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Novel Insights into Exosomes and Cardiovascular Disorders Review

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Abstract: Extracellular vesicles (EVs) are tiny biological entities, ranging from micro to nano size, surrounded by lipid layers that influence processes like cell movement, new blood vessel formation, and cancer cell proliferation by conveying various biomolecules such as DNA and proteins. EVs consist of exosomes, microparticles, and apoptotic bodies. Their advantages include their ability to pass through biofilms without being broken down by different enzymes, which makes them valuable potential biomarkers for diseases, garnering significant interest from the scientific community. Recent investigations indicate that EVs play a role in the progression of several cardiovascular diseases (CVD), including heart failure and injuries related to myocardial ischemia-reperfusion. Furthermore, EVs derived from stem cells are significant in diagnosing and treating different CVDs. This review discusses the biological characteristics of EVs, their involvement in various CVDs, and the hurdles they face in CVD treatment.

Keywords: Exosomes, Cardiovascular Disorders, Diagnosis, Treatment.

I.Introduction

Extracellular vesicles (EVs) represent a wide-ranging collection of nanoscale particles featuring lipid bilayer membranes, originating from cells.[1-3] Exosomes, in particular, are often recognized for facilitating communication and long-range interactions among cells. Cardiovascular diseases (CVD) rank among the top causes of mortality globally[4], where effective communication and coordination among different cell types are crucial for maintaining organ health and functionality. The heart's cell communication relies on interactions between cells, their connections to the extracellular matrix, and biochemical signals present outside cells. EVs, such as exosomes, microvesicles (MVs), and apoptotic bodies(2-4), are responsible for moving functional components including nucleic acids, proteins, and lipids from donor cells to recipient cells, playing an essential role in cardiovascular interactions and disease progression[5]. The generation and size differences of EVs contribute to their specific roles in disease processes like heart failure, atherosclerosis, and myocardial infarction[6,7].

Recent progress in cardiovascular studies has shed light on EVs produced by various kinds of cells, including cardiac muscle cells, endothelial cells (ECs), and smooth muscle cells (SMCs) [2,8]. These EVs are vital in managing the intricate mechanisms that drive CVD. The components carried within EVs, safeguarded by their lipid layers, are closely associated with CVD disorders, vary considerably among patients, and can be found in bodily fluids such as serum and urine, making EVs attractive options for non-invasive biomarkers in diagnosing and predicting CVD[9]. Additionally, the potential therapeutic applications of EVs in treating CVD are under thorough investigation. Their beneficial qualities, such as easy access, ability to be modified,

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stability during storage, low immunogenic response, and capacity to overcome biological barriers, make them promising tools for managing CVD. The main focus of this review is to clarify the significance of EVs in CVD, particularly highlighting their function in cell communication and their therapeutic potential (Figure1)[26] Subsequently, we will explain how EVs derived from cardiomyocytes, endothelial cells, and macrophages influence the pathophysiology of major cardiovascular diseases such as atherosclerosis, myocardial infarction, and heart failure through the transfer of proteins, lipids, and microRNAs (miRNAs). The protein content of EVs encompasses a glycosidases, which provide valuable information for diagnostics, the development of therapies, and predictive biomarkers in cardiovascular disease. We will also briefly discuss the twofold role of miRNA- loaded EVs in cardiovascular disease , where they regulate gene expressionin receiving cells, affect cardiac remodeling, and act as potential therapeutic targets. Furthermore,wewill examine the possibilities of utilizing EVs as innovative carriers for therapy and predictive biomarkers, emphasizing their significance in the improvement of cardiovascular treatment and diagnostics.

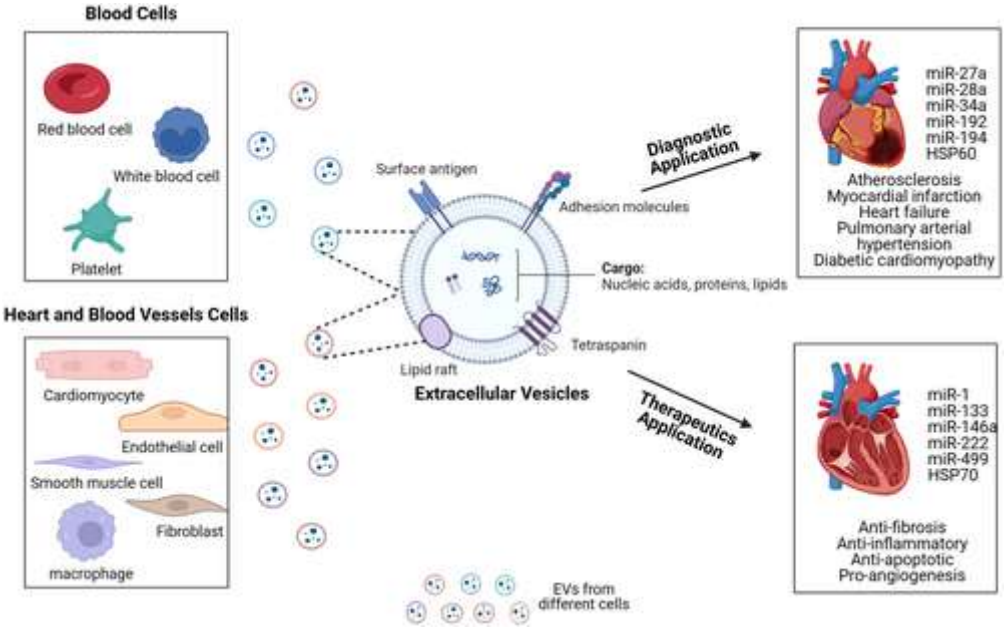

Figure 1 Origins, contents and roles of extracellular vesicles in cardiovascular diseases. Cardiovascular system- related cells, including cardio myocytes, endothelial cells, fibroblasts, platelets, smooth muscle cells, macrophages, red blood cells and white blood cells, can release extracellular vesicles (EVs). Under physiological conditions, EVs play vital roles in preserving normal cardiac structure and function. However, under pathological conditions, their composition changes, contributing to the progression of cardiovascular diseases (CVD). As a result, EVs offer significant potential for monitoring and treating CVD.

Finally, we outlined the existing constraints of extracellular vesicles (EVs) within healthcare settings. Although EVs hold significant promise for cardiovascular disease (CVD) applications, several obstacles impede their practical application. Major deficiencies comprise the absence of standardized manufacturing processes, challenges related to storage, uncertainty about biodistribution and targeted delivery, and the necessity for high-quality clinical trials to confirm safetyand effectiveness. Addressing these challenges is crucial for connecting basic research with clinical practice.

