Cell-of-origin-dependent extracellular vesicle signaling in the cardiac microenvironment: Pathological roles and targeted therapeutic strategies (Review)

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Abstract. Extracellular vesicles (EVs) are critical mediators of intercellular communication in the cardiac microenvironment by transferring bioactive cargo. Their effects on cardiac injury and repair depend on cellular origin, donor-cell state, recipient cells and disease context. The present review describes the beneficial and detrimental roles of EVs from several cell types in the cardiac microenvironment, with emphasis on cell types, experimental models and the evidence supporting specific cargo-dependent mechanisms. Heterogeneous EV preparations and the difficulty of controlling single-cargo content constrain the interpretation of EV-mediated mechanisms. Therapeutic EV sources, engineering methods and strategies for myocardial targeting are also discussed. Further translation remains limited by inefficient cardiac delivery and by the challenges of reproducible large-scale production. Most therapeutic evidence remains preclinical, and efficacy and safety require further clinical validation. Understanding the cellular context and the strength of the evidence for EV activity is therefore essential for selecting therapeutic sources and designing cardiac-targeted interventions.

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Abbreviations: AF, atrial fibrillation; BMDCs, bone marrow-derived dendritic cells; CPCs, cardiac progenitor cells; CTP, cardiac-targeting peptide; DCs, dendritic cells; DOX, doxorubicin; EAT, epicardial adipose tissue; EVs, extracellular vesicles; HCAECs, human coronary artery endothelial cells; HCMECs, human cardiac microvascular endothelial cells; HFpEF, heart failure with preserved ejection fraction; hiPSC-CMs, human induced pluripotent stem cell-derived cardiomyocytes; HUVECs, human umbilical vein endothelial cells; I/R, ischemia/reperfusion; MI, myocardial infarction; MSCs, mesenchymal stem cells; NRCFs, neonatal rat cardiac fibroblasts; NRVMs, neonatal rat ventricular myocytes; sEVs, small extracellular vesicles; Treg, regulatory T cell

Key words: EVs, cardiac microenvironment, intercellular communication, cardiac injury and repair, EV engineering, targeted delivery

1. Introduction

Extracellular vesicles (EVs) are a heterogeneous family of lipid-membrane-limited vesicles first described in 1983 (1,2). Initially, the EVs were considered as a disposal mechanism for cells to eliminate waste products. However, further exploration has revealed that these vesicles, which carry various biologically active molecules, are now recognized as critical mediators of cellular communication (3,4). Generally, EVs can be divided into three subtypes based on their biogenesis pathways: exosomes, microvesicles and apoptotic bodies. Exosomes, the smallest EVs (50-150 nm), originate from inward budding of multivesicular bodies and subsequent fusion with the plasma membrane. By contrast, microvesicles (200-800 nm) are formed through direct outward budding of the plasma membrane, while apoptotic bodies (≥1 μm) are produced during programmed cell death (5-7). However, due to technological limitations, isolating EV subtypes based on their biogenesis pathways is difficult. According to the 2023 Minimal Information for Studies of Extracellular Vesicles (MISEV2023) guidelines, EVs should be classified by size, including small EVs (sEVs; <200 nm) and large EVs (>200 nm) (8). In addition, this guideline also recommends approaches for EV characterization, including transmission electron microscopy, nanoparticle tracking analysis and

EV-related markers (8). Although some early studies lacked such comprehensive characterization, they contributed to our understanding of EV biological activities and molecular contents.

The cardiac microenvironment includes resident cardiac cell populations and cells recruited from the circulation following cardiac injury. Although cardiomyocytes are the predominant cell type in the heart and their dysfunction causes various cardiac diseases, growing evidence indicates that other constituent cells also play a critical role in cardiac function and homeostasis (9-11). In addition to their autonomous functions, these cells actively remodel the cardiac microenvironment through intercellular communication mechanisms, including paracrine signaling, direct cell-cell contact and EV-mediated cargo transfer (12,13). Therefore, recognizing intercellular crosstalk in the heart is critical for understanding the pathophysiological processes of heart disease and for developing novel therapeutics, as previous studies have reported the functional roles of EVs produced by different cell types in heart disease (14,15). In addition, due to their specific characteristics, EVs are engineered to carry various types of molecules, including drugs and microRNAs (miRNAs or miRs), which have shown promising effects in cardiac diseases. A previous review highlighted the roles of EVs in intercellular communication between cardiomyocytes and non-cardiomyocytes, together with their implications for cardiovascular diagnosis and therapy (16). Building on this work, the present review discusses the beneficial and detrimental effects of EVs from selected cellular sources in the cardiac microenvironment, including T-cell- and dendritic-cell-derived EVs in cardiac repair and allograft immunity. Particular attention is paid to the identity and source of donor and recipient cells, the experimental models used, and the strength and limitations of the evidence. The present review also discusses EV engineering approaches and strategies for cardiac targeting.

