



Review

Advances in Exosome Engineering for Cardiovascular Applications

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Abstract

Exosomes have emerged as favorable natural carriers of theragnostic molecules. Once considered the “disposal bags of the cells” for cellular metabolic waste, they have emerged as central players in intercellular communication. They carry bioactive-molecule cargo from donor cells to recipient cells, which respond to these signals. The cargo delivered in this process is diverse, encompassing proteins, nucleic acids, small molecules, and even gene-editing tools. Recipient cells take up cargo via one or more mechanisms, including receptor-mediated endocytosis, membrane fusion, or phagocytosis. To enhance their therapeutic efficacy, researchers have developed protocols to engineer exosomes by modifying their cargo profile and surface characteristics for site-specific delivery of bioactive molecules. Compared with their native counterparts, engineered exosomes are expected to be more therapeutically efficacious at lower doses while minimizing the undesired effects often caused by non-specific tissue uptake. While the exosomal cargo profile depends on pre- and post-loading strategies (e.g., electroporation, sonication, membrane permeabilization, or chemical transfection), multiple approaches can modify surface membrane characteristics, including genetic modulation, chemical modification, and physical and hybrid approaches. Each cargo-profile modulation and surface-modification approach has respective advantages and limitations. This narrative review summarizes exosome engineering strategies and their therapeutic use in cell-free therapy. It also examines preclinical and clinical data comparing engineered exosomes with naïve exosomes to demonstrate their superiority.

Keywords

Cargo, Cell-free, Exosome, Engineering, Payload, Preloading, Post-loading

Article History

Received: 11 August 2026

Accepted: 12 October 2026

Revised: 14 September 2026

Available Online: 20 October 2026

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1. Introduction

Exosomes are 30-130 nm in size, lipid bilayer-membranous nanovesicles, and contain various molecular signatures such as miRNAs, mRNAs, DNA, lipids, proteins, nucleic acids, etc. [1]. Almost all cell types in the body, including stem/progenitor cells, secrete them as part of paracrine activity [2]. They constitute the insoluble fraction of the secretome and carry a specific cargo profile [3]. Produced via the endosomal pathway and released by exocytosis from the cell surface, they primarily facilitate intercellular communication by initiating signal transduction for cellular functions ranging from cell survival to metabolic activity and differentiation. Because they can carry a diverse profile of bioactive molecules and withstand harsh manipulation protocols to engineer their cargo and membrane characteristics, researchers are exploring their theragnostic use for targeted delivery of bioactive molecules, drugs, genetic materials, genetic engineering tools, etc. [4]. This narrative review provides an overview of advances in engineering strategies to modulate cargo profiles to deliver selective biomolecules and drugs and to modify membrane surface characteristics for targeted delivery to cells and tissues of interest.

2. Isolation and Characterization of Exosomes

Researchers use diverse methods to isolate exosomes and improve recovery, purity, sensitivity, and specificity [5]. The most commonly used method is isolating exosomes based on shape, size, mass, and density. Other common methods include size-exclusion using ultrafiltration (much faster than ultracentrifugation) or size-exclusion chromatography (which can be combined with other methods), precipitation, microfluidic-based methods, and immunoaffinity; each method has advantages and limitations [6].

After isolation and purification, researchers characterize and quantify exosomes by morphology, particle size, membrane protein composition, and lipid rafts [6]. Commonly used characterization techniques include nanoparticle tracking analysis (NTA), dynamic light scattering (DLS), tunable resistive pulse sensing (TRPS), Western blot, atomic force microscopy (AFM), spectrophotometry, and transmission electron microscopy (TEM). Physicochemical techniques such as NTA, DLS, and TRPS provide information on particle size distribution and concentration [7]. At the same time, electron microscopy (TEM, SEM, or cryo-EM) allows direct detection of their morphology and fine structure. On the molecular level, exosomes are identified by the presence of conserved marker proteins, including tetraspanins (CD9, CD63, CD81, CD151), ESCRT-associated proteins (TSG101, ALIX), and heat shock proteins (HSP70, HSP90), detected using Western blotting, ELISA, or flow cytometry [8]. These complementary characterization methods not only distinguish exosomes from other extracellular vesicles but also provide insights into their functional properties.

3. Typical Exosome and Its Cargo Profile

Typically, exosomes have a composite structure of lipids, nucleic acids, and proteins [9]. They are surrounded by a phospholipid membrane rich in cholesterol, phosphatidylserine, sphingomyelin, ceramides, and fatty acids that primarily contribute to their structural rigidity. Additionally, nucleic acids (e.g., RNA, DNA, mRNA, miRNA, and non-coding RNA) are present in their lumen as cargo for intercellular communication (Figure 1).

