] This design prolongs the retention time of external bodily secretions and facilitates intermittent mixing within the circular spaces created by direct pathways.[166] Furthermore, the ExoChip incorporates geometric design principles, enabling analysis using standard enzyme-linked instrumentation.[156,160] Additionally, Liu et al. developed a size-based exosome separation chip, termed ExoTIC, designed to enrich and purify exosomes ranging from30 to200 nm through multiple nanoporous membranes.[168] While this approach enhances the yield of secreted exosomes, the obstruction of membrane pores significantly impedes the continuous collection of these vesicles.[161] To address this challenge, Chen et al. developed an innovative rapid isolation system that integrates a double-coupled harmonic oscillator within a dual membrane filter setup.[169] This system generates periodic negative pressure oscillations across a nanoporous anodic alumina membrane through meticulous modulation of both negative and positive air pressures. This process facilitates the passage of smaller particles, such as proteins and nucleic acids, along with fluid, while larger exosomes remain trapped in the central compartment.[161] While these systems appear highly promising, they necessitate additional implementation to achieve their complete potential.[156] Furthermore, the elution of trapped exosomes from the wells is a time-consuming process, which limits the utility of this method in diagnostic applications.[160]

Liu and his colleagues reported another microfluidic device that first establishes a Mag-CD63-exosome complex by premixing with the capture agent Mag-CD63.[170] This complex is introduced through inlet 1, while the primary antibody is introduced through inlet 2, forming a Mag-CD63-Exo-Ab1 complex. Subsequently, a fluorescently labeled secondary antibody is introduced through inlet 3 to capture the targeted exosomes, which are then detected using inverted fluorescence microscopy. The results indicated that the number of EpCAM-positive exosomes in the plasma of breast cancer patients was significantly higher than that in the control group. In another study, Castro and his colleagues developed an immunomagnetic exosomal RNA (iMER) microfluidic platform for the isolation and enrichment of cancer-specific exosomes coated with antistatic magnetic beads featuring EGFR/EGFRvIII.[170] The enriched and separated exosome subpopulations are lysed within the chip and subsequently introduced into a glass bead filter. Next, the mRNA from the exosomes is adsorbed onto the glass beads through electrostatic interactions between the glass substrate and the mRNA. The isolated mRNA is then reverse-transcribed, amplified, and quantified using qPCR.[171]

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ACS Omega 2025, 10, 18145−18169

4.1.4. New Technologies of Exosome Characterization. There are many new technologies of exosome characterization, including flow cytometry, nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM), etc. (Table 3)

TEM is the most widely used imaging method for accessing morphological structure information of extracellular substances. However, Preparation of samples for TEM typically involves dehydration, chemical fixation, or staining, which can alter the morphology of exosomes.[156] Cryo-electron microscopy (cryo-EM) is a specialized form of electron microscopy that analyzes samples at low temperatures. In contrast to TEM, cryo-EM is regarded as the superior technique for visualizing nanoparticles without the complications associated with dehydration and fixation artifacts.[156,172] Furthermore, cryo-electron microscopy offers enhanced overall resolution and is capable of capturing detailed images of the lipid bilayer of exosomes. However, the limited availability of instruments and the time-intensive nature of sample analysis have hindered the broader application of cryo-electron microscopy.[156]

NTA offers straightforward sample preparation and rapid analysis, alongside high resolution that enables precise measurement of nanovesicles. Notably, sample collection occurs in the liquid phase, minimizing alterations to the vesicles under investigation. Nevertheless, NTA has its limitations. To achieve reliable measurements, it is essential to use the "correct" dilution factor. The NTA camera should identify every vesicle in the sample, ensuring that caution is exercised to prevent the exaggerated measurement of larger vesicles, as they may conceal the smaller ones.[156,173,174] Moreover, NTA is constrained by challenges associated with fluorescence signal detection.