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1. The Physiology of EVs in CVD

1.1The Creation andReleaseofEVs

The production of exosomes is a specific event that occurs within the cell. Initially, the creation of exosomes starts with the endocytosis of the cell membrane, allowing external materials to enter the cell and form early-sorting endosomes (ESEs), which can exchange materials with the trans-Golgi network and endoplasmic reticulum[10]. As these endosomes develop, they slowly evolve into late-sorting endosomes (LSEs), which subsequently progress to multivesicular bodies (MVBs) and generate intraluminal vesicles (ILVs) within the endosomal membrane. ILVs are essential precursors to exosomes[11], making their formation a crucial stage in the creation of exosomes. The generation of ILVs depends on the Endosomal Sorting Complex Required for Transport (ESCRT)[12] complexes (ESCRT-0, -I, -II, and -III) along with key proteins such as ESCRT-III, ALIX, and tumour susceptibility gene 101 (TSG101), which facilitate the development of ILVs by controlling the inward folding of membranes and the separation of vesicles. Following this, mature MVBs either move to the cell membrane or lysosomes, aided by molecules like Ras-associated binding (Rab) GTPases. When MVBs merge with lysosomes, ILVs are broken down; however, if they connect with membranes, endosomes are released into the extracellular environment, forming exosomes. Besides the traditional ESCRT-mediated method, exosomes can also be produced via ESCRT-independent mechanisms involving lipids and Rab GTPases[13]. For instance, lipid raft-enriched areas of the cell membrane may directly fold inward during endocytosis, creating ILVs. This pathway operates independently of ESCRT complexes but still needs specific lipids and membrane proteins, including tetraspanins (like CD63, CD81, etc.), which typically play a significant role in exosome production and function. Likewise, Rab GTPases[14] is engaged in the ESCRT-mediated synthesis of exosomes but also influence exosome production outside the ESCRT pathway. Rab proteins, such as Rab27a, Rab35, and Rab11, facilitate the release of exosomes by managing the transport of MVBs and their fusion with cell membranes. In the absence of ESCRT, these Rab proteins may also directly aid in forming or releasing endosomal vesicles by interacting with membrane proteins and other transport elements.

The formation of microvesicles (MVs) seems to occur through the outward budding and fission of the plasma membrane, generally not involving endosome creation and requiring focused molecular rearrangements within the plasma membrane, including shifts in lipid makeup, proteins, and calcium ions. For instance, enzymes that transfer phospholipids, scramblases, and calpains drive the asymmetric reshaping of membrane phospholipids, culminating in the physical bending of the membrane and the remodeling of the actin cytoskeleton, which promotes membrane budding and MV formation. Cytoskeletal components and their controlling factors are essential for the production of MVs. Growing evidence indicates that the generation of MVs is closely linked to the modulation of cytoskeletal components by small GTPases[15], such as the Rho family and ADP-ribosylation factor (ARF). In malignancies, proteins like Rho-associated coiled protein kinases and the GTP-binding protein ARF6 act as facilitators of vesicle budding[16]. This process bears resemblance to the described formation of retroviral particles externally, focusing on the region where MVs appear due to their affinity for lipids or by anchoring directly to the plasma membrane's constituents.Apoptotic bodies represent another subtype of EVs formed by the budding off of dying cells from the plasma membrane during the process of apoptosis and are typically identified and engulfed by phagocytes[17]. Research has indicated that the creation of apoptotic bodies is influenced by the breakdown of apoptotic cells, which involves factors like ROCK, Pannexin-1, and PlexinB2[18]. In comparison to exosomes, understanding of apoptotic bodies as a subtype of EVs has seen limited exploration.

1.2The Contents inCVD

The relevance of EVs in cardiovascular disease (CVD) largely stems from the nucleic acids, proteins, and lipids that they transport. Notably, miRNAs enriched in EVs have surfaced as significant biomarkers for CVD[19]. Research has revealed that certain miRNAs display atypical expression patterns in conditions like atherosclerosis and myocardial infarction, with the ability to

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facilitate intercellular signaling through EVs[20,21]. These miRNAs influence disease development and progression by regulating gene expression in target cells and managing biological functions including cell growth, programmed cell death, and cellular movement[22]. Furthermore, the process by which miRNAs are sorted into EVs is a precise mechanism that involves the interaction of RNA-binding proteins (like Argonaute and HNRNPA2B1) with miRNAs, facilitating their transport from the cytoplasm and their encapsulation in EVs by recognizing specific sequences, often regulated by lipid microdomains and tetraspanin-rich areas.The creation and release of EVs alongside the miRNA. It depicts the formation, sorting, and release of miRNAs through apoptotic bodies, microvesicles, and exosomes. The journey of miRNAs begins within the nucleus, where primary miRNAs (pri-miRNAs) are synthesized by RNA polymerase II and processed into precursor miRNAs (pre-miRNAs) via Drosha. Following this, pre-miRNAs are transferred to the cytoplasm with the help of Exportin-5 and are further cleaved into mature miRNAs by Dicer. Once matured, these miRNAs may either remain in the cytoplasm for cellular activities or be directed into extracellular vesicles. miRNAs with CELL motifs or 3′-adenylation remain in the cytoplasm and associate with AGO proteins to create the RNA-induced silencing complex (RISC), which regulates mRNA degradation or inhibits translation. In contrast, miRNAs with EXO motifs or 3′-uridylation are recognized by RNA-binding proteins (RNPs) like hnRNPA2B1, YBX1, and SYNCRIP, allowing for their selective sorting into exosomes or microvesicles. Group 1 RNPs (such as hnRNPA2B1, YBX1, MVP) assist in integrating miRNAs into exosomes via multivesicular bodies (MVBs), while Group 2 RNPs (like hnRNPU, Alyref, Cav-1) guide miRNAs into microvesicles through budding from the plasma membrane. Exosomes, ranging from 30 to 150 nanometers, are expelled into the extracellular space through the fusion of multivesicular bodies with the plasma membrane, whereas microvesicles, measuring between 100 and 1000 nanometers, are released directly from the plasma membrane. Furthermore, apoptotic bodies, which are sized between 1 to 5 micrometers and arise during the process of apoptosis, contain miRNAs alongside other cellular materials. The release of miRNAs is also regulated by particular enzymes like nSMase2, which plays a crucial role in the formation of exosomes. This intricate system guarantees the careful sorting and release of miRNAs, facilitating communication between cells and the regulation of genes.

The proteins found in extracellular vesicles, including Alix, TSG101, HSP70, Syntenin-1, and tetraspanins (for instance, CD63 and CD81), not only indicate the traits of their original cells but also contribute to the development and functionality of the vesicles. For instance, HSP70 assists in the recovery process following myocardial damage by serving a protective function in the cardiac cells' stress response[23]. Additionally, Post-Translational Modifications such as phosphorylation and glycosylation of proteins within the extracellular vesicles are closely related to the advancement of cardiovascular disease and may act as potential indicators for early detection and ongoing monitoring[24]. Lastly, the lipid makeup of extracellular vesicles enhances their stability and facilitates their uptake by cells. The membranes of these vesicles are abundant in cholesterol, sphingomyelin, and phosphatidylserine, which not only strengthen their membrane stability but also aid in targeting specific recipient cells within the cardiovascular system. Lipid elements in extracellular vesicles are crucial for the transfer of signals between cells, particularly in the context of cardiovascular disease, as they can influence pathological changes and support tissue repair through the modulation of cell-to-cell signaling[25].