2. Literature search methodology

For the present review, PubMed was searched using 'EVs', 'microvesicle' and 'exosome' in combination with terms for relevant cell types, such as 'cardiomyocyte', covering articles searched up to September 2026. For the discussion of EV-mediated regulation of the cardiac microenvironment, focus was addressed on basic experimental studies investigating effects on the heart. No studies directly examining the regulation of the cardiac microenvironment by pericyte-, vascular smooth muscle cell- or B-cell-derived EVs were identified within this search. Registered clinical trials of EV-based therapies were identified by searching ClinicalTrials.gov using the same EV-related keywords. Additional PubMed searches combined EV-related keywords with terms specific to the relevant research fields to identify emerging therapeutic and engineering approaches. The present review did not include any preprints. The present review also included non-English studies that contain English abstracts (Fig. 1).

3. Function of EVs in different cardiac diseases

EVs play critical regulatory roles in the heart, contributing to both microenvironmental homeostasis and disease progression. In various disease models, genetic or pharmacological inhibition of EV biogenesis has been shown to ameliorate disease, whereas administration of native EVs with protective properties can also confer therapeutic benefits. These divergent effects may reflect differences in EV cellular origin, overall physiological or pathological state, and cardiac disease type. In this section, the opposing roles of EVs across different diseases were summarized and the potential factors underlying these differences were discussed.

Following myocardial infarction (MI), EV-mediated communication has been associated with cardiomyocyte survival, regenerative responses and immune-mediated repair (17-23). However, EVs have also been implicated in adverse outcomes, including impaired cardiomyocyte proliferation and increased fibrosis (24,25). Notably, M2-like macrophage-derived EVs have been reported to have both reparative and profibrotic functions (20,26), suggesting that cellular origin alone may not fully determine their effects. Similarly, in ischemia/reperfusion (I/R) injury, EVs isolated from injured hearts exacerbated inflammation and myocardial injury, whereas pre-treatment with an EV biogenesis inhibitor attenuated injury (27). By contrast, protective effects have been reported for selected EV preparations derived from cardiac and immune cells (28-31). Furthermore, inflammatory stimulation can impair the protective effects of endothelial cell-derived EVs (32), suggesting that the state of the source cells may influence EV function. Collectively, these findings highlight the context-dependent roles of EVs in ischemic injury.

Diabetic cardiomyopathy models provide further evidence of EV-associated cardiac remodeling. Under diabetic or high-glucose conditions, EV-mediated communication has been linked to impaired angiogenesis, cardiomyocyte dysfunction and fibroblast activation (33-35). In heart failure with preserved ejection fraction (HFpEF), systemic inhibition of EV secretion and adipose tissue-specific intervention attenuated disease phenotypes (36). Experimental studies have further implicated that EV-mediated communication promoted disease progression in pathological cardiac hypertrophy and remodeling (37-40). However, protective EV activity has also been identified alongside disease-promoting signaling (41). Notably, inhibiting EV release by GW4869 from mechanically stressed cardiomyocytes weakened the anti-fibrotic activity of their conditioned medium *in vitro* (42), implying stress-associated EV signaling is not uniformly detrimental.

The involvement of EVs in atrial fibrillation (AF) has been investigated across several experimental models with distinct substrates for atrial remodeling. In a canine rapid-pacing model, inhibition of EV release reduced atrial fibrosis and AF inducibility (43). Conversely, administered human cardiac-derived EVs reduced inflammation, fibrosis and AF susceptibility in a rat sterile-pericarditis model (44). EVs from immortalized cardiosphere-derived cells also reduced HFpEF-associated AF, although diastolic function remained unchanged (45). Thus, both inhibition and supplementation approaches have shown benefits, but in different models and with different EV populations.