While next-generation flow cytometers implement various angles for forward scatter detection, thereby enhancing the resolution of particles, exosomes typically fall beneath the resolution threshold of flow cytometry.[156,175,176] To overcome this limitation, Suarez and associates employed a bead-assisted technique for the semiquantitative assessment of exosomes.[177] This approach entails combining exosomes with aldehyde/sulfate-latex beads, followed by incubation with antibodies before analysis.[156]

4.2. Exosome Proteomics and Transcriptomics Analysis. Nearly all standard proteomic methods have been employed to analyze secreted proteins, ranging from two-dimensional gel electrophoresis to advanced quantitative LC-MS/MS techniques.[178] Proteomics approaches facilitate in-depth protein analysis of various biological samples, including tissue, plasma, serum, and cerebrospinal fluid (CSF), in order to identify potential biomarkers.[179] For instance, Fernanda G. Kugeratski et al. employed quantitative proteomics to elucidate the core exosome proteome, revailing that Syntenin-1 is the most prevalent protein, suggesting it may serve as a universal biomarker.[180] Transcriptomics has also played a significant role in exosome research about CVD. For instance, exosomal miR-21-3p derived from macrophages exposed to nicotine, may facilitate the progression of atherosclerosis by promoting the migration and proliferation of vascular smooth muscle cells (VSMCs) through its influence on the PTEN gene.[78] Furthermore, the expression of M2E in VSMCs facilitates softening and promotes the vascular tissue repair process. The differentiation and softening of VSMCs are also supported by the isolation of M2E. Additionally, M2E activates the c-Jun/Activator Protein 1 (AP-1) pathway, resulting in an increase in c-KIT expression in VSMCs, which further augments vascular tissue repair.[181]

4.3. Exosome miRNA, lncRNA, and Other Noncoding RNA as Biomarkers. Exosomes and other EVs serve as primary sources of circulating miRNAs, with exosome-bound circulating miRNAs demonstrating significant potential as novel diagnostic biomarkers for heart disease.[1,182] Matsumoto et al. proposed that exosome-bound miRNAs could effectively predict indicators of ischemic heart failure in patients following AMI.[156,183] Furthermore, the content of exosomes may also prove valuable in diagnosing cerebrovascular diseases. For instance, in patients experiencing acute ischemic stroke, serum concentrations of secreted body and brain-specific miRNAs were notably elevated when compared to a control group.[156] Moreover, a positive correlation was observed between the levels of miR-9 and miR-124, and the National Institutes of Health Stroke Scale (NIHSS) score, infarct size, as well as the serum concentrations of interleukin-6 (IL-6).[184,185] Additionally, specific secreted miRNAs or long noncoding RNAs (lncRNAs) have also been suggested to play roles in cardiovascular development and disease, potentially serving as biomarkers for CAD.[156,186,187]

While the field of clinical biomarkers is currently dominated by studies focusing on microRNAs miRNAs, the biomarkers that have successfully undergone validation and are utilized in clinical settings are predominantly mRNAs.[188] The heightened interest in miRNAs largely stems from their prevalence and established regulatory roles. Nonetheless, from the perspective of liquid biopsies, longer RNA types—particularly mRNAs with identified mutations and actionable variations—have emerged as more accessible and practical targets. Beyond merely measuring gene expression levels and detecting tumor-specific somatic alterations, long RNAs offer additional avenues to explore various biological processes which may serve as indicators of disease state or progression, offering valuable insights into the underlying mechanisms of diseases.[188]

4.4. Potential of Exosome DNA as a Liquid Biopsy Marker. Exosomes exhibit stable circulation in bodily fluids, prompting investigations into their potential use as diagnostic and prognostic biomarkers for a wide range of diseases.[104,161,189,190]

Exosomes are notable for their ability to transport DNA, with the circulating DNA accompanying with exosomes reflecting tumor DNA across various types of cancer.[188] The utilization of exosome-derived DNA as cancer biomarkers with potential clinical applications has risen significantly, encompassing genomic rearrangements,[191] mutations,[192] and alterations in copy number.[188,193] For instance, whole-genome sequencing demonstrated that exosomes present in the serum of patients with pancreatic cancer contain complete double-stranded DNA genomes that include all chromosomes.[194] Furthermore, Mutations that drive the development of pancreatic ductal adenocarcinoma (PDAC) were detected within the exosomal DNA analyzed.[194,195]

5. EXOSOMES AS THERAPEUTIC DRUG CARRIERS FOR CARDIOVASCULAR DISEASES

Exosomes, whether functioning independently or as carriers for drug payloads, possess the capability to transport bioactive molecules, including proteins, genes, RNA, viruses, and other therapeutic agents, thereby facilitating the treatment of diseases and the prevention of disease progression.[196]

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Furthermore, exosomes are particularly appealing for therapeutics aimed at drug delivery systems due to their minimal immunogenicity, low toxicity, and exceptional biocompatibility.[197,198] Below, we will introduce exosomes from the aspects of their pharmacokinetic properties, safety and immunogenicity, mechanisms of drug delivery systems, engineered modifications, and cardiovascular regeneration.