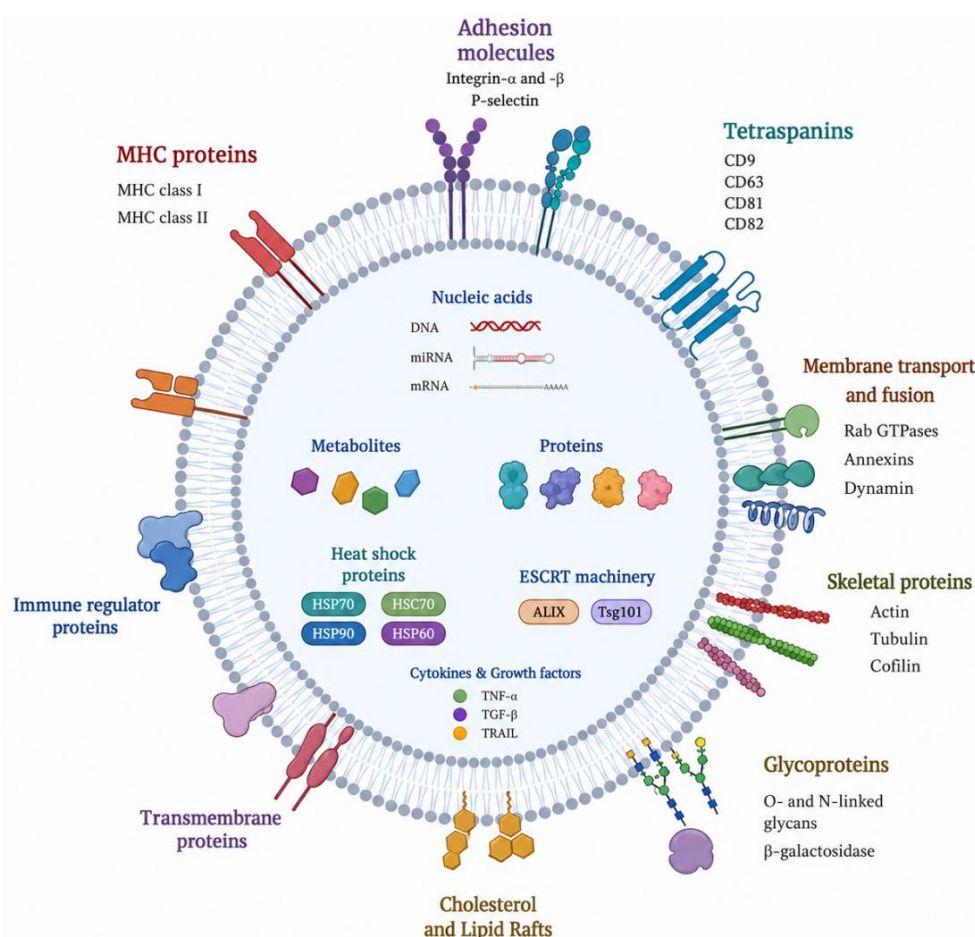


Figure 1. Typical exosome and its cargo.

Credit: <https://app.biorender.com/illustrations/6a64d9d0224364da05fa0cbb?slideId=b31d71e6-712b-4ced-b00d-fce1d79a3ffc>

In addition, exosome protein composition varies by cell type and body fluid. They carry proteins involved in biogenesis, sorting and signaling, respectively, like ESCRT machinery (ALIX, Tsg101), Tetraspanins (CD9, CD63, CD81, CD82), Heat shock proteins (HSP70, HSC70, HSP90, HSP60), others Membrane transport and fusion (Rab GTPases, Annexins, Dynamin), Adhesion molecules (Integrin- α and - β , P-selectin), Skeletal proteins (Actin, Tubulin, Cofilin) which has role in trafficking and secretion, Glycoproteins (O- and N-linked glycans, β -galactosidase), and Growth factors and cytokine (tumor necrosis factor- α (TNF- α), transforming growth factor β (TGF- β), TNF-related apoptosis-inducing ligand (TRAIL)) [9]. Exosomes also carry metabolites and active enzymes, including amino acids, nucleotides, sugars, catalase, and superoxide dismutase, which allow them to influence local metabolism and oxidative stress. The cargo profile of exosomes varies with cell source and cell component, as well as how their parent cells have been manipulated and cultured. For example, MSC-derived exosomes are enriched in anti-inflammatory proteins, angiogenic factors, and regenerative miRNAs [10]. In contrast, tumor-derived exosomes contain oncogenic proteins, glycolytic enzymes, immune-evasive molecules, and pro-metastatic miRNAs. Endothelial cell-derived exosomes transfer molecules that support angiogenesis and cardioprotection [11].

4. Exosome Engineering Approaches

4.1 Exosome Payload Engineering Approaches

Like traditional polymeric nanoparticles and liposomes, exosomes and their hybrid derivatives have been used as delivery vehicles, but with superior stability, biocompatibility, and lower immunogenicity [12]. Moreover, they offer advantages including reduced toxicity, cell-specific targeting capability, an optimal size for uptake via plasma membrane fusion with recipient cells for cargo delivery, extended circulation half-life, and cargo-delivery efficiency [13]. These features make them potential biological carriers for delivering diverse therapeutic cargoes. They can circulate stably in body fluids and can cross physiological barriers, including the blood-brain and myocardial endothelium, positioning them as a potential platform for therapeutic delivery in cardiovascular diseases [14].

Although exosomes are inherently effective because of their natural cargo of bioactive molecules, they can be modulated to efficiently encapsulate small molecules, improving their pharmacokinetic and pharmacodynamic profiles, or surface-engineered for targeted cargo delivery [15] (Figure 2). For instance, in experimental cardiovascular models, exosomes carrying anti-inflammatory compounds such as curcumin reduced infarct size, mitigated oxidative stress, and enhanced

myocardial recovery following ischemia–reperfusion injury [16]. Endothelial cell-derived exosomes loaded with atorvastatin restored endothelial nitric oxide synthase activity, suppressed vascular inflammation, and reduced atherosclerotic plaque formation [17]. Because cardiac progenitor cell-derived and MSC-derived exosomes display natural tropism toward injured myocardium, they preferentially accumulate in ischemic tissue, prolonging local drug exposure and minimizing off-target effects [18].