Inflammatory, systemic and immune-mediated conditions reveal further disease-specific roles. In sepsis-associated cardiac dysfunction, EV inhibition and administration of

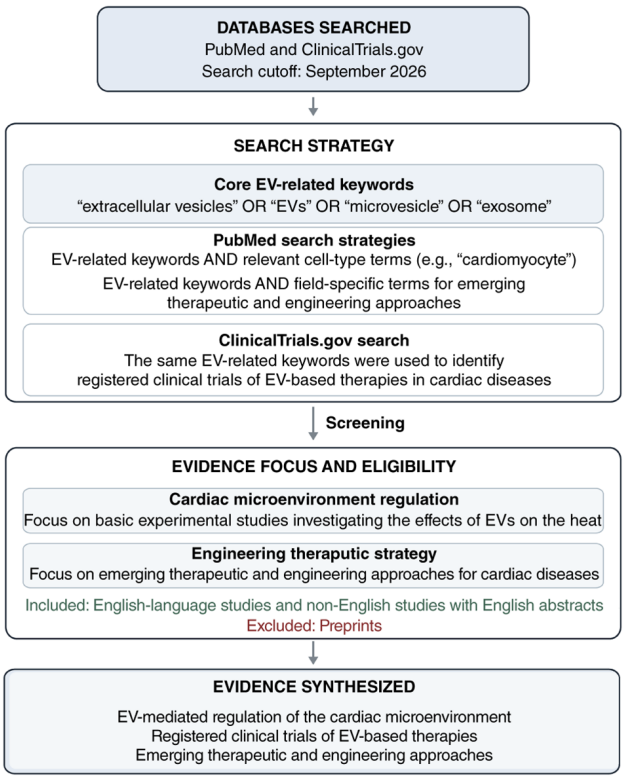


Figure 1. Literature search strategy and evidence synthesis. PubMed and ClinicalTrials.gov were searched through September 2026. PubMed was used to identify experimental studies and studies exploring emerging therapeutic and engineering approaches involving EVs in cardiac diseases, while ClinicalTrials.gov was used to identify registered clinical trials of EV-based therapies for cardiac diseases. EVs, extracellular vesicles.

selected protective EV preparations have both shown benefits in experimental studies (46,47). In uremic cardiomyopathy, macrophage-derived EV signaling has been linked to cardiomyocyte injury and hypertrophy (48). Similarly, EVs facilitate viral transmission in coxsackievirus B3 (CVB3) myocarditis, and reduced secretion in *RAB27A*-deficient mice attenuated disease (49). In cardiac transplantation, EVs can promote donor-cell injury, vascular dysfunction and alloimmune activation (50-52), whereas selected dendritic-cell EV preparations have shown immunomodulatory benefits (53,54).

Drug-induced and genetic cardiomyopathies further show the importance of exposure conditions for EV function. In doxorubicin (DOX)-induced cardiotoxicity, EVs have been implicated in cellular stress responses (55), and pharmacological inhibition of EV release by GW4869 attenuated cardiac injury in mice (56). In Duchenne muscular dystrophy (DMD), cardiomyocyte-derived EVs exerted short-term protective effects in cellular models (57). However, this protection did not persist after prolonged exposure to EVs derived from DMD cardiomyocytes. In addition, GW4869 reduced cardiac fibrosis in isoproterenol-treated DMD model mice (58). These studies suggest that EV function depends on both the disease status of the source cells and the duration of exposure.

Collectively, these studies show that EV effects vary across cardiac diseases and experimental conditions. Interpreting these findings requires consideration of EV source, donor-cell state, disease stage, intervention timing and the outcome assessed. The following cell-type sections examine the cargo, recipient cells and signaling mechanisms underlying these disease-associated effects.