5.1. Pharmacokinetic Properties of Exosomes. Pharmacokinetics primarily examines the dynamic alterations in the body's handling of drugs, offering critical insights into their therapeutic efficacy.[199] The administration routes of exosomes are categorized into local and systemic administration. The most straightforward treatment approach involves the local application of exosomes to the targeted tissue. This can be achieved, for instance, by delivering them to brain tissue via the nasal cavity or by directly injecting them into subcutaneous tumors.[200,201] Meanwhile, systemic administration exhibits complex interactions characterized by blood circulation, biological barriers, and immune clearance.[202] Intravenous injection is the most commonly used route for systemic administration of exosomes, and its main problems are liver clearance and short half-life.[203,204] A new type of nanovesicle (hGLV) with overexpression of CD47 has the ability to reduce the clearance rate mediated by the mononuclear phagocyte system (MPS) and thus extend the duration of blood circulation.[205] It is noteworthy that exosomes naturally occurring in the bloodstream can likewise influence the elimination rate of therapeutically administered exosomes. Endogenous exosomes inhibit the uptake of exogenous exosomes by macrophages through activation of the CD36-mediated pathway, thus acting as competitors in the clearance of exosomes in the liver.[206] During their distribution in the body, exosomes exhibit cell-selective fusion, tissue-specific tropism, and the capacity to traverse the blood-brain barrier and dense tissue structures.[207] Most unmodified exosomes distribute to the liver, lungs, and spleen following intravenous injection.[208] In a murine model, blood cell-derived exosomes carrying dopamine showed more than 15 times higher brain distribution, effectiveness, and lower toxicity than free dopamine.[209]

5.2. Safety and Immunogenicity Assessment of Exosomes. Exosomes represent a promising class of next-generation therapeutics and vehicles for drug delivery. Compared with liposomes, exosomes have better stability and circulation capacity. Compared with synthetic nanoparticles, exosomes can efficiently interact with targets in a shorter blood circulation time due to their superior immune escape and targeting properties, avoiding blood toxicity caused by leukocyte damage.[210] Compared with cell therapy, it avoids the risks of malignancy and vascular occlusion and improves safety.[211] It is noteworthy that clinical blood transfusions also result in the transfer of trillions of exosomes without eliciting adverse events, further attesting to the safety of exosomes. Fortunately, both autologous and allogeneic experiments have shown that exosomes are nontoxic.[211]

Despite demonstrating favorable safety profiles in preclinical and clinical investigations, the unexpected immunogenic properties of exosomes remain incompletely understood.[212] Studies have shown that microvesicles derived from pathogenic bacteria carry antigenic determinants and virulence factors, and their immunomodulatory effects can be studied as emerging vaccine vectors, showing the favorable side of exosome immunogenicity.[213] However, exosomes also have adverse immunological interactions. Compared with autologous EVs, xenogeneic and allogeneic EVs show higher clearance rates, which may be related to the polymorphism of self-recognition ligand-handle pairs on macrophages, which can recognize xenogeneic and allogeneic cells as foreign cells.[214] For autologous EVs with low immune clearance rates, EV heterogeneity seen in different individuals and pathophysiological conditions may lead to large differences in treatment and drug delivery effects.[215] It is necessary to focus on the differences between batches of allogeneic EVs to ensure consistency of efficacy.

5.3. Exosomes as Delivery Systems for Cardiovascular Drugs. By incorporating cardiovascular drugs into exosomes, their in vivo stability, blood circulation time, and cellular targeting efficacy can be enhanced. Exosomes, through surface molecular recognition, undergo endocytosis in a highly specific manner that targets particular cell types.[216] The pathogenesis of ischemic cerebrovascular disease suggests that I/R injury frequently results in heightened oxidative stress, characterized by the accumulation of reactive ROS that activate mitochondria-induced apoptosis, thereby compromising the blood-brain barrier (BBB) and ultimately leading to secondary damage to brain tissue. Some drugs can be loaded into exosomes. For example, curcumin, a natural antioxidant derived from the turmeric plant, exhibits antioxidant, anti-inflammatory, and free radical-quenching capabilities.[217,218] Nonetheless, the clinical utilization of curcumin is hampered by its instability and the challenge of accessing brain lesion sites. Macrophage-derived exosomes loaded with curcumin(Ex-cur), serving as a versatile biomimetic delivery system, rely on the inflammation-homing properties mediated by exosomes and the antioxidant properties of curcumin to not only cross the BBB but also carry a significant amount of curcumin into the lesion area.[219] Ex-cur can decrease the accumulation of ROS, elevate the mitochondrial membrane potential, and inhibit the release of cytochrome c from mitochondria. By inhibiting mitochondria-mediated neuronal apoptosis within the lesion area, Ex-cur alleviates cerebral ischemia-reperfusion injury.[219,220]