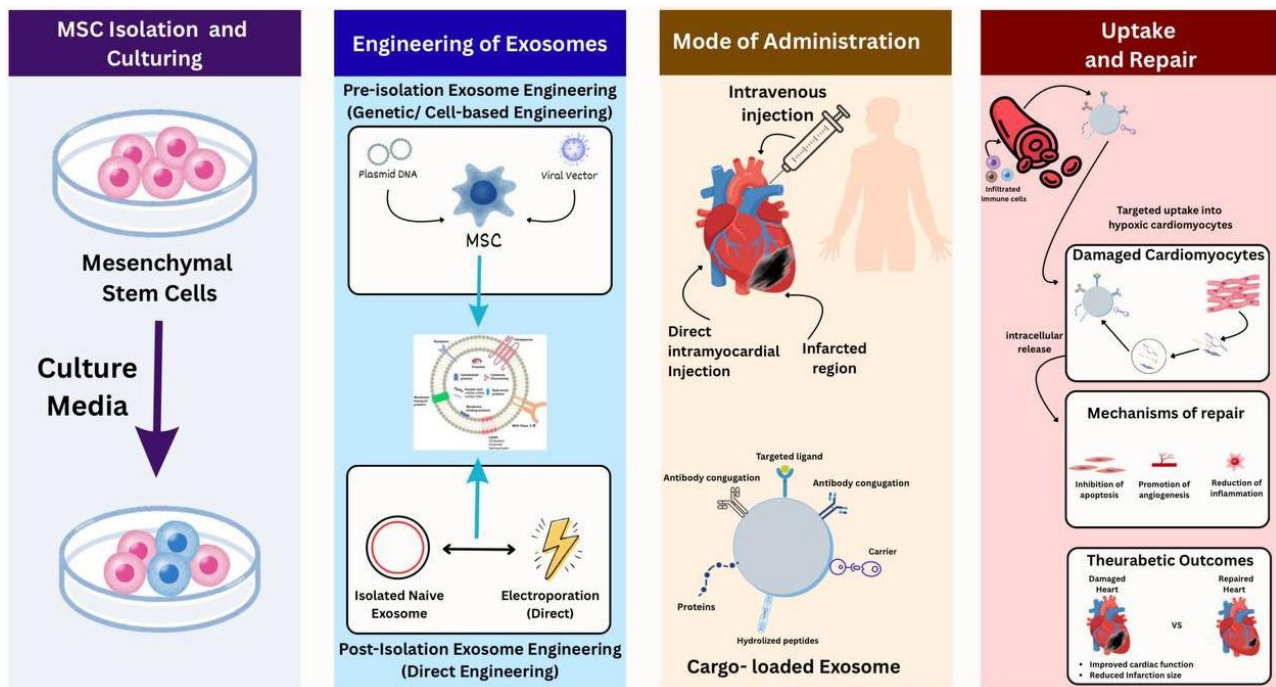


Figure 2. Depiction of exosome engineering strategies for cardiovascular applications.

Because exosomes have a biocompatible membrane that protects large biomolecules from proteolysis and facilitates fusion with target cells to deliver their contents, researchers have used them to deliver bioactive molecules for myocardial protection and repair. For example, MSC-derived exosomes enriched with vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), and hepatocyte growth factor (HGF) have been used for angiomyogenic repair of the ischemically damaged heart. For example, Zhang et al. reported a pre-loading approach to enrich human bone marrow MSC-derived exosomes for VEGF content by genetically modifying the cells to overexpress a VEGF transgene [19]. The authors reported successful VEGF cargo loading in the engineered exosomes with preserved size and surface markers. The authors later used VEGF-loaded MSC-derived exosomes to reduce endothelial cell apoptosis and proangiogenic activity *in vitro* [19]. Likewise, given the significant role of HSPs in exosome biogenesis and as cardiokines, exosomes containing HSP27 and HSP70 exhibited cytoprotective effects in hypoxic cardiomyocytes by stabilizing mitochondrial membranes and maintaining calcium homeostasis [20]. These findings highlight exosomes' intrinsic cardioprotective capacity and their ability to deliver bioactive proteins directly to the injury site.

Exosomes can encapsulate mRNA, plasmid DNA, antisense oligonucleotides, and non-coding RNAs through pre-loading and post-loading strategies, protecting these molecules from nuclease degradation and promoting efficient intracellular delivery [21,22]. As described earlier, exosomes engineered to carry VEGF-mRNA or fibroblast growth factor 2 (FGF2) mRNA enhanced angiogenesis and tissue regeneration in ischemic myocardium [19]. Exosomes post-loaded with mRNA encoding different bioactive molecules are also gaining popularity. Dendritic-cell-derived exosomes expressing the Lamp2b-RVG fusion peptide successfully transported small interfering RNA (siRNA) across the blood–brain barrier to silence the BACE1 gene, marking a milestone in exosome-mediated gene therapy [23]. In cardiovascular disease, exosomes loaded with siRNA against TNF- α , nuclear factor- κ B (NF- κ B), or vascular cell adhesion molecule-1 (VCAM-1) reduced endothelial inflammation and vascular remodeling in atherosclerotic models [24].