4. Diverse EVs in the heart

Cardiac EVs function as multimodal molecular repositories, encapsulating a dynamic repertoire of proteins, nucleic acids and even mitochondria that collectively govern cardiac physiology and pathology. Cargo is the most critical factor determining EV function. The sorting of cargo into EVs is highly dependent on the types and contexts of donor cells, which is why EVs from different sources play distinct roles in the heart (59). Blocking EV production during cardiac disease is beneficial, while reinfusing EVs from a healthy heart could also ameliorate cardiac disease. So far, numerous EVs carrying various cargoes from different cell types have been identified in the heart (Table I and Fig. 2). However, current isolation methods (such as differential ultracentrifugation) struggle to extract EVs derived from a specific cellular origin *in vivo*, forcing reliance on *in vitro* cell lines that inadequately recapitulate the *in vivo* condition. In addition, current approaches have limited capacity to selectively manipulate a specific EV cargo component without affecting other cargo, complicating the interpretation of the mechanisms underlying EV-mediated physiological effects. Moreover, differences in recipient cells, exposure duration and disease models may lead to opposing effects of EVs derived from the same cell type. Despite these limitations, current research continues to uncover the critical roles of EVs in cardiac pathophysiology. The following sections review the functions of EVs from major cardiac cellular players, including cardiomyocytes, fibroblasts, immune cells and vascular components.

Across the studies discussed below, evidence supports a role for EVs in cardiac intercellular communication, but the strength of support for specific cellular sources and causal cargo varies. Cargo enrichment, vesicle uptake and recipient-cell responses suggest potential communication pathways but do not alone establish that a particular cargo mediates an endogenous effect *in vivo*. This distinction is important because conventional isolation methods may recover heterogeneous EV populations and non-vesicular components, while cultured cells only partially reproduce the multicellular and metabolic context of disease. Differences in donor and recipient models, EV preparation, dose and exposure duration further limit comparisons and may contribute to divergent outcomes. Convergent evidence linking defined donor-cell origins to EV-associated activity and cargo-dependent effects, particularly through appropriately controlled loss-of-function and rescue experiments and validation in relevant *in vivo* models, strengthens confidence in specific mechanisms. Human studies provide additional evidence of disease relevance, although clinical associations alone do not establish causality.

Cardiomyocyte-derived EVs. Cardiomyocytes constitute a predominant cellular population in the human heart, representing 30.1% of all profiled atrial nuclei and 49.2% of all profiled ventricular nuclei in an established adult human heart atlas (60). Because cardiomyocytes primarily drive heart contractility, abnormalities in cardiomyocytes are closely associated with heart disease. Previous research found that cardiomyocytes can communicate with other cells by releasing

Table I. EVs in the cardiac microenvironment and their cargo.

Donor cell type	Recipient cell type	Cargo linked to beneficial effects (reference)	Cargo linked to detrimental effects (reference)
Cardiomyocytes	Cardiomyocytes	miR-21-5p (18) HSP20 (64)	miR-339-5p (70) miR-30a (71)
	Cardiac fibroblasts	miR-24-3p (66) miR-30d (67)	miR-92a (73) miR-208a (74) miR-494-3p (75)
	Endothelial cells	linc-ROR (28) HSP20 (64) GLUT1, GLUT4 (65)	miR-320 (33)
	Macrophages	miR-199b-3p (68)	-
Cardiac fibroblasts	Cardiomyocytes	miR-133a (83)	miR-21-3p (39,87) miR-27a* (40) miR-27a, miR-28-3p, miR-34a (85) miR-23a-3p (86)
	Cardiac fibroblasts	piRNA-62788 (PIWIL2 also detected) (84)	-
	Endothelial cells	-	miR-200a-3p (88)
Endothelial cells	Cardiomyocytes	MEK1/2, HSP90 (32)	MST1 (34) miR-126-3p (94)
	Cardiac fibroblasts	-	TGFB1 mRNA (35)
	Endothelial cells	let-7b-5p, miR-126-3p (92)	-
Macrophages	Cardiomyocytes	miR-1271-5p (19) miR-148a (100) miR-378a-3p (101) miR-145-5p (102) miR-146a-5p (103) miR-124-3p (104) miR-146a (105) Surface transferrin receptor (106) miR-181a-5p (110) miR-29b-3p (113)	miR-155 (24,48) SNHG14 (110) miR-122-5p (111) miR-29a (112) piR-50971 (114) miR-34a-5p (115) TNF-α (116)
	Cardiac fibroblasts	miR-124-3p (104)	circUbe3a (26) miR-210-3p (120) miR-155-5p (122) miR-155 (123) ELAVL1 mRNA/protein (121)
	Endothelial cells	miR-132-3p (20) let-7-family miRNAs, miR-126-3p (108) miR-23b-3p (119)	miR-155 (117) MALAT1 (118)
	Macrophages	miR-146b, miR-378a, miR-23a/b (99) lncRNA AK083884 (107) miR-181b-5p (31)	-
	Cardiac tissue	miR-24-3p (47)	miR-155 (117) ELAVL1 mRNA/ protein (121)
T cells	Cardiomyocytes	FOXP3 (41)	FASL, granzyme B, perforin, TCR and miRNAs (50)
	Cardiac fibroblasts	-	miR-142-3p (25)
	Endothelial cells	SHH (128)	miR-142-3p (51) IFN-γ and TNF (131) MEK1/2 and ERK1/2 (132)
	Cardiac tissue	SHH (130)	-
Dendritic cells	Endothelial cells	miR-494-3p (enriched) (137)	-
	T cells	IDO1 (136) PD-L1 together with donor MHC-I (141)	-
	Dendritic cells	PD-L1 together with donor MHC-I (141)	CD86 (52) CLIC1 (143) MHC-I/II from cardiac donor (52,143)
	Immune responses	CCR7 (22) MHC-I/II from immature dendritic cells (53,138,139)	METTL3 RNA/protein, ICAM-1 (54)