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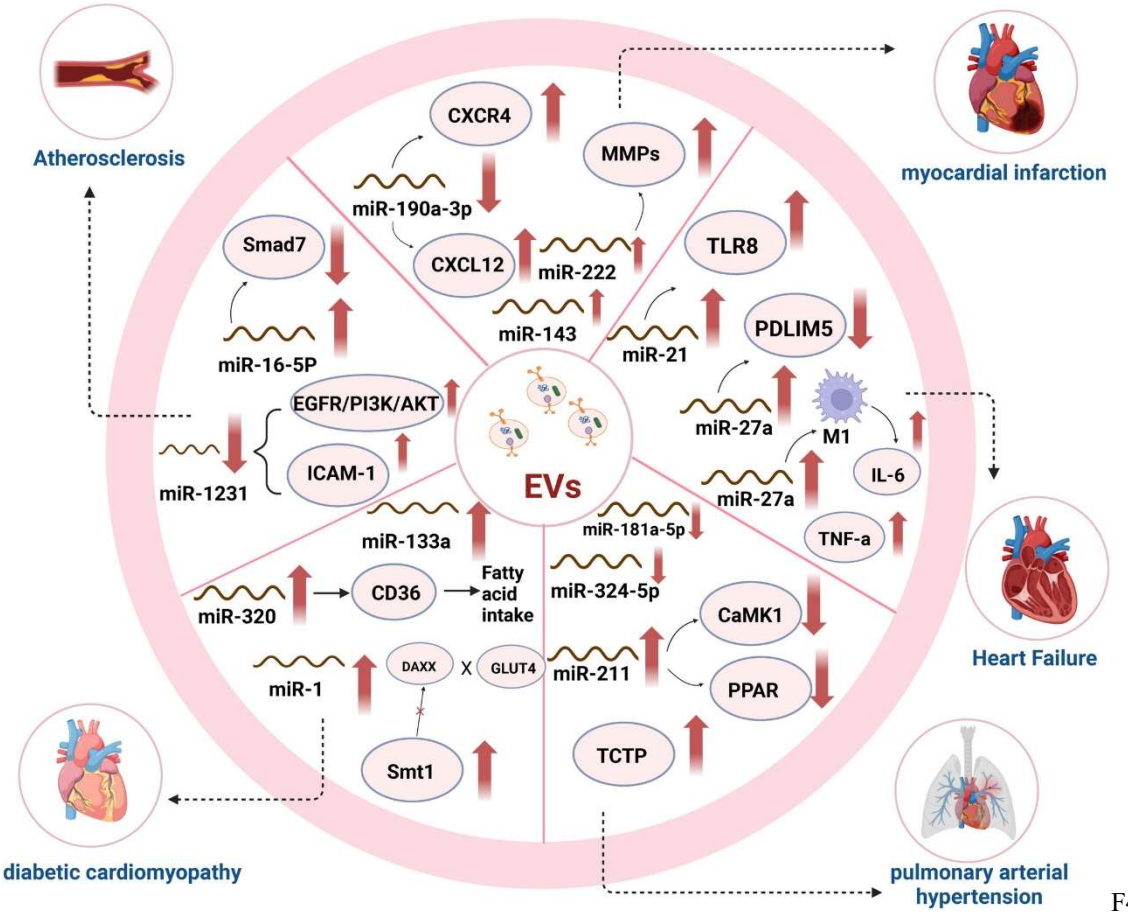

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Figure 2 EVs in CVD. EVs originate from cardiovascular or non- cardiovascular systems construct a complex modulating network for CVDs. The pathophysiological processes of CVDs are mainly influenced by diverse bioactive cargoes of EVs, such as nucleic acids, proteins and metabolites[26]

2. Exosomes as Indicators in Liquid Biopsies for Heart Disease

By examining the origins and components of exosomes, along with their selectivity in targeting cells, we can understand their possible roles as indicators for diagnosis and forecasting. Exosomes play a crucial role in preserving vascular health by enabling communication between cells through the transfer of biomolecules to specific target cells. The quantity and components of exosomes can vary based on different physiological conditions and can be detected in various body fluids[27,28]. Consequently, exosomes found in circulation along with their molecular content, which includes proteins and nucleic acids, might aid in diagnosing cardiovascular disease (CVD) and could act as indicators for these health issues [27,29].

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2.1. Collecting and Analyzing Circulating Exosomes

Collecting high-quality exosomes is essential for clarifying their functions in both normal and abnormal conditions, as well as for investigating their potential clinical uses in various diseases, particularly those affecting the cardiovascular system. For instance, in a model of heart attack, high-quality exosomes can clearly show that their contributions to heart repair come from the exosomes themselves, rather than from misleading cytokines or apoptic bodies[30]. Furthermore, utilizing highquality exosomes improves the consistency of experiments, reduces variability between batches,and provides a solidbasis formechanisticresearch. However, creating a dependablmethod for effectively distinguishing exosomes from other extracellular vesicles (EVs) within biological samples continues to be a major hurdle in the field. This issue is especially evident when analyzing biological fluids, as it often becomes necessary to concentrate or purify exosome samples to enhance the signal-to-noise ratio for identifying exosomes among non-exosomal contaminants. Each existing technique for purifying exosomes introduces particular biases that can be influenced by the sample type and the co-purified contaminants present[29,31,32]. Additionally, the method chosen for isolating exosomes significantly affects the quality of the exosomes obtained, which consequently influences subsequent analyses. Therefore, developing a strong protocol for exosome isolation is critical for ensuring reliable data in exosome research [29].

2.1.1. Ultracentrifugation Methods

Ultracentrifugation methods are currently the most commonly used isolation technique and are regarded as the standard for collecting and isolating exosomes. This process is mainly divided into two stages: first, a series of medium to low-speed centrifugation steps are performed to remove dead cells, cell debris, and larger EVs. After this, a centrifugation force of 100,000g is employed to extract exosomes at a higher velocity, followed by washing the exosomes with phosphate-buffered saline (PBS) to eliminate impurities, including contaminating proteins[32]. This method does not require the labeling of exosomes, decreasing the chances of cross-contamination. For example, Paul and his team have utilized ultracentrifugation to isolate EVs in order to study their role in epicardial signaling following heart injury. This study aims to uncover how these vesicles assist in heart repair and regeneration, which may lead to improved treatment methods for heart-related issues[33].

Moreover, ultracentrifugation (UC) may not be optimal for clinical samples and has difficulty in effectively isolating exosomes amidst other collected biomolecules. The repeated washing involved in ultracentrifugation can diminish coisolated contaminants, but it also risks damaging the vesicles, which could lead to reduced yields[29]. Density gradient centrifugation is tailor-made for exosome purification and is commonly combined with ultracentrifugation to improve their purity. In this method, exosomes are initially concentrated using a 60% iodixanol buffer, which enhances recovery while preserving their biological and physical characteristics more effectively. After this concentration phase, density gradient ultracentrifugation is used to separate the exosomes, successfully removing nonexosomal nanoparticles and protein impurities. Despite the considerable time required for this method, it enables the extraction of highly pure exosomes, and the bioinert nature of iodixanol makes it appropriate for subsequent functional analyses[29,32].In comparison to differential centrifugation, density gradient ultracentrifugation yields exosome preparations with superior puritybut resultsin lower yields andthroughput. Additionally, this method is time-consuming and costly due to the necessity of forming polymer density layers. Thus, it is typically not advised for processing exosomes on alargescale[34,35]. Recently, various new methods have emerged to enhance exosome isolation, including the use ofdensitygradientsandsucrosebasedcentrifugationtechniques. This strategyfacilitates the ascent of vesicles within the sucrose gradient, effectively enabling the precipitation of proteins and impurities at the bottom of the tube. As a result, this method simplifies the elimination of contaminants, allowing for the isolation of exosomes that are free of aggregates[36].