Another interesting avenue in exosome cargo engineering is loading miRNAs, given their multifunctional roles in cardiovascular homeostasis and disease [25]. Exosomes carrying specific miRNAs such as miR-21, miR-126, miR-132, and miR-210 have been shown to activate pro-survival and pro-angiogenic signaling cascades, including PI3K/Akt and ERK pathways, thereby enhancing endothelial repair, promoting new vessel formation, and supporting cardiomyocyte proliferation after ischemic injury [26]. In contrast, exosomal delivery of miRNAs such as miR-24 and miR-214 can attenuate excessive extracellular matrix deposition and fibroblast activation, reduce cardiac fibrosis, and mitigate adverse ventricular remodeling [27]. Moreover, endothelial cell–derived exosomal miRNAs such as miR-146a and miR-155 are crucial regulators of immune cell behavior, including macrophage polarization, which can suppress chronic vascular inflammation and contribute to plaque stabilization in atherosclerosis [28]. Clinical studies have also shown correlations between circulating exosomal miRNAs (e.g., miR-21 and miR-126) and endothelial function and disease prognosis in patients with coronary artery disease [28]. The recent integration of CRISPR/Cas9 genome-editing technologies with

exosome delivery has opened new avenues for gene correction in stem cells and cardiovascular disorders [29]. Exosomes can encapsulate Cas9 mRNA and guide RNA complexes, enabling precise gene editing without the safety concerns associated with viral vectors [30]. Viral vectors, particularly adeno-associated viruses (AAVs), and non-viral vectors, such as lipid nanoparticles and polymer-based nanocarriers, are increasingly attractive for cardiovascular applications [31].

In summary, engineering the exosome profile to modulate its properties offers a next-generation approach to developing delivery vehicles that carry treatment-specific cargo and bridge nanotechnology and regenerative medicine. Their endogenous origin, natural cell-specific tropism, and ability to carry a broad range of therapeutic cargoes—from small molecules and peptides to mRNAs, miRNAs, and CRISPR/Cas9 complexes—give them a unique advantage over synthetically developed vectors. Refining exosome profile engineering, scaling manufacturing, and standardizing characterization will accelerate their translation into cardiovascular clinical practice. Moreover, researchers must decide whether to use pre-loading or post-loading approaches, because neither strategy suits many cargo types.

Cargo profile engineering uses different protocols, each with its own advantages and limitations in efficiency, cargo stability, scalability, and the preservation of EVs' stability and integrity. Table 1 summarizes commonly used methods for exosome profile engineering. The passive loading approach uses the simplest protocol, generally exploits the physicochemical properties of cargo molecules, and is gentle on extracellular vesicles (EV) structure and integrity but is relatively inefficient [32]. In contrast, physical methods such as electroporation, sonication, extrusion, or freeze-thaw cycles temporarily alter membrane porosity to allow loading of large cargo. However, the harshness of the protocol may compromise membrane integrity and, at times, cause aggregation of EVs and cargo molecules such as nucleic acids. In contrast, the endogenous loading approach involving donor cell priming is complex and may induce significant batch-to-batch variation, but it remains efficient. Similarly, chemical permeabilization of the donor cell membrane is efficient but may leave difficult-to-remove chemical residues in the final product, raising safety concerns [33]. Therefore, when selecting an EV cargo-loading protocol, one should seek an intelligent compromise between loading efficiency and the stability, integrity, and natural characteristics of EVs and cargo molecules from a translational perspective.

Table 1. Exosome payload engineering approaches.

Modification Strategy	Mechanistic insight	Advantages and limitations	Ref.
Pre-loading approach			
Endogenous	Parent cells are primed or genetically modified to load therapeutic molecules of interest (e.g., miRNAs, mRNAs, proteins, therapeutic peptides) into exosomes during biogenesis.	Exosomes sustain their natural surface characteristics and surface markers, with stable cargo integration. Cargo loading follows a natural process. The limitation is the loss of dosage control of the molecules of interest.	[34]
Post-loading approach			
Exogenous	Isolated and purified exosome preparations are modified for their cargo profile (e.g., small molecules, siRNA, drugs, nanoparticles, etc.) by chemical and physical methods.	Post-loading strategies are flexible, allowing loading of different cargo types and desired dosages. A major limitation is that post-loading protocols may compromise vesicle integrity and cause undesired surface marker modifications, reducing targeting efficiency.	[35, 36]
Electroporation	Electric pulses temporarily increase exosome membrane porosity, allowing loading of cargo molecules such as siRNA, miRNA, and mRNA.	High loading efficiency for nucleic acids; however, the method requires careful optimization to avoid exosome aggregation.	[37]
Sonication	Enhances exosome membrane porosity to facilitate small-molecule uptake.	This strategy is used for loading drugs or proteins. The primary limitation is the risk of vesicle membrane damage if overapplied.	[37]
Chemical modulation/ Transfection	Facilitates entry into exosomes (nucleic acids, small molecules, etc.).	Simple strategy but needs extensive purification to ensure complete removal of residual reagents.	[38]
Hybrid	Combining exosomes with liposomes or other nanoparticles to carry large or multifunctional cargo, i.e., Drugs, imaging agents, nanoparticles.	Increases loaded cargo capacity and multifunctionality; may alter immunogenicity or targeting.	[35]
Click Chemistry	Attachment of exosomes to molecules by covalent bonds after isolation to carry Peptides, small molecules, etc.	Precise, modular modifications can be integrated with targeting ligands, but careful optimization is required to preserve vesicle integrity.	[36]