Table I. Continued.

Donor cell type	Recipient cell type	Cargo linked to beneficial effects (reference)	Cargo linked to detrimental effects (reference)
Neutrophils	Endothelial cells	-	miR-155 (144)
	Vascular tissue	-	miR-155 (144)
Platelets	Cardiomyocytes	-	ACSL1 (157)
	Macrophages	miR-4306 (153)	-
Adipocytes/ adipose tissue	Cardiomyocytes	Surface adiponectin (159) VDAC/COXIV (161)	miR-130b-3p (163) miR-130b (164) miR-802-5p (165) ACADM/ACSL1 (166)
	Cardiac fibroblasts	-	miR-23a-3p (167)
	Cardiac tissue	Adipsin (160)	-
Epicardial cells	Cardiomyocytes	miR-30a, miR-100, miR-27a, miR-30e (171)	-

EV cargoes are grouped by donor and recipient cell types and their reported beneficial or detrimental effects. A dash indicates that no specific cargo was reported. Numbers in parentheses refer to the corresponding references. EV, extracellular vesicle; miR, microRNA; HSP20, heat shock protein 20; linc-ROR, long intergenic non-protein-coding RNA, regulator of reprogramming; GLUT1/4, glucose transporters 1 and 4; piR, PIWI-interacting RNA; PIWIL2, PIWI-like protein 2; MEK1/2, mitogen-activated protein kinase kinases 1 and 2; HSP90, heat shock protein 90; MST1, mammalian STE20-like protein kinase 1; TGF-β1, transforming growth factor-β1; mRNA, messenger RNA; SNHG14, small nucleolar RNA host gene 14; circUbe3a, circular RNA derived from the ubiquitin-protein ligase E3A gene; ELAVL1, ELAV-like RNA-binding protein 1; MALAT1, metastasis-associated lung adenocarcinoma transcript 1; lncRNA, long non-coding RNA; FOXP3, forkhead box P3; FASL, Fas ligand; TCR, T-cell receptor; SHH, sonic hedgehog; IFN-γ, interferon-γ; TNF, tumor necrosis factor; ERK1/2, extracellular signal-regulated kinases 1 and 2; IDO1, indoleamine 2,3-dioxygenase 1; PD-L1, programmed death-ligand 1; MHC, major histocompatibility complex; CD86, cluster of differentiation 86; CLIC1, chloride intracellular channel 1; CCR7, C-C motif chemokine receptor 7; METTL3, methyltransferase-like 3; ICAM-1, intercellular adhesion molecule 1; ACSL1, acyl-coenzyme A synthetase long-chain family member 1; VDAC, voltage-dependent anion channel; COXIV, cytochrome *c* oxidase subunit IV; ACADM, medium-chain acyl-coenzyme A dehydrogenase.

sEVs containing proteins and nucleic acids to modify recipient-cell responses and cardiac function (61). Early studies showed that heat shock protein 60 (HSP60) associates with primary adult rat cardiomyocyte sEVs and that HL-1 cardiomyocyte sEVs transfer nucleic acids to NIH-3T3 fibroblasts, establishing a basis for intercellular communication without defining a uniformly protective or detrimental function (62,63). Subsequent research has shown that the activity of sEVs from cardiomyocytes depends on the donor-cell state, recipient cell and duration of exposure. These conditions are critical when interpreting the contribution of cardiomyocyte-derived sEVs to cardiac repair and disease.