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2.1.2. Immunoaffinity Capture

Immunoaffinity capture efficiently extracts specific exosomes from mixed groups by targeting particular surface markers. This method generally involves magnetic beads that are chemically modified with streptavidin, providing strong binding to any biotinylated capture antibody. This technique demonstrates effective results in isolating specific subsets of exosomes from different cell types[29]. Additionally, it is a gentle approach likely to preserve the biological functionality of exosomes after isolation and is frequently used alongside size-exclusion chromatography (SEC) and other preparatory techniques[34]. Recent advancements in exosome isolation have indicated that antibody-coated magnetic beads can effectively retrieve exosomes from antigen-presenting cells. Selecting appropriate membrane markers for exosomes, like CD81, CD9, and CD63, aids in successful immune capture. Unlike traditional exosome separation methods, magnetic beads coated with antibodies facilitate direct extraction of exosomes from biological fluids, thus minimizing the need for long centrifugation processes. For example, Zarovni et al. utilized antibody-functionalized magnetic beads to isolate exosomes from both cell culture and plasma samples[35].

Recently, a new immunological technique involving a lipid nanoprobe has been introduced, which is designed to separate exosomes from cell culture supernatants free of serum and plasma. In this approach, exosomes are initially tagged with biotin-labeled 1,2-distearoyl-SN-glycero-3-phosphate ethanolamine-poly(ethylene glycol) to focus on the lipid membrane. The labeled exosomal vesicles are subsequently gathered using NeutrAvidin-coated magnetic submicron particles, which enables further extraction and examination of the exosomes[36].

A strategy for directly obtaining exosomal media from intricate samples has also been outlined. This method utilizes Gold-Loaded Nanoporous Ferric Oxide Nanozymes that are first modified with CD63 and suspended in the sample fluid. These nanozymes function as "dispersible nanocarriers," allowing the effective capture of a large number of exosomes. After their magnetic retrieval and purification, the exosomes linked to AU-NPFe2O3NC are moved to screen-printed electrodes that are tailored with antibodies specific to tissue types[37]. Moreover, a new and straightforward technique for exosome separation employing a novel category of carboxyl-functionalized magnetic iron oxide nanoparticles (C-IONPs) has been reported[38]. These nanoparticles were crafted using a method aided by starch, followed by modifications to their surface and characterization. C-IONPs were initially altered with the universal CD9 antibody, acting as a "dispersible nanocore" for the direct retention of exosomes from cell culture. The captured exosomes were then anchored onto screen-printed carbon electrodes (SPCE) that had been previously activated with CA125 antibodies, specific to ovarian cancer. Following this, chronoamperometric tests were performed using a hydrogen peroxide/hydroquinone (HO/HQ) system, taking advantage of the peroxidase-like properties of C-IONPs.

2.1.3 Microfluidic Chip Technology

A microfluidic device, known as a lab-on-a-chip, utilizes fluid dynamics concepts to improve traditional separation techniques. This method lowers costs and maximizes time efficiency while addressing the discontinuous separation methods typical of other prevalent techniques[39]. This technology is also highly beneficial for enhancing biomarker detection by delivering sensitive readings over a wide spectrum, all contained within a compact design[40].For example, the ExoChip that Kanwar and colleagues developed features a microfluidic layout consisting of numerous circular capture chambers interconnected through slender channels[41]. This configuration extends the retention duration of external bodily fluids and supports periodic mixing within the circular areas formed by these direct channels[40]. Additionally, the ExoChip adopts geometric design strategies, allowing for analysis using standard enzyme-linked equipment[29,34]. Moreover, Liu and associates created a size-specific exosome separation chip called ExoTIC, aimed at

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concentrating and purifying exosomes between 30 to 200 nm through several nanoporous membranes[42]. Although this method increases the yield of secreted exosomes, the clogging of membrane pores significantly hinders the ongoing collection of these vesicles[35]. To combat this issue, Chen and team proposed an inventive rapid isolation system that combines a double-coupled harmonic oscillator within a dual membrane filtration framework[43]. This setup produces periodic negative pressure oscillations over a nanoporous anodic alumina membrane by carefully regulating both negative and positive air pressures. This mechanism allows the movement of smaller particles, like proteins and nucleic acids, along with the fluid, while larger exosomes staycaptured in the central section[161]. Although these systems show great potential, they require further implementation to fully realize their capabilities[29]. Additionally, the process of extracting trapped exosomes from the wells is quite lengthy, which restricts the effectiveness of this method in diagnostic uses[34]. Liu and his team introduced a different microfluidic apparatus that initially creates a Mag-CD63-exosome complex by combining with the capture agent Mag-CD63[44]. This complex is then fed in through inlet 1, while the primary antibody enters through inlet 2, resulting in the formation of a Mag-CD63-Exo-Ab1 complex. After that, a secondary antibody that is labeled with a fluorescent tag is introduced via inlet 3 to bind with the specific exosomes, which are later identified using inverted fluorescence microscopy. The findings revealed that breast cancer patients had a considerably higher number of EpCAM-positive exosomes in their plasma compared to the control participants. In another investigation, Castro and his team created an immunomagnetic exosomal RNA (iMER) microfluidic system designed for the extraction and enrichment of cancer specific exosomes coated with antistatic magnetic beads that feature EGFR/EGFRvIII [44]. The segregated exosome subgroups undergo lysis within the chip before being introduced into a glass bead filter. Following this, the mRNA from these exosomes adheres to the glass beads due to electrostatic interactions between the substrate and the mRNA. The collected mRNA is then reverse-transcribed, amplified, and quantified through qPCR[45].

2.1.4. Innovative Techniques for Exosome Characterization

Numerous recent methods for exosome characterization have emerged, including flow cytometry, nanoparticle tracking analysis (NTA), and transmission electron microscopy (TEM).TEM stands as the most commonly employed imaging technique for examining the morphological details of extracellular materials. However, preparing samples for TEM often includes processes like dehydration, chemical fixation, or staining, which can modify the exosome morphology[29]. Cryo-electron microscopy (cryo-EM) is a specific variant of electron microscopy that studies samples at low temperatures. Unlike TEM, cryo-EM is viewed as a superior approach for visualizing nanoparticles, avoiding issues linked to dehydration and fixation artifacts[29,46]. Moreover, cryo-electron microscopy provides improved overall resolution, allowing for the capture of intricate images of the exosome's lipid bilayer. However, the limited availability of equipment and the labor-intensive analysis process have restricted the wider use of cryo-electron microscopy[29].

NTA facilitates simple sample preparation and swift analysis, along with high resolution that permits accurate measurement of nanovesicles. Notably, the collection of samples happens in a liquid phase, which reduces the risk of altering the vesicles being examined. Nonetheless, NTA has certain drawbacks. To achieve accurate results, it is crucial to utilize the “appropriate” dilution factor. The NTA camera must detect each vesicle within the sample, necessitating careful handling to avoid inflating the measurement of larger vesicles, which may overshadow the smaller ones[29,47,48]. Furthermore, NTA faces challenges related to fluorescence signal detection.While next-generation flow cytometers[29,49,50] utilize various angles for detecting forward scatter, thus improving particle resolution, exosomes generally remain below the detection resolution of flow cytometry. To address this challenge, Suarez and colleagues adopted a bead-assisted method for the semiquantitative evaluation of exosomes[51].This method involves the combination of exosomes with aldehyde/sulfate-latex beads, followed by the addition of antibodies and subsequent examination [29156].