4.2 Exosome Surface Engineering Approaches

As with exosome engineering for payload profiles, researchers are also developing surface-engineering protocols to improve immunocompatibility, enhance targeted payload delivery, and retain exosomes at the target site long enough to participate in the repair process [39]. Surface engineering modifies exosome membrane characteristics and is expected to significantly improve therapeutic outcomes by enabling homing and docking on target cells to deliver their payload (Figure 2). Leading engineering approaches include LAMP-2B-mediated genetic display of targeting ligands, click-chemistry conjugation, and hybrid Exosome-Liposome Nanoparticles that markedly enhance tissue selectivity and therapeutic potency [40]. Table 2 summarizes widely used surface-engineering approaches.

Table 2. Exosome surface modification approaches.

Modification Approach	Reagent used and Mechanism	Purpose	Ref.
Genetic (Donor-cell engineering)	Donor cell genetically modified to express a fusion protein or ligand on the exosome surface. For example, Lamp2b fused to neuron-targeting RVG peptide; integrin-specific peptides fused to membrane proteins.	This allows high-specificity targeting and uniform modification throughout biogenesis.	[34]
Chemical/Covalent conjugation	Chemical linkers bind ligands, PEG, antibodies, or aptamers to the exosome surface (via amine, carboxyl, or click chemistry). Examples include EDC/NHS coupling, maleimide-thiol reactions, PEGylation, and click chemistry.	It extends circulation time and enables flexible functionalization for imaging or targeting.	[41]
Physical/Hybrid methods	Incorporation of nanoparticles, coatings, or membranes onto exosomes or fusion with synthetic vesicles.	Adding magnetic guidance, imaging contrast, or hybrid delivery properties.	[35]
Glycan/Ligand display modification	Modifies carbohydrate and ligand composition on the surface to facilitate uptake or targeting: Glycan modifications, aptamer display, peptide ligands (iRGD).	Improves selective uptake by target cells, reduces off-target accumulation.	[42]
PEGylation or 'stealth' coating	Applying PEG or polymers to decrease immune clearance by the RES system. For example, PEG grafting, polymer coatings.	It increases circulation time and reduces opsonization and macrophage uptake.	[43]

Genetic modulation to overexpress homing peptides or ligands on the EV membrane remains the most commonly used method for exosome surface engineering. The protocol generally involves delivery of a vector carrying the gene of interest to the parent cells, which then serve as tiny factories producing exosomes with the desired surface characteristics. For example, Alvarez-Erviti et al. transfected a plasmid encoding a neuron-specific rabies virus glycoprotein RVG fused to LAMP-2B into dendritic cells, which subsequently released exosomes with brain tissue-specific uptake [23]. The authors later used the surface-modified exosomes to deliver siRNA to neurons, microglia, and oligodendrocytes. In another interesting development, Wang et al. reported a cardiac muscle-targeting peptide (SKTFNTHPQSTP) fused with LAMP-2B to transfect satellite cells. The genetically modified cells were cultured to isolate cardiac-specific exosomes and were loaded with miR-26a (Exo/miR-26a) to treat complications of chronic kidney disease [44]. In a similar study, Wang et al. developed cardiac cell-targeted exosomes expressing an ischemic myocardium-targeting peptide (IMPT, CSTSMLKAC) in MSCs. These MSC-derived exosomes exerted cardioprotective effects in acute myocardial infarction (MI) [45]. Metkowsky and Kang engineered Cardiosphere cells (CDCs) to express Lamp2b fused to a cardiomyocyte-specific peptide (CMP; WLSEAGPVVTVRALRGTSW). Exosomes isolated from engineered CDCs expressed CMP on their surface, retained their native physical properties, and showed increased uptake by cardiomyocytes [46]. Although it is easy to alter the surface display of peptides and proteins, this approach is limited to targeting motifs that are genetically encodable [47].

Beyond genetic engineering, the exosome surface can be selectively engineered with various chemical molecules or functional groups, such as saponin, carboxyl, amino, azide, alkyne, maleimide, thiol, disulfide, biotin, and many others [48]. This approach to exosome surface modification uses native groups and foreign moieties for both therapeutic and diagnostic purposes. Usually, researchers intentionally embed foreign moieties into membranes through metabolic engineering. More recently, Zhang et al. have reported a technology for site-specific functionalization of exosomes with different synthetic groups by exploiting surface-expressed CD38, an ADP-ribosyl cyclase (ARC), and its covalent inhibitor derived from nicotinamide adenine dinucleotide (NAD⁺) [49]. These specially designed exosomes (ARC Exos), loaded with fluorescent imaging probes, small-molecule ligands, cytotoxic drugs, and bone-targeting agents, have shown activity and specificity *in vitro* and/or *in vivo*. Advances in click chemistry offer a precision-based, versatile approach to exosome surface engineering for site-specific targeting [50]. For example, using concepts from materials science, magnetic particles can functionalize exosomes to home them to the target site with their payload [51]. Similarly, click-chemistry conjugation of antibodies to the exosome membrane is widely used in cancer immunotherapy [52]. Despite immense progress and significant outcomes, the approach has limitations. For example, chemical surface engineering

may modify membrane characteristics by changing the original distribution and density of membrane proteins on the exosomal surface or even denaturing them, leading to loss of intrinsic functionality and affecting therapeutic efficacy.