After a heart attack or procedures such as coronary artery bypass surgery, patients are highly likely to experience myocardial ischemia-reperfusion injury (MIRI).[221] Targeted delivery of microRNA-146a (miR-146a) via milk exosomes modified with the peptide sequence CSTSMLKAC (IMTP) can exert cardioprotective effects.[222] IMTP directs exosomes to preferentially target ischemic myocardial regions. MiR-146a acts as a negative feedback regulator to modulate the nuclear factor-κB (NF-κB) signaling pathway by suppressing the expression of crucial adapter proteins like tumor necrosis factor receptor-associated factor 6 (TRAF6) and interleukin-1 receptor-associated kinase 1(IRAK1), thereby reducing scar tissue and increasing viable myocardial tissue.[223]

5.4. Engineering Exosomes for Targeted Delivery of Therapeutic Molecules. While natural exosomes have undergone preliminary verification in clinical studies, controlling their targeting and functionality has emerged as a challenge. Fortunately, exosome engineering is advancing rapidly, offering a range of techniques such as genetic engineering, chemical modification, and physical methods to modify the surface or cargo of exosomes for practical clinical applications (Figure 2).

Through the strategy of endogenous loading of therapeutic drugs, cells release exosomes loaded with target molecules.

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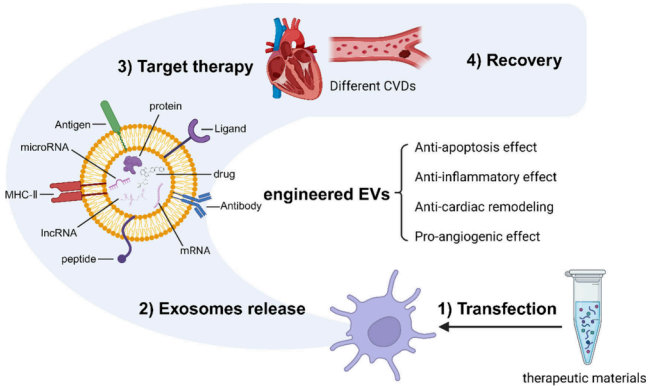

Figure 2. Engineered exosomes can be potential drugs for CVDs.

These exosomes accumulate in the heart and blood vessels, delivering their cargo to damaged tissues, thereby exerting protective effects in various CVDs. Created with biorender

5.4.1. Surface Modification Strategies. In the targeted exosome delivery system, engineering modifications to the exosome outer membrane are essential to improve their biodistribution within the body and enhance their targeted therapeutic efficacy.[224] We will introduce strategies for surface modification of exosomes in two aspects: genetic engineering and chemical modification.

Genetic engineering editing involves fusing the gene sequence of the target protein or peptide with the gene sequence of a selected exosome membrane protein, thereby displaying it on the exosome surface and directing the exosomes to a specific location in the body. Xu Wang et al. first used the ischemic myocardium targeting peptide CSTSMLKAC and the exosome-enriched membrane protein (Lamp2b) to reconstruct MSC exosomes so that they preferentially target the ischemic myocardium.[225]

Researchers use chemical methods, that is, by combining additional materials with exosome surface components through connectors, to design exosomes that target specific tissues and cell types. Tian et al. used azide-functionalized c(RGDyK)-conjugated exosomes (cRGD-Exo) to target lesions in cerebral ischemia. Curcumin-loaded cRGD-Exo can effectively inhibit inflammatory responses and cell apoptosis in lesions in ischemic brain mouse models.[226] Recently, a team from Fudan University constructed platelet membrane-modified extracellular vesicles (P-EVs) and connected peripheral monocytes to achieve targeted therapy for cardiac repair, which provides potential for future immunomodulation of myocardial ischemia-reperfusion and other immune-related disorders[227]