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2.2. Analysis of Exosomal Proteomics and Transcriptomics

A wide range of typical proteomic techniques has been utilized to assess proteins that are secreted, including everything from two-dimensional gel electrophoresis to sophisticated quantitative LC-MS/MS methods [52]. These proteomic techniques allow for detailed analysis of proteins found in various biological samples, such as tissues, plasma, serum, and cerebrospinal fluid (CSF), to help identify possible biomarkers [53]. For example, Fernanda G. Kugeratski and colleagues used quantitative proteomics to map out the fundamental exosome proteome, discovering that Syntenin-1 is the most abundant protein, which suggests it could act as a universal biomarker [54]. Transcriptomics has also been crucial in studying exosomes related to cardiovascular diseases (CVD). For instance, miR-21-3p from exosomes of macrophages exposed to nicotine may enhance atherosclerosis progression by fostering the migration and growth of vascular smooth muscle cells (VSMCs) through its effect on the PTEN gene [55]. Additionally, the expression of M2E in VSMCs aids in softening and assisting the repair of vascular tissues. The isolation of M2E also contributes to the differentiation and softening processes of VSMCs. Moreover, M2E stimulates the c-Jun/AP-1 pathway, leading to increased c-KIT expression in VSMCs, which further supports vascular tissue repair [56].

2.3. Exosomal miRNA,lncRNA,andOtherNon-codingRNAsas Indicators

Exosomes and other extracellular vesicles (EVs) are key sources of circulating miRNAs, with those attached to exosomes showing significant promise as new diagnostic markers for heart diseases [27,57]. Matsumoto and associates suggested that miRNAs attached to exosomes could reliably forecast signs of ischemic heart failure in individuals after acute myocardial infarction (AMI) [29,58]. Furthermore, the composition of exosomes may also be crucial for diagnosing cerebrovascular conditions. For example, in patients who have suffered an acute ischemic stroke, serum levels of specific body and brain miRNAs were found to be significantly higher than in a control group [29]. In addition, there was a notable link between the concentrations of miR-9 and miR-124 and the scores on the National Institutes of Health Stroke Scale (NIHSS), the size of the infarct, as well as the levels of interleukin-6 (IL-6) in serum [59,60]. Moreover, certain secreted miRNAs or long noncoding RNAs (lncRNAs) have been proposed to be involved in cardiovascular development and diseases, possibly acting as indicators for coronary artery disease (CAD) [29,61,62].

While current clinical biomarker research primarily concentrates on studies of microRNAs, the biomarkers that have been effectively validated and are used clinically are mainly mRNAs [63]. The significant focus on miRNAs is due to their widespread prevalence and known regulatory functions. Nevertheless, when considering liquid biopsies, longer RNA types—especially mRNAs with recognized mutations and actionable changes—have proven to be more attainable and viable targets. In addition to evaluating gene expression levels and identifying tumor-specific genetic alterations, longer RNA forms provide additional pathways to investigate various biological processes that may reflect disease states or progressions, offering valuable insights into the fundamental mechanisms behind diseases [63].

2.4. The Potentialof Exosomal DNAasaMarkerfor LiquidBiopsies

Exosomes circulate stably within bodily fluids, leading to research regarding their potential as biomarkers for diagnosing and predicting various diseases. [35,64,65] They are particularly recognized for their role in transporting DNA, with the DNA present in exosomes reflecting tumor DNA in multiple cancer types. [63] The increasing application of DNA derived from exosomes as cancer biomarkers for clinical use encompasses genomic changes, [66] mutations, [67] and variations in copy numbers. (63,68) For example, comprehensive genome sequencing revealed that exosomes found in the serum of pancreatic

cancer patients carry a complete set of double-stranded DNA genomes that include all chromosomes. [69] Additionally, mutations responsible for pancreatic ductal adenocarcinoma (PDAC) were found within the examined exosomal DNA. [69,70]

3. Exosomes:asCarriersfor TherapeuticDrugs in CardiovascularDisorders

Exosomes, serving either independently or as vehicles for drug delivery, have the ability to carry bioactive substances such as proteins, genes, RNA, viruses, and other therapeutic materials, thus supporting disease treatment and preventing its progression. [71] Moreover, exosomes are particularly attractive for therapeutic drug delivery due to their low immunogenicity, minimal toxicity, and excellent compatibility with biological systems. [72,73] Below, we will explore the pharmacokinetic aspects of exosomes, their safety and immunogenicity profiles, drug delivery mechanisms, engineered alterations, and contributions to cardiovascular regeneration.

3.1. Pharmacokinetic Characteristics ofExosomes

Pharmacokinetics focuses on how drugs change within the body over time, providing essential insights into their effectiveness in therapy. [74] Administration methods for exosomes are classified into local and systemic approaches. The simplest treatment method involves applying exosomes directly to the targeted tissue. For instance, they can be delivered to brain tissue through the nasal passages or through direct injection into subcutaneous tumors. [75] In contrast, systemic administration involves complex interactions affected by blood circulation, biological barriers, and immune system clearance. [76] Intravenous injection is the preferred method for systemic delivery of exosomes, though this approach faces challenges related to liver clearance and a short duration of action. [77,78] A novel nanovesicle type (hGLV) featuring CD47 overexpression can decrease the clearance rate caused by the mononuclear phagocyte system (MPS), thus prolonging blood circulation time. [79] It is important to point out that endogenous exosomes present in the bloodstream can affect the clearance of administered exosomes. These natural exosomes can hinder the uptake of added exogenous exosomes by macrophages through the activation of the CD36-mediated pathway, serving as competitors in exosome removal by the liver. (80) As they travel throughout the body, exosomes display selective fusion with specific cells, tissue-targeting preferences, and an ability to cross the blood-brain barrier and dense tissue. [81] Most unmodified exosomes migrate to the liver, lungs, and spleen after intravenous administration. [82] In studies using murine models, exosomes derived from blood cells that carried dopamine demonstrated over 15 times greater distribution to the brain, improved effectiveness, and reduced toxicity compared to free dopamine. (83)

3.2. Evaluation of Safety and Immunogenicity of Exosomes

Exosomes are viewed as an encouraging type of advanced therapeutics and drug delivery systems. In comparison to liposomes, they exhibit enhanced stability and better circulation capabilities. When compared to synthetic nanoparticles, exosomes can effectively engage with targets in a reduced blood circulation duration due to their exceptional ability to evade immune responses and their targeting efficiency, thereby decreasing the risk of blood toxicity linked to leukocyte damage. [84] Unlike cell therapy, they mitigate the dangers of cancer and vascular blockage, leading to improved safety. [85] It is important to note that clinical blood transfusions also involve the transfer of billions of exosomes without causing any adverse effects, which further supports the safety profile of exosomes. Luckily, both autologous and allogeneic studies have indicated that exosomes do not exhibit toxicity. [85]

While promising safety profiles have been noted in both preclinical and clinical studies, the unexpected immunogenic traits of exosomes are still not fully understood. [86] Research indicates that microvesicles from pathogenic bacteria carry antigenic markers and harmful factors, and their immunomodulatory capabilities could be explored as innovative vaccine

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vectors, highlighting the positive side of exosome immunogenicity. [87] Conversely, exosomes may also provoke negative immunological responses. Xenogeneic and allogeneic extracellular vesicles (EVs) have higher clearance rates compared to autologous EVs, possibly due to the variability in self-recognition ligand pairs on macrophages that identify foreign cells. [88] For autologous EVs with lower immune clearance rates, the variability of EVs among different individuals and under varying pathophysiological conditions could lead to substantial differences in their therapeutic and drug delivery effects. [89] Emphasis should be placed on the variation between batches of allogeneic EVs to ensure effectiveness remains consistent.