Physical manipulation and hybrid approaches are also gaining popularity because of their safety profile and because they offer the best of a combinatorial approach. For example, hybrid nanosystems for exosome engineering combine nanoparticles with exosomes to exploit the benefits of both while overcoming the limitations of using each alone [53]. The resulting “hybrid exosomes” combine the inherent biocompatibility of natural exosomes with the versatility of synthetic nanoparticles, improving post-delivery acceptability and enabling superior drug-loading capacity and targeted delivery to tumor sites, resulting in minimized toxicity profiles [54]. Additionally, hybrid EVs are easily modifiable for cargo loading, stability, and targeted delivery, with multifunctional activity. However, in more extensively engineered hybrid exosomes, hybridization can severely alter the product's natural surface characteristics, compromising membrane properties through reorganization of structural and functional proteins, lipid components, membrane fluidity and integrity, and even targeting properties. Moreover, these molecular and structural changes may alter immunogenicity and material-associated toxicity, leading to an altered clinical safety profile. For example, genetic modification can alter membrane-associated proteins, potentially rendering them immunogenic. Depending on the genetic modulation strategy, the residual genetic material in the EV product also requires strict control to prevent potential horizontal transfer. Similarly, chemical modification may introduce extraneous chemical moieties into the membrane structure, making EVs more likely to be recognized by the recipient's immune system [55]. This risk may be accentuated with repeated administration of surface-engineered EV preparations compared with naïve, unmodified EVs [56]. Therefore, protocols must be developed to evaluate responses, complement activation, and immunogenicity, especially after repeated dosing. Additionally, standardized manufacturing, rigorous characterization, and long-term preclinical monitoring will be critical to establish the safety profile of engineered exosomes and facilitate regulatory approval and clinical translation.

Therefore, it is imperative to balance the advantages and limitations of the end product arising from the hybrid approach by using optimized protocols that strike an intelligent compromise between the benefits of developing hybrid products and their limitations. Put together, the hybrid approach may address challenges related to rapid clearance and insufficient therapeutic payload observed in native exosomes.

However, these protocols need optimization to ensure that EVs retain their natural characteristics while benefiting from engineering for targeted delivery to the site of action and payload delivery.

5. Mechanism of EV Delivery

Unlike passive targeting with naïve EVs, targeted delivery requires surface modification so EVs carrying the desired cargo can reach and interact with target cells. Because naïve exosomes passively reach the target site after systemic delivery, they follow complex distribution kinetics affected by several factors, including EV size, membrane proteins, lipid composition, and immune cell surveillance. Much of the delivered dose ends up in non-target clearance tissues such as the liver, kidneys, and spleen. This nonspecific uptake significantly reduces efficacy and, hence, the therapeutic outcome. Surface engineering overcomes these limitations by modifying surface properties through impregnation with peptides, antibodies, or receptors that recognize specific motifs on target myocardial tissue [57]. Upon reaching and binding to target cells, EVs either fuse with them or are internalized to deliver their cargo. Together, surface-engineered exosomes show reduced nonspecific uptake, curtailing undesired outcomes while enhancing target tissue-specific uptake, leading to improved therapeutic efficacy with lower dose requirements [58]. In summary, naïve exosomes rely primarily on intrinsic biological properties and the disease microenvironment for tissue localization, whereas engineered exosomes introduce additional molecular recognition mechanisms to increase target specificity [59].

6. Engineered Exosomes in Experimental Animal Studies and Clinics

6.1 Engineered Exosomes in Experimental Animal Studies

Recent studies report that exosomes contain disease-specific molecular cargo, especially microRNAs, long non-coding RNAs, proteins, and other bioactive molecules [60]. Alternatively, researchers can modulate them to alter their cargo profile and surface characteristics for use in treating cardiac remodeling [61]. Across cardiovascular-focused reviews, many published *in vivo* studies used engineered exosomes in different experimental animal models of cardiac pathologies (Table 3). Engineered exosome products were surface-decorated with targeting ligands, such as peptides that recognize ischemic myocardium and antibodies against cardiac fibroblasts and cardiomyocytes, to enhance homing of stem/progenitor cell-derived exosomes to the injured heart.

Table 3. Experimental studies with engineered exosomes.