5.4.2. Endogenously Loaded Therapeutic Nucleic Acid Molecules. Drug loading into exosomes can be categorized into exogenous and endogenous approaches. Exogenous methods encompass electroporation, coincubation, ultrasound, and freeze−thaw techniques.[228] Endogenous loading of drugs uses the biogenesis of exosomes to integrate the target molecule into the exosomes, which are then secreted from the donor cells and finally separated and purified back into the drug-loaded exosomes.[224] Since Valadi et al. first reported in 2007 that the presence of mRNA and microRNA-mediated transfer in exosomes is a new mechanism for intercellular gene exchange, research on endogenous loading of therapeutic nucleic acid molecules in exosomes has developed rapidly.[229] Rongchuan Yue et al. transfected miR-182-5p into MSCs and verified that the derived exosomes can target GSDMD, reduce inflammation and apoptosis in vivo, and have a salvaging influence on ischemia-reperfusion injury of cardiomyocytes.[104] Li Qiao et al. added miR-21-5p to cell culture to obtain exosomes that can rescue cardiac repair function in patients with heart failure. The miR-21-5p carried by exosomes enhances angiogenesis and cardiomyocyte survival by enhancing Akt kinase activity.[230]

Based on the characteristics of exosomes that can protect nucleic acid molecules from degradation and can target receptor cells, exosome engineering that delivers specific miRNA or small interfering RNA (siRNA) payloads is being developed in an orderly manner aiming at the management of cardiovascular diseases. Based on the strategy of in vivo self-assembled exosomal siRNA, Fu et al. achieved the fully automated in vivo process of siRNA from expression to loading and then to delivery. They constructed a gene circuit capable of expressing siRNA in vitro, allowing flexible insertion of multiple siRNAs and targeting peptides.[231]

5.5. Exosomes from Stem Cells for Cardiovascular Regenerative Medicine. MSC are a specific type of stem cell. Although their multilineage differentiation potential is expected to help cardiovascular regenerative medicine, the clinical application of transplanted cells is limited by their low survival rates, differentiation challenges, and tumorigenic potential.[232,233] Fortunately, exosomes derived from mesenchymal stem cells possess advantages such as carrying parental beneficial factors, nonimmunogenicity, nontumorigenicity, and robust stability, thereby exhibiting promising clinical application potential. A large number of studies are forming a consensus that exosomes are the key paracrine components that mediate the repair function of cardiovascular stem cells.

Insufficient proliferation of cardiomyocytes after cardiac injury can lead to pathological healing processes and fibrotic scar formation, ultimately resulting in heart failure.[234] Exosomes originating from human umbilical cord mesenchymal stem cells can safeguard cardiomyocytes against apoptosis and stimulate angiogenesis via the Wnt/β-catenin signaling pathway.[235] Exosomes obtained from CPCs enhance the viability and proliferation of H9C2 cells by promoting the expression of Akt and activating the Akt/mTOR pathway.[234] Additionally, exosomes sourced from adipose mesenchymal stem cells overexpressing glyoxalase-1 (GLO-1) can enhance neovascularization in ischemic hindlimbs and hearts of mice, mediated by the miRNA-31/FIH1/HIF-1α pathway, thereby improving MI injury.[236] Exosomes produced by CD34+ hematopoietic stem cells (HSCs) exhibit significant expression of angiogenic miRNAs, such as miR-126 and miR-130, which promote the development of blood vessels in damaged hearts.[237] Hypertension is a significant contributor to the morbidity and mortality associated with cardiovascular diseases., and EVs are currently in the early stages of development as a therapeutic option for hypertension. Studies have shown that circulating EVs with lower plasma levels in WKY (Wistar-Kyoto rats) and SHR (spontaneously hypertensive rats) may uniquely alter vascular reactivity in isolated mesenteric arteries.[238] Exosomes derived from endothelial progenitor cells (EPCs) have demonstrated the ability to expedite wound healing and regeneration by enhancing the angiogenic activity of endothelial cells via the Erk1/2 signaling pathway.[239]

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ACS Omega 2025, 10, 18145−18169