Beneficial roles of cardiomyocyte-derived EVs in cardiac repair. Cardiomyocyte EVs can promote myocardial repair by influencing other cardiomyocytes. sEVs from neonatal rat ventricular myocytes (NRVMs) targeted cardiomyocytes to improve cardiac function and reduce scarring after MI in mice. Depleting miR-21-5p in donor-derived sEVs attenuated their activity, supporting a contribution of this cargo to the response (18). Similarly, sEVs from primary adult cardiomyocytes from mice overexpressing heat shock protein 20 (HSP20) improved cardiac function after MI in mice (17). These HSP20-enriched sEVs from adult mouse cardiomyocytes also exerted similar cardioprotective effects in diabetic mice (64).

Beyond targeting cardiomyocytes, cardiomyocyte-derived sEVs also act on endothelial cells to ameliorate several cardiac injuries. After glucose deprivation, NRVMs transferred glucose transporters and glycolytic proteins to endothelial cells through sEVs, thereby increasing glucose uptake and pyruvate production (65). In a hypoxia/reoxygenation model, sEVs from Tongxinluo-pre-treated human ventricular cardiomyocytes reduced injury in human cardiac microvascular endothelial cells (HCMECs) through a response involving long intergenic non-protein coding RNA, regulator of reprogramming (linc-ROR). linc-ROR downregulated miR-145-5p to increase 70-kDa ribosomal protein S6 kinase 1 expression and activate the endothelial nitric oxide synthase (eNOS) signaling pathway. Donor linc-ROR knockdown or interventions in the recipient eNOS pathway attenuated the protective function of sEVs (28).

Cardiomyocyte EVs also inhibit fibroblast activation. sEVs from human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) reduced transforming growth factor-β1 (TGF-β1)-induced myofibroblast differentiation in human primary cardiac fibroblasts, while inhibition of miR-24-3p in donors weakened this effect. Under simulated ischemia, the content of miR-24-3p in sEVs decreased, resulting in the loss of anti-fibrotic activity (66). In addition, miR-378 in cardiomyocyte-derived sEVs has also been reported to restrain fibrosis. During mechanical stress, NRVMs released sEVs enriched with miR-378 to inhibit collagen expression in neonatal rat cardiac fibroblasts (NRCFs). Further experiments identified MKK6 as a direct target of miR-378 and showed that miR-378 reduced P38 mitogen-activated protein kinase (MAPK)/SMAD2/3 activation in fibroblasts, supporting an anti-fibrotic mechanism for this intercellular