3.3. Exosomes as Transport Mechanisms for Cardiovascular Pharmaceuticals

Integrating cardiovascular medications into exosomes can improve their stability in the body, extend their circulation duration, and enhance their ability to target specific cells. Exosomes utilize surface molecular recognition for a highly selective endocytosis process, aimed at specific cell types. [90] The development of ischemic cerebrovascular disease indicates that ischemia/reperfusion (I/R) injury often triggers increased oxidative stress, marked by the build-up of reactive oxygen species (ROS) that activate apoptosis through mitochondria, disrupting the blood-brain barrier (BBB) and ultimately resulting in further damage to brain tissue. Certain pharmaceuticals can be encapsulated in exosomes. For instance, curcumin, a natural antioxidant sourced from turmeric, possesses antioxidant, anti-inflammatory, and free radical-scavenging properties. [91,92] However, clinical applications of curcumin are limited due to its instability and difficulties in reaching brain injury sites. Exosomes derived from macrophages infused with curcumin (Ex-cur) function as a versatile biomimetic delivery platform, utilizing inflammation-targeting capabilities facilitated by the exosomes alongside the antioxidant characteristics of curcumin to not only cross the BBB but also deliver a notable quantity of curcumin to the affected area. [93] Ex-cur can reduce ROS accumulation, enhance the mitochondrial membrane potential, and prevent the escape of cytochrome c from mitochondria. By suppressing mitochondria-induced neuronal apoptosis within the affected area, Ex-cur mitigates ischemic-reperfusion injury in the brain. [93,94]Following a heart attack or interventions like coronary artery bypass surgery, patients are at a high risk for myocardial ischemia-reperfusion injury (MIRI) [95].

The focused delivery of microRNA-146a (miR-146a) utilizing milk-derived exosomes enhanced with the peptide sequence CSTSMLKAC (IMTP) can have protective benefits for the heart [96]. IMTP allows exosomes to specifically target areas of the heart affected by ischemia. MiR-146a serves as a negative feedback regulator in the nuclear factor-κB (NF-κB) signaling pathway by inhibiting essential adapter proteins such as tumor necrosis factor receptor-associated factor 6 (TRAF6) and interleukin-1 receptor-associated kinase 1 (IRAK1). This action helps to diminish scar tissue and foster more viable cardiac tissue [97].

3.4. Engineering Exosomes for Targeted Delivery of Therapeutic Molecules

Although natural exosomes have shown initial promise in clinical trials, managing their targeting and effectiveness has posed difficulties. Fortunately, advancements in exosome engineering are rapidly emerging, presenting various methods like genetic modifications, chemical alterations, and physical techniques aimed at enhancing the surface properties or the contents of exosomes for clinical use.

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3.4.1. Surface Modification Strategies

To optimize targeted delivery of exosomes, altering the outer membrane of exosomes is crucial for improving their distribution throughout the body and augmenting their therapeutic effectiveness [98]. This section will explore two approaches to modify the surface of exosomes: genetic engineering and chemical modifications.

Genetic editing entails merging the gene sequence of the desired protein or peptide with that of a selected exosomal membrane protein, thus showcasing it on the exosome's surface and guiding them to specific body regions. Xu Wang and colleagues initially applied the ischemic myocardium-targeting peptide CSTSMLKAC alongside the exosome-enriched membrane protein Lamp2b to engineer MSC exosomes for better targeting of ischemic myocardial regions [99].

Chemical techniques involve attaching additional materials to the components of the exosomal surface via linkers, aiming to create exosomes that target distinct tissues and cell types. For instance, Tian and co-workers utilized azide-functionalized c(RGDyK)-conjugated exosomes (cRGD-Exo) to focus on lesions in cases of cerebral ischemia. Curcumin-loaded cRGD-Exo demonstrated significant effectiveness in reducing inflammatory reactions and preventing cell death in ischemic brain mouse model lesions. [100]

Not long ago, researchers from Fudan University created platelet membrane-modified extracellular vesicles, referred to as P-EVs, and connected them with peripheral monocytes to facilitate targeted treatment for heart repair. This advancement holds promise for future immunomodulatory approaches to myocardial ischemia-reperfusion and various immune-related conditions [101].

3.4.2. Therapeutic Nucleic Acid Molecules Loaded Endogenously

The introduction of drugs into exosomes can be divided into two categories: exogenous and endogenous methods. Exogenous techniques include electroporation, coincubation, ultrasound application, and freeze-thaw methods [102]. In contrast, endogenous drug loading utilizes the natural formation process of exosomes to incorporate the desired molecule into exosomes, which are secreted from donor cells and subsequently isolated and purified back into drug-loaded exosomes. Since Valadi and colleagues first documented in 2007 the transport of mRNA and microRNA through exosomes as a novel means for gene exchange between cells, studies on the endogenous loading of therapeutic nucleic acids into exosomes have progressed significantly [103]. Rongchuan Yue and colleagues introduced miR-182-5p into mesenchymal stem cells (MSCs) and demonstrated that the resultant exosomes could target GSDMD, reduce inflammation and cell death in vivo, and positively affect ischemia-reperfusion injury in cardiomyocytes [104]. Li Qiao and team incorporated miR-21-5p into cell culture to generate exosomes that could restore cardiac repair capabilities in individuals suffering from heart failure. The miR-21-5p contained in these exosomes promotes new blood vessel formation and enhances the survival of cardiomyocytes by increasing Akt kinase activity [105].

Given that exosomes can protect nucleic acid molecules from degradation while effectively targeting receptor cells, efforts are being made to engineer exosomes capable of delivering specific miRNA or small interfering RNA (siRNA) payloads for treating cardiovascular diseases. Utilizing a strategy of in vivo self-assembly for exosomal siRNA, Fu and colleagues successfully automated the process of siRNA from expression to loading and delivery. They devised a gene circuit that can express siRNA in vitro, making it possible to insert multiple siRNAs and targeting peptides flexibly [106].

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3.5. Stem Cell-Derived Exosomes for Cardiovascular Regenerative Medicine

Mesenchymal stem cells (MSCs) represent a particular form of stem cell. While their ability to differentiate into multiple lineages is promising for cardiovascular regenerative medicine, the practical use of transplanted cells is restricted by low survival rates, challenges in differentiation, and the risk of causing tumors [107,108]. Fortunately, exosomes extracted from mesenchymal stem cells come with benefits like the transportation of advantageous factors from parent cells, lack of immunogenicity, non-tumorigenic properties, and high stability, indicating strong potential for clinical applications. Numerous investigations are reaching a common understanding that exosomes play a crucial role in mediating the repair effects of cardiovascular stem cells.

Insufficient cardiomyocyte proliferation following heart damage can result in undesirable healing patterns and the formation of fibrotic scars, ultimately leading to heart failure [109]. Exosomes derived from human umbilical cord mesenchymal stem cells help protect cardiomyocytes from cell death and promote angiogenesis through the Wnt/β-catenin signaling pathway [110]. Exosomes sourced from cardiac progenitor cells (CPCs) boost the survival and growth of H9C2 cells by enhancing Akt expression and activating the Akt/mTOR pathway [234]. Furthermore, exosomes derived from adipose tissue mesenchymal stem cells that overexpress glyoxalase-1 (GLO-1) can improve the formation of new blood vessels in the ischemic hindlimbs and hearts of mice through the miRNA-31/FIH1/HIF-1α pathway, thus alleviating myocardial infarction damage. [111] Exosomes from CD34+ hematopoietic stem cells show a significant presence of angiogenic miRNAs, including miR-126 and miR-130, which aid in vascular development in injured cardiac tissues. [112] High blood pressure significantly raises the risk of health issues and fatalities linked to heart diseases, leading extracellular vesicles to be explored as a potential treatment for hypertension at an early stage. Research indicates that circulating extracellular vesicles with diminished plasma levels found in WKY (Wistar-Kyoto rats) and SHR (spontaneously hypertensive rats) may distinctly modify vascular responses in isolated mesenteric arteries. [113] Exosomes from endothelial progenitor cells (EPCs) have shown effectiveness in promoting wound healing and regeneration by boosting the angiogenic function of endothelial cells through the Erk1/2 signaling pathway. [114]