6. CHALLENGES IN EXOSOME RESEARCH AND APPLICATION

As a natural drug delivery carrier, exosomes can be genetically engineered and chemically modified to enhance the targeting capability of drugs to specific cells or organs. However, due to the rich and diverse sources of exosomes, in order to put exosomes into clinical use, it is necessary to continuously monitor the production links such as the selection of donor cells, the standardization of exosome isolation and purification and characterization, preparation of preparations, and quality control, analyze the individualized and disease-specific exosome spectrum, and strictly control the regulation of exosomes.[240,241]

6.1. Standardization of Exosome Isolation, Purification, and Characterization. Due to the small size, large number, lack of specific markers and complexity of surrounding biological fluids, the separation and purification of exosomes has always been a highly concerned issue in exosome research.[215,242] Ultracentrifugation is currently regarded as the gold standard in the industry due to its reliable reproducibility, high efficiency, and applicability to various sample types. However, the equipment is expensive, the operation is cumbersome, and repeated ultracentrifugation steps can damage the structure and biological integrity of exosomes, leading to exosome aggregation and coisolation of nonexosomal components, which may affect exosomal proteomics, RNA analysis, in vivo experiments, and subsequent clinical applications.[243] Ultrafiltration serves as a faster alternative to ultracentrifugation, but the shear stress induced by the applied pressure often damages exosomes, and the use of dead-end filtration can result in vesicle clogging and membrane damage.[244] Although slower, size-exclusion chromatography allows exosomes to retain their integrity, biological activity, and initial abundance during gel filtration, with minimal interaction with the stationary phase. It also facilitates more precise separation of molecules of different sizes, effectively compensating for the shortcomings of ultrafiltration. The combined use of ultrafiltration and size-exclusion chromatography represents an efficient isolation method, enhancing yields, quality, purity, time efficiency, and reproducibility levels.[245] However, considering the susceptibility of ultrafiltration membranes to clogging and their higher sensitivity to impurities, this combination is more suitable for cells grown in serum-free media.[246] Affinity-based capture, leveraging the binding affinity between proteins and protein receptors, is commonly used to produce high-purity exosomes. This technique meets the stringent requirements for isolating exosomes containing specific target proteins and is primarily used in liquid biopsies.[247] However, solid matrix-based separation strategies, damage to exosomes by elution buffers, isolation of only specific exosome subsets, high costs, and low yields significantly hinder their applications, particularly for large-scale exosome preparation. Precipitation, unlike immunoaffinity-based capture, is a technique that uses polymers to precipitate exosomes, yielding high volumes. However, polymers can precipitate not only exosomes but also various water-soluble substances, resulting in lower purity of the obtained product and even cytotoxicity during cancer treatment.[248] Microfluidics-based technologies are rapidly gaining popularity due to their high speed, precision, and high yields. Currently, widely used microfluidic tools fully integrate size-based separation,[249] immunoaffinity-based separation,[250] and dynamic separation.[165,251]

Establishing standardized methods for the characterization of exosomes is crucial for their safe application in clinical practice. Flow cytometry can detect the particle size, concentration, and surface protein markers of exosomes. Traditional flow cytometry struggles to detect particles smaller than 300 nm. The new generation of flow cytometry employs multiaxis lasers to detect exosomes attached to secondary fluorophore-conjugated antibodies, significantly improving particle resolution; however, it tends to mistake clusters of vesicles with high concentrations of exosomes as single events, leading to misleading conclusions.[252] NTA analyzes the concentration and particle size of exosomes based on variations in light scattered by suspended particles and their Brownian motion, without relying on specific labeling and altering the material in any way, albeit the equipment is costly.[253] Tunable resistive pulse sensing (TRP) can identify the number and size of exosomes by monitoring voltage changes transmitted across membrane pores, but it faces challenges such as pore clogging, membrane contamination, and the requirement for more specialized equipment, which hinder the development of this technology.[254] Electron microscopy provides a direct means to observe the morphology and size of individual exosomes and is widely used for qualitative analysis and quality assessment of isolated exosomes.[255] Microfluidics is also applied in exosome characterization analysis. A novel immunomicrofluidic device with a mica channel surface can directly quantify circular exosomes from 30 μL plasma samples within 100 min.[256] In addition, for the identification and quantification of protein markers, Western blotting is the most commonly used method to detect protein concentrations. Enzyme-linked immunosorbent assay (ELISA) is employed for cost-effective analysis of specific protein indicators, while mass spectrometry is used for protein analysis in complex biological samples. Lipid quantification commonly involves fluorescence microscopy and sulfo-phospho-vanillin (SPV) assays. PCR is the gold standard for quantifying targeted DNA/RNA sequences. The International Vesicle Association stipulates the minimum experimental requirements for exosome identification, including overall characterization and individual characterization.[257] However, our understanding of the specific biosynthetic and release mechanisms of exosomes still remains limited.