Surface Modification	Source of Exo	Summary Results	Ref.
SKOV3- and CPC-derived exosome-liposome hybrids were prepared to combine the properties of liposomes and exosome-encapsulated siRNA.	SKOV3 and cardiac CPC-derived exosome-liposome hybrid nanoparticles encapsulating siRNA.	The functional behavior of hybrids, in terms of cellular uptake, toxicity, and gene-silencing efficacy, was altered. They retained endothelial signaling activation and migration properties.	[62]
CHP-linked EVs for IRI in canine and murine IRI models, as well as H9 human ESC-induced cardiomyocytes or neonatal rat cardiomyocytes.	Healthy human donor plasma-derived miR-486-deleted EVs	EVs with miR-486 attenuated IRI-induced MI and cardiomyocyte apoptosis. The CHP strategy improved EV retention in mice (<i>in vivo</i>) and dogs (<i>ex vivo</i>).	[63]
The nanoparticles consist of an Fe ₃ O ₄ core and a silica shell and carry two types of CD63-specific antibodies.	The two antibodies bind either to CD63 on the EV surface or to myosin light chain on injured cardiomyocytes.	In rabbit and rat MI models, magnetically guided accumulation of captured CD63-expressing exosomes reduced infarct size, improved LVEF, and promoted angiogenesis.	[64]
Mon-EVs for <i>in vitro</i> angiogenic response and in a mouse myocardial ischemia-reperfusion injury model.	Rat BM-derived MSCs fused with Monocyte membrane vesicles prepared by an incubation-extrusion method.	Mon-EVs showed affinity for injured myocardium after MI/RI via the Mac1/LFA1-ICAM-1 interaction. Mon-EVs promoted endothelial maturation, modulated macrophage sub-populations, and improved heart function in a mouse MI/IRI model.	[65]
Using a CHP (CSTSMKAC) to target intravenously infused exosomes.	CDCs and HT1080-derived exosomes	Increased retention of the exosomes within heart sections <i>ex vivo</i> and <i>in vitro</i> with neonatal rat cardiomyocytes.	[66]
EV membrane anchoring platform termed “cloaking” to directly embed tissue-specific antibodies or homing peptides on the EV membrane. ^{Ctrl} CDC-EVs + Qdot 655 or ischemia-targeted exosomes (^{Isch} CDC-EVs + Qdot 655) in IRI mouse model.	^{Ctrl} CDC-EVs + Qdot 655 or ischemia-targeted exosomes (^{Isch} CDC-EVs + Qdot 655)	Primary biodistribution of CDC-EVs was to the liver and kidneys, independent of whether I/R was modeled—significant uptake of the ^{Isch} CDC-EVs in the heart only when I/R was applied vs ^{Ctrl} CDC-EVs.	[67]
Engineered exosomes in a rat AMI model and a hypoxia-induced H9c2 model.	Exosomes from wild-type or miR-146a-modified ADSCs.	miR-146a-rich exosomes suppressed AMI-induced apoptosis and fibrosis. Both <i>in vivo</i> and <i>in vitro</i> results showed that miR-146a suppressed EGR1 expression, which reversed AMI- or hypoxia-induced TLR4/NFκB activation.	[68]

Note: ADSCs: Adipose-derived stem cells; AMI: Acute myocardial infarction; BM: Bone marrow; CDCs: Cardiosphere cells; CHP: cardiac homing peptide; CPC: cardiac progenitor cell; ESCs: Embryonic stem cells; EV: Extracellular vesicles; MI: Myocardial infarction; Mon-EVs: Monocyte mimic-bioinspired MSC-EVs; MSCs: Mesenchymal stem cells; IRI: ischemia-reperfusion injury.

These cardiac-specific modifications enhanced their myocardial tropism, increased their accumulation and retention in the infarct border zone, and participated in the intrinsic repair process by delivering pro-angiogenic, anti-inflammatory, or anti-apoptotic cargo, leading to attenuated infarct size, increased capillary density, and globally preserved cardiac pump function compared with native counterparts (Table 3). Biomaterial-assisted surface engineering, such as exosomes immobilized on injectable hydrogels or cardiac patches, or coupled to adhesive peptides, shows prolonged retention in the myocardium of small-animal models and more durable improvements in ventricular remodeling and fibrosis.

Despite encouraging results from experimental animal studies demonstrating the cardioprotective and reparative potential of engineered EV preparations, their translational potential for routine clinical use remains uncertain because of several limitations, unless they are tested in translational experimental settings [69]. For example, experimental small-animal models differ significantly from their human counterparts. Similarly, the experimental animal models often use acute myocardial injury or relatively homogeneous disease phenotypes, while the patients commonly present with complex, chronic, and multifactorial cardiovascular conditions that may be accompanied by comorbidities such as diabetes, obesity, renal dysfunction, and advanced age. EV pharmacokinetics and tissue distribution in humans may differ from those in the animal model used. The situation may be even more complex, as engineered exosomes may also exhibit batch-to-batch variability and altered biological behavior after surface changes induced during the engineering process. Given these factors, interpret and extrapolate experimental animal data cautiously to clinical settings.

6.2 Engineered EVs in Clinical Perspective

As of 1st August, 2026, search terms “Extracellular vesicles” and “Exosomes” fetched, respectively, 419 records (33 recruiting, 79 completed, and 9 terminated; with 252 interventional and 164 observational studies) and 593 records of clinical trials (40 recruiting, 147 completed, and 18 terminated; with 356 interventional and 236 observational studies)

assessing their use in diverse clinical conditions for theragnostic applications (Clinicaltrials.gov). However, only a few interventional studies assess the safety and efficacy of exosomes in patients with MI and heart failure. One registered phase I study (ClinicalTrials.gov ID: NCT04327635) evaluates the safety of intracoronary EV infusion in patients undergoing intracoronary stenting (EV-CSI). The study uses a single-dose administration of a preparation labeled PEP for intracoronary delivery during percutaneous coronary intervention. An ongoing clinical trial (ClinicalTrials.gov ID: NCT05669144) is designed to co-transplant MSC-derived exosomes and mitochondria to treat heart failure in patients undergoing coronary artery bypass grafting (CABG). The study results are awaited. Despite this progress, we have not found any records of FDA regulatory approval for EV- and exosome-based products.