4. Challenges in Exosome Research and Application

Exosomes can serve as natural carriers for drug delivery and can be genetically modified or chemically altered to improve the precision of drug targeting to specific cells or organs. Nevertheless, because exosomes originate from various rich sources, ensuring their clinical application requires ongoing monitoring of various production processes. This includes selecting appropriate donor cells, standardizing exosome isolation and purification, characterizing their properties, preparing formulations, and implementing quality control measures, analyzing the specific spectrum of exosomes related to individual diseases, and ensuring strict regulatory oversight. [115,116]

4.1. Standardization of Exosome Isolation, Purification, and Characterization

The challenges associated with the isolation and purification of exosomes stem from their tiny size, high quantity, absent specific markers, and the complexity of biological fluids in which they are found, making this a significant focus within exosome research. [89,117] Currently, ultracentrifugation is seen as the industry standard due to its dependable reproducibility, effectiveness, and versatility across different sample types. However, this method requires expensive equipment, is labor-intensive, and the repeated steps of ultracentrifugation may compromise the structural and biological integrity of exosomes, causing their aggregation and the co-purification of non-exosomal materials, potentially

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impacting exosomal proteomics, RNA analysis, in vivo studies, and future clinical uses. [118] Ultrafiltration presents a quicker option compared to ultracentrifugation; however, the shear forces from the applied pressure can often harm exosomes, and dead-end filtration methods may lead to clogging of vesicles and damage to membranes. [119] While slower in pace, size-exclusion chromatography allows exosomes to maintain their structure and biological function during gel filtration, with minimal interaction with the stationary phase, enabling better separation of molecules by size, thus compensating for ultrafiltration's limitations. Combining ultrafiltration with size-exclusion chromatography offers a robust isolation approach, improving yields, quality, purity, efficiency, and consistency. [120] Nonetheless, due to ultrafiltration membranes' vulnerability to clogging and their heightened sensitivity to contaminants, this combined technique is best suited for cells cultured in serum-free conditions. [121]

Affinity-based capture, which utilizes the binding affinity between proteins and their receptors, is a prevalent method for generating exosomes of high purity[122]. This approach satisfies the demanding criteria for isolating exosomes with particular target proteins and is mainly applied in liquid biopsy procedures. However, strategies involving solid matrix separation face numerous challenges, such as damage to exosomes from elution buffers, the capability of isolating only certain exosome subsets, high expenses, and low yield amounts. These issues significantly limit their use, especially for large-scale exosome extraction. In contrast to immunoaffinity capture, precipitation employs polymers to extract exosomes, leading to large quantities. Nonetheless, these polymers can also precipitate a range of water-soluble compounds, reduce the purity of the final product and potentially cause cytotoxicity during cancer treatment [123]. Technologies based on microfluidics are swiftly becoming more popular because of their speed, accuracy, and high output. Presently, common microfluidic instruments seamlessly incorporate size-based separation, immunoaffinity separation, and dynamic separation [39,124].

It's essential to develop standardized techniques for exosome characterization to ensure their safe use in clinical settings. Flow cytometry is capable of measuring exosome particle size, concentration, and surface protein markers. Conventional flow cytometry has difficulty detecting particles smaller than 300 nm. The latest flow cytometry generation utilizes multiple lasers to identify exosomes bound to secondary antibodies linked with fluorophores, greatly enhancing particle resolution; however, it may inaccurately interpret clusters of vesicles with high exosome concentrations as single entities, leading to incorrect conclusions. Nanoparticle tracking analysis [125](NTA) assesses the concentration and size of exosomes based on differences in light scattered by suspended particles and their Brownian motion, requiring no specific labeling and not altering the material, although the equipment is expensive.[126] Tenable resistive pulse sensing (TRP) determines the count and size of exosomes by observing voltage shifts across pore membranes, yet it encounters problems such as clogging, membrane contamination, and the need for specialized equipment, which slows the advancement of this technique[127]. Electron microscopy offers a direct observation method for assessing the morphology and dimensions of individual exosomes and is frequently employed for qualitative evaluation and quality control of isolated samples [128]. Microfluidics is also utilized in the analysis of exosome characterization. A new immunomicrofluidic device featuring a mica channel surface enables the direct quantification of circular exosomes from 30 μL plasma samples within 100 minutes [129]. Furthermore, Western blotting remains the most prevalent technique for identifying and measuring protein markers. Enzyme-linked immunosorbent assay (ELISA) is utilized for economical assessment of specific protein indicators, while mass spectrometry is preferred for the analysis of proteins in complex biological samples. The quantification of lipids generally involves fluorescence microscopy and sulfo-phospho-vanillin assays. For targeted DNA or RNA sequence quantification, PCR is considered the gold standard. The International Vesicle Association has established minimum experimental criteria for exosome identification, which includes both general and individual characterization [130]. Nevertheless, our comprehension of the particular biosynthetic and release processes concerning exosomes continues to be limited.

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4.2. Examination of Customized andDisease-Related Exosome Characteristics

Exosomes act as vital information transporters and significantly influence essential physiological and pathological processes. By assessing their lipid, protein, and nucleic acid compositions, unique and disease-oriented biomarkers can be identified. Nonetheless, conventional methods that rely on integrated and batch analyses often obscure the diversity of exosomes, complicating accurate evaluations and obstructing their utilization in disease assessment. Recently, significant advancements have occurred in the exploration of exosome profiles within academic research. A research group has developed an enhanced technique to detect circulating exosomal mRNA (emRNA), distinguishing it from tissue mRNA, and has characterized new emRNA markers for prostate cancer (PCa) identification, creating a novel emRNA-dependent PCa detection system. Proximity encoding technology (PBA) has emerged as a highly sensitive and specific method for analyzing individual exosomes, capable of encoding and tagging all surface proteins present on single exosomes in high throughput samples. This technology assesses single exosome protein compositions and identifies unique surface proteins that can serve as biomarkers for detecting exosomes released from specific tissues into the bloodstream [131]. Through the use of proximity coding technology, research has indicated that exosome subpopulations expressing ITGB3+ and ITGAM+ show potential as early diagnostic markers and therapeutic targets for colorectal cancer.

4.3. ExosomeBatch Production andQuality Assurance

It is essential to enhance cell culture, isolation, and purification techniques to facilitate the integration of extracellular vesicle (EV) therapy into clinical studies and large-scale manufacturing. The production of exosomes is largely dependent on the source cells, which limits the capacity for secretion and poses challenges along with the costs involved in extensive cell culturing. Efforts to boost exosome output mainly focus on enlarging cell culture dimensions, with three-dimensional (3D) culture techniques proving to be a breakthrough in increasing exosome yield in laboratory settings. The hanging drop approach can bring cells together in the centre of a droplet, encouraging them to naturally create multicellular spheroids. However, in spheroid cultivation, the center often faces low oxygen levels, and the inherent complexity of the hanging drop method restricts overall exosome production [132]. In contrast, the microwell array technique allows cells to be placed in an array of tiny wells, producing numerous 3D spheroids, which enhances exosome production[133]. Unlikeearlier methods,scaffolds replicate invivo environments by creating a supportive microenvironment for cell attachment, which mitigates cell death. Fiber bioreactors consist of multiple hollows, semipermeable fiber membranes attached to a large bioreactor, making them ideal for automated and high-capacity exosome production [134].