6.2. Analysis of Personalized and Disease-Specific Exosome Profiles. Exosomes serve as carriers of information and play a crucial role in regulating vital physiological and pathological activities. Individualized and disease-specific biomarkers can be obtained by analyzing their lipid profiles, protein profiles, and nucleic acid profiles. However, traditional methods based on integrated and batch detection usually mask the heterogeneity of exosomes, making it difficult to perform accurate analysis, thus hindering their development and application in disease diagnosis. In recent years, there have been major breakthroughs in the analysis of exosome profiles in the academic field. Based on the different characteristics of circulating exosomal mRNA (emRNA) and tissue mRNA, a team has devised an improved strategy for detecting emRNA, further characterized novel emRNA markers for PCa detection, and established a new emRNA-based PCa detection platform. Proximity encoding technology(PBA), as an emerging single exosome analysis technology with high sensitivity and good specificity, can encode and label all single exosome surface proteins in samples with high throughput, analyze single

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exosome protein profiles, and find specific surface proteins as biomarkers for identifying exosomes released from specific tissues into the blood.[258] With the help of proximity coding technology, studies have shown that exosome subpopulations expressing ITGB3+ and ITGAM+ hold promise as early diagnostic biomarkers and therapeutic targets for colorectal cancer.[259]

6.3. Exosome Batch Preparation and Quality Control. Efforts need to be made to improve the scale of cell culture, separation, and purification methods to introduce EV therapy into clinical trials and industrial-scale production. Since the production of exosomes is heavily reliant on their originating cells, it is constrained by the capacity of various cells to secrete exosomes and the challenges and expense associated with large-scale cell culture. Attempts to enhance exosome production are primarily reflected in the expansion of cell culture scales, with three-dimensional (3D) culture methods serving as a breakthrough for increasing exosome yields in vitro. The hanging drop method can aggregate cells at the center of a droplet, prompting them to spontaneously form multicellular spheroids. During spheroid culture, the central region of the spheroid is prone to hypoxia, and the complexity of the hanging drop method itself limits exosome production.[260] In contrast, the microwell array allows cells to be seeded into an array of small wells, and this continuous pattern can generate a large number of 3D spheroids, thereby enhancing exosome yields.[261] Unlike the first two techniques, scaffolds simulate in vivo conditions by providing a sufficient microenvironment for cell adhesion, which reduces cell necrosis.[262] Fiber bioreactors, consisting of multiple hollow semipermeable fiber membranes connected to a large-scale bioreactor, are suitable for automated and large-scale production of exosomes.[263]

Following large-scale isolation and purification, identifying an appropriate container closure system for storing EV products is equally crucial. The current consensus appears to favor storing EVs at − 80 °C.[264] Cryopreservation utilizing cryoprotectants (CPAs) is a commonly used method to alleviate osmotic damage and bolster the stability of proteins and cells during the freezing procedure. For clinical-grade EVs, Plasma-Lyte A, an isotonic solution that mimics the salt composition of plasma but lacks proteins or sugars, has been suggested as a suitable storage solution.[265] Ultimately, all aforementioned steps must adhere to current Good Manufacturing Practice (cGMP) guidelines, and manufacturing personnel must comply with cGMP protocols.[266]

In the batch preparation process, the absence of sensitive high-throughput analysis methods, the diversity of exosome sources and their own high heterogeneity make the quality control of exosomes quite difficult.[267] In addition, since exosomes have harmful substances excreted by parent cells, precise control of the contents of exosomes has also become a major problem in the quality control of exosomes.[268] Blood is the most frequently utilized bodily fluid in EVs research. To improve the reproducibility of blood EV research, it is necessary to consider not only donor issues but also address the challenge that the physical properties of soluble macromolecules, cells, and nonvesicular nucleic acids abundant in blood may significantly overlap with those of EVs.[269] The International Society for Extracellular Vesicles (ISEV) Blood EV Working Group has created the Minimum Information for Studies of Extracellular Vesicles in Blood(MIBlood-EV), which covers three parts: general study information, blood collection and processing, and quality control.[270] MIBlood-EV serves as a tool for documenting and reporting preanalytical parameters related to blood collection, processing, and storage, as well as detection methods for assessing the quality of these preparations. It aims to provide guidelines for improving the quality of blood extracellular vesicle research through transparent and collaborative processes.[270]