A major challenge hampering the progress of engineered EV-based therapies in routine clinical applications is the lack of standardization, quality control, and GMP manufacturing to develop clinical-grade engineered EV products. EV-based product quality depends on several factors, including tissue and cell source, *in vitro* cell handling, cell passage, and EV isolation and purification [70]. Standardization becomes even more complex when purified EVs undergo rigorous chemical or genetic engineering protocols to modify their cargo profile and alter surface characteristics. To achieve uniform, clinical-grade preparations, standardized manufacturing protocols combined with robust quality control that meets GMP requirements are essential to ensure batch-to-batch consistency and produce a safe, therapeutically efficacious product. A robust quality-control framework remains central to product development. This also includes microbiological safety parameters such as sterility, endotoxin, and mycoplasma testing. Physicochemical characterization alone may be inadequate to predict therapeutic activity; hence, validated potency assays that reflect the intended biological mechanism are warranted. Well-characterized cell banks, controlled culture conditions, standardized media, validated isolation and purification processes, and predefined acceptance criteria can minimize this variability. In this context, AI, with its transformative potential, can play a pivotal role in process optimization, characterization, and quality control of exosome products to ensure product safety and the therapeutic efficacy required for clinical use [71].

7. Conclusion and Future Perspective

Exosomes are nature's nanotechnology, mediating intercellular communication by delivering bioactive molecular cargo with high precision from donor cells to recipient cells. Moreover, they are robust enough to withstand the procedural rigors of cargo modification and surface-engineering protocols. This enables targeted delivery of the desired cargo for desired and predictable biological responses. The engineered vesicles represent next-generation delivery vehicles and have consistently shown improved therapeutic potential compared with their native counterparts in diverse cardiovascular-related experimental settings. However, standardized, GMP-compliant manufacturing platforms are needed to define their patient safety profile, along with pharmacokinetic and pharmacodynamic parameters to better understand their *in vivo* behavior. Establishing protocols for exosome engineering to home to particular tissues or release payloads in a controlled manner is one of the exciting opportunities and significant challenges the field will face in the future. Ischemia-responsive engineered exosomes, for example, represent a step towards programmable advanced medicinal therapy products. Similarly, hybrid biomimetic nanoparticles and surface-modified vesicles carrying disease-specific ligands represent a leap forward in targeted cargo delivery.

From a clinical and regulatory perspective, a comprehensive understanding of post-delivery pharmacokinetics and pharmacodynamics, biodistribution and tissue-specific targeting for site-specific cargo delivery, immunogenicity, toxicity, and an acceptable safety profile is essential. Beyond scalable production, logistical considerations such as long-term storage and stability of the exosome preparation, cost-effective production, and ready-to-use, off-the-shelf availability must be addressed for routine clinical applicability. Similarly, achieving GMP standards and meeting regulatory requirements are mandatory for producing clinical-grade exosome preparations. Despite its immense potential, judicious AI use requires validation, transparency, and regulatory oversight to move engineered exosomes from experimental status to clinical-grade products for patient use.

Acknowledgments

None.

Funding

No funding supported this publication.

Ethical Statement

Not applicable.

Data Availability Statement

No new datasets were generated or analyzed in this review.

Author Contributions

Khawaja Husnain Haider designed the study protocol, participated in drafting and finalizing the manuscript, and handled the manuscript submission to the journal. Ahmed Rabee, Nouredin Alibhasha, and Mohammad Hamadan reviewed the database screening and data extraction processes and helped draft the manuscript. Ahmed Rabee and Nouredin Alibhasha made Figure 1. Kh Muhammad Mowahid Haider and Adeena Imran included studies, helped revise the draft, and prepared the Tables. All authors reviewed the final manuscript and approved its contents for publication.

Conflict of Interest

All the authors declare no conflict of interest.

Generative AI Statement

The authors declare that they have not used AI-generated work in this manuscript. Grammarly was used for spell check and language revision.

Abbreviations

AAVs: Adeno-associated viruses
ADSCs: Adipose-derived stem cells
AFM: Atomic force microscopy
AMI: Acute myocardial infarction
BM: Bone marrow
CDCs: Cardiosphere cells
CHP: Cardiac homing peptide
CPC: Cardiac progenitor cell
CSP: Cardiomyocyte-specific peptide
DLS: Dynamic light scattering
ELISA: Enzyme-linked immunosorbent assay
ESCs: Embryonic stem cells
EV: Extracellular vesicles
FGF2: Fibroblast growth factor 2
HGF: Hepatocyte growth factor
HSP: Heat shock proteins
IGF-1: Insulin-like growth factor-1
IRI: Ischemia-reperfusion injury
MI: Myocardial infarction
Mon-EVs: Monocyte mimic-bioinspired MSC-EVs
MSC: Mesenchymal stem cells
NF- κ B: Nuclear factor- κ B
NTA: Nanoparticle tracking analysis
siRNA: Small interfering RNA
TEM: Transmission electron microscopy
TGF- β : Transforming growth factor β
TNF- α : Tumor necrosis factor- α
TRAIL: TNF-related apoptosis-inducing ligand
TRPS: Tunable resistive pulse sensing

VCAM-1: Vascular cell adhesion molecule-1

VEGF: Vascular endothelial growth factor

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