Following extensive isolation and purification, selecting a suitable container closure system for the storage of EV products is equally important. Current agreement indicates a preference for maintaining EVs at -80 °C [135]. Cryopreservation employing cryoprotectants is a widely accepted technique aimed at reducing osmotic harm and enhancing protein and cell stability during the freezing process. For EVs intended for clinical use, Plasma-Lyte A, which is an isotonic solution that replicates the salt makeup of plasma but does not contain proteins or sugars, has been proposed as an appropriate storage medium [136]. Ultimately, all previously mentioned procedures must comply with current Good Manufacturing Practice (cGMP) standards, and those involved in manufacturing must adhere to cGMP practices [137].

In the batch preparation phase, the lack of high-throughput analysis techniques, along with the variety of exosome origins and their inherent high variability, complicates the quality assessment of exosomes [138]. Furthermore, exosomes can contain harmful elements released by their parent cells, making it a significant challenge to accurately regulate their contents during quality assurance processes [139]. Blood is the most commonly used bodily fluid in EV research. To enhance the consistency of studies on blood EVs, it is vital to consider not only factors related to donors but also to tackle the issue that the physical characteristics of soluble macromolecules, cells, and non-vesicular nucleic acids that are

prevalent in blood may substantially overlap with those of EVs [140]. The International Society for Extracellular Vesicles (ISEV) Blood EV Working Group has developed the Minimum Information for Studies of Extracellular Vesicles in Blood (MIBlood-EV), featuring three sections: general research information, blood collection and processing details, and quality control measures [141]. Mixblood-EV acts as a framework for recording and reporting preanalytical factors linked to blood collection, processing, and storage, as well as evaluation techniques to determine the quality of these preparations. Its objective is to establish guidelines to enhance the quality of blood extracellular vesicle research through transparent and cooperative methods [142].

4.4. Drug Regulation and Ethical Considerations for Exosome Therapy

Regarding drug regulation, a definitive regulatory structure for exosomes as a novel kind of biological tissue product is absent both locally and globally. In the sphere of exosome therapy, the U. S. Food and Drug Administration (FDA) issued a public safety notice about exosome-based products in 2019, followed by a consumer warning in 2020 concerning regenerative medicine products containing both stem cells and exosomes [142]. However, regulatory agencies have not yet provided guidance on how to evaluate the safety and efficacy of exosomes. Since exosomes are products of cells, their quality control can temporarily reference applicable principles associated with cell products.From an ethical perspective, choosing autologous or allogeneic cells with low immunogenic potential as donor cell sources requires compliance with relevant regulations and meeting ethical review criteria. Moreover, due to the complexities and potential dangers associated with exosomes, healthcare professionals must also take action to address societal concerns while advocating for the clinical application of synthetic exosomes. Such restrictions can impede the advancement of exosome research and therapy.

5. Summary

5.1. Benefits of Exosomes as Indicators and Treatment Targets for Cardiovascular Conditions

Exosomes, which exhibit variations in their origins, amounts, and contents during pathological states, indicate their potential to serve as specific biomarkers for diseases, providing real-time insights into patients' statuses. Complex biomarkers derived from exosomes offer a thorough multiparameter assessment for monitoring diseases and hold great promise for diagnosing a wide array of health issues.

Moreover, exosomes can circulate throughout various cells, tissues, and organs via multiple bodily fluids and engage in numerous pathophysiological functions as diseases progress, highlighting their potential as therapeutic targets. The engineering of exosomes opens up exciting possibilities to enhance their therapeutic roles beyond their inherent capabilities. As research on exosomes advances, they have emerged as an optimal option for drug delivery due to their low immunogenicity and toxicity, stability, exceptional compatibility with biological systems, and encouraging research prospects.

5.2. Future Application of Precision Medicine Utilizing Exosomes

With the increasing focus on precision medicine, the exosome sector has witnessed rapid advancements in recent years. Currently, more than 600 clinical trials worldwide are investigating extracellular vesicles (EVs) for diagnostic and therapeutic purposes, laying a foundation for their potential incorporation into standard clinical procedures [143]. Exosomes have found applications across various areas, including neurodegenerative diseases, organ damage, degenerative issues, infections, regenerative therapies, cancer treatment, and immune responses. The combination of exosome research with emerging fields such as nanotechnology, bioengineering, and artificial intelligence (AI) has produced innovative, customized methods for addressing a variety of health challenges. Ketki Kalele and colleagues have utilized machine learning and AI technologies to quickly and effectively analyse key

molecular expressions found in cancer-related exosomes, helping to resolve the difficulty of identifying early cancer biomarkers and enhance precision medicine [144]. Additionally, exosome barcode technology allows for the identification of distinct groups of surface proteins associated with exosomes from different sources, which could serve as valuable indicators of tissue-specific involvement in diseases [145].

5.3. Scientific Challenges Requiring Further Investigation in Exosome Research

Currently, the clinical application of exosomes encounters numerous obstacles and difficulties. Given the variability of exosomes, it is essential to standardize the methods for their isolation, extraction, and characterization to enhance their clinical application. The accurate diagnosis using exosomes necessitates reliable biomarkers. Improvements in loading capacity and targeting of exosomes are needed before they can be widely used in clinical environments. During the process of engineering and modifying exosomes, researchers must not only identify suitable sources for synthetic exosomes and consider the distinctiveness of various biological targets but also address challenges such as safety, stability, and compatibility with biological systems.

As a developing area of study, exosomes have gained considerable interest and can serve various purposes in regenerative medicine without cell involvement, treatment of heart, brain, and tumor-related diseases, as well as for immunomodulation and drug delivery systems. It is anticipated that the progression of innovative platforms and sophisticated technologies, along with the persistent efforts of pharmaceutical companies and research institutions, will accelerate both the basic research and practical uses of exosomes, ultimately aiding many patients.

II.Conclusion

In recent decades, therapies based on extracellular vesicles (EVs) have seen transformative progress, especially in understanding the biology of EVs, their crucial roles in cardiovascular disease (CVD) dynamics, and their possible applications in diagnosis and therapy. Nonetheless, additional research is required to gain deeper insights into the specific sorting of cargo, internalization mechanisms, and release of cargo by EVs in cardiovascular settings; this knowledge will help clarify inconsistencies in the functionality of similar EVs reported across various studies. This field is still developing, and upcoming mechanistic investigations will reveal factors influencing EV functionality. Differences in the components of EVs under varying pathophysiological states indicate that EVs could identify particular types or stages of cardiovascular disease. As a result, assessing the utility of EVs for a more accurate categorization of CVD stages is necessary. Although the therapeutic advantages of EVs for heart disease have been validated in large animal studies, their use in treating CVD is still at a preliminary stage. Further investigation is required to optimize the efficiency of loading EVs, improve target specificity, and integrate EVs with traditional therapeutic agents.

Abbreviations

Drosha : is a **Class 2 ribonuclease III enzyme** that in humans is encoded by the DROSHA (formerly RNASEN) gene.

Dicer : is an enzyme that plays a crucial role in the RNA interference (RNAi) pathway. It processes double-stranded RNA (dsRNA) into small interfering RNAs (siRNAs) or microRNAs (miRNAs).

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