6.4. Drug Regulation and Ethical Considerations for Exosome Therapy. In terms of drug regulation, there is no clear regulatory framework for exosomes as a new type of biological tissue product both domestically and internationally. In the context of exosome therapy, the United States Food and Drug Administration (FDA) released a public safety announcement concerning exosome-based products in 2019, followed by a consumer warning in 2020 about regenerative medicine products that incorporate both stem cells and exosomes.[271] However, to date, regulatory bodies have yet to provide directions on assessing the safety and effectiveness of exosomes. Given that exosomes are cell products, their quality control and guidelines can temporarily refer to the relevant principles of cell products.

From an ethical standpoint, selecting autologous cells or allogeneic cells with minimal immunogenicity as the source for donor cells necessitates adherence to pertinent regulations and the fulfillment of ethical review criteria. In addition, due to the complexity and potential risks of exosomes, medical experts must also take measures to address social concerns when promoting the clinical use of artificial exosomes. These limitations hinder the further development of exosomes.

7. SUMMARY AND OUTLOOK

7.1. Advantages of Exosomes as Markers and Therapeutic Targets for Cardiovascular Diseases. The advantage of exosomes, whose sources, quantities, and cargoes vary under pathological conditions, suggests that they can become disease-specific biomarkers, reflecting the disease status of patients in real time and dynamically. Exosome-based complex biomarkers provide a comprehensive multiparameter diagnostic test for disease monitoring and have significant potential in diagnosing a wide range of diseases.

At the same time, exosomes can be distributed throughout the body's cells, tissues, and organs through a variety of body fluids, and participate in a variety of pathophysiological processes during the development of the disease, which determines its potential as a therapeutic target. The strategy of engineering exosomes provides exciting opportunities to expand the therapeutic capabilities of exosomes beyond their natural functions. With the exploration and development of exosomes, exosomes have become a better choice for drug delivery due to their low immunogenicity, toxicity, stability, excellent biocompatibility, and promising scientific research platforms.

7.2. Application Prospects of Precision Medicine Based on Exosomes. Driven by the trend of precision medicine, the field of exosomes has developed rapidly in recent years. To date, more than 600 clinical trials worldwide have utilized EVs for diagnosis and treatment, paving the way for their future use in standard clinical practice.[272] Exosomes have been applied in a myriad of contexts, encompassing neurodegenerative disorders, damaged organs, degenerative processes, infectious diseases, regenerative procedures, cancer therapy, and immune function. The amalgamation of exosome studies with nascent domains like nanotechnology, bioengin-

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eering, and artificial intelligence (AI) has yielded novel, tailored approaches to addressing diverse health challenges. Ketki Kalele et al. have integrated machine learning and AI technologies to swiftly and comprehensively analyze pivotal molecular expressions within cancer-associated exosomes to tackle the challenge of early cancer biomarker detection and facilitate precision medicine.[273] Additionally, exosome barcode technology facilitates the discrimination of unique sets of surface proteins linked to exosomes originating from various sources, which may serve as indicative markers for tissue-specific involvement in diseases.[274]

7.3. Scientific Issues That Need Further Exploration in Exosome Research. At present, the clinical transformation of exosomes faces many problems and challenges. Considering the diversity of exosomes, the processes of isolation, extraction, and characterization of exosomes must be standardized to advance their clinical utilization. The diagnosis of exosomes requires reliable biomarkers. Exosomes need to be optimized and improved in loading capacity and targeting before they can be used on a large scale in clinical practice. In the process of engineering and modifying exosomes, researchers must not only consider the appropriate source of artificial exosomes and the uniqueness of various biological targets, but also solve problems such as safety, instability and biocompatibility.

As an emerging field, exosomes have attracted much attention and can be used for cell-free regenerative medicine, treatment of cardiovascular, central nervous system and tumor diseases, immunomodulators and drug delivery carriers. It is believed that the development of new platforms and advanced technologies and the unremitting exploration of pharmaceutical companies and scientific research institutions will expedite both the foundational research and clinical application of exosomes, ultimately benefiting a substantial number of patients.

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