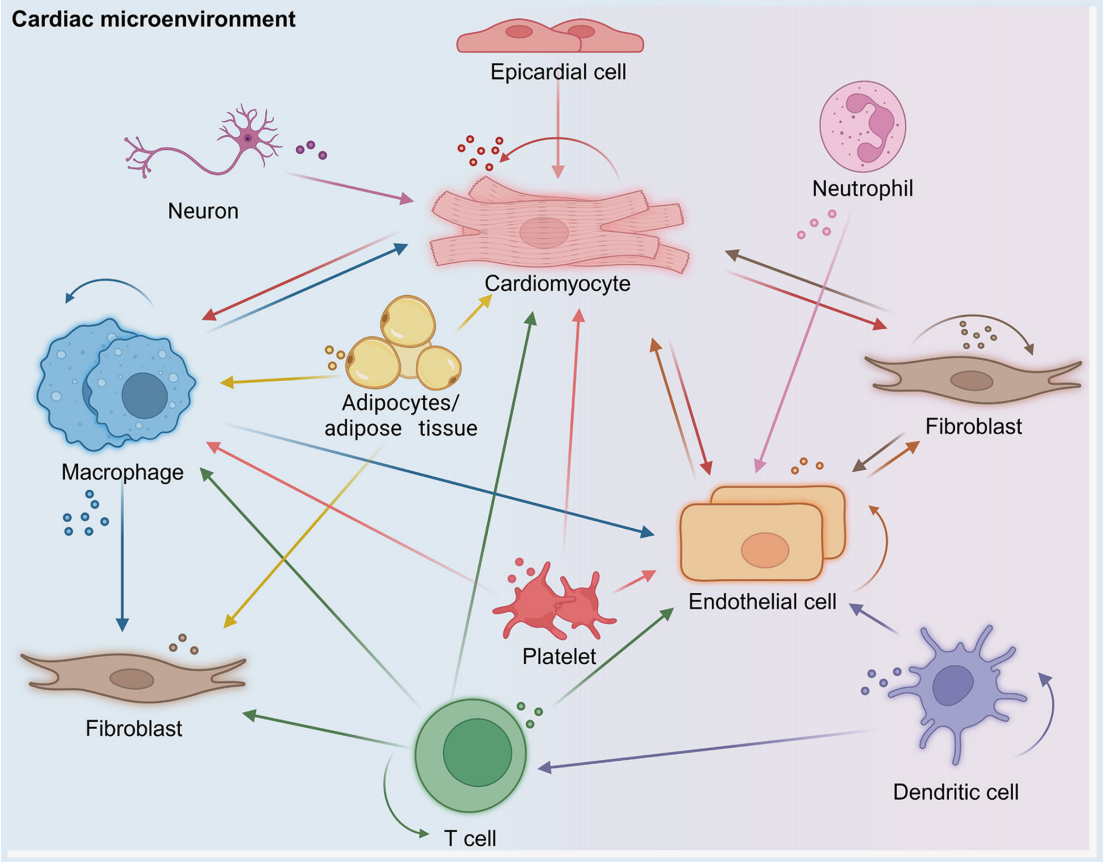


Figure 2. EVs mediate intercellular communication within the cardiac microenvironment. This schematic illustrates the network of EV-mediated communication among major cell types in the cardiac microenvironment, including cardiomyocytes, endothelial cells, fibroblasts, macrophages, dendritic cells, adipocytes/adipose tissue, neurons, neutrophils, epicardial cells, T cells and platelets. EV, extracellular vesicle. The figure was created using BioRender; https://BioRender.com/g8vlt92.

communication (42). Similarly, sEV-associated miR-30d contributes to anti-fibrotic activity. The abundance of miR-30d increased in NRVM-derived sEVs following hypoxia. Transfer of miR-30d reduced expression of its target integrin α5 (ITGA5) in recipient NRCFs, as confirmed by reporter assays. Fibroblast proliferation induced by angiotensin II (Ang II) and TGF-β was also attenuated by miR-30d-enriched sEVs (67).

Macrophages are another target of cardiomyocyte EVs and may influence repair. sEVs from hypoxia/reoxygenation-preconditioned HL-1 cells promoted an anti-inflammatory response in RAW264.7 macrophages by transferring miR-199b-3p. This miRNA further directly decreased FGL2 expression to suppress nuclear factor kappa B (NF-κB) signaling, while *FGL2* overexpression reversed these sEV-induced effects (68). Using Myh6-CreERT2/CD63-GFP mice, Chen *et al* (69) also found that cardiomyocyte sEVs could be taken up by cardiac macrophages. In addition, they used a cardiomyocytic *KIT* mutation to enhance M2-like macrophage polarization. After DOX treatment, miR-142-3p and miR-135a-5p levels were increased in cardiac-derived sEVs and isolated cardiomyocytes of *KIT*-mutation mice compared with DOX-treated wild-type controls (69). Both miRNAs promoted M2-like polarization in *in vitro* transfection experiments using mouse peritoneal macrophages. These results indicate that cardiomyocytes may transfer miR-142-3p- and miR-135a-5p-enriched sEVs to macrophages, thereby ameliorating cardiac injury (69). However, miRNA levels were measured in whole-heart sEV preparations without specifically isolating cardiomyocyte-derived sEVs. Thus, the parallel increases in these miRNAs in cardiomyocytes and cardiac sEVs do not establish their enrichment in cardiomyocyte-derived sEVs.

Detrimental roles of cardiomyocyte-derived EVs in cardiac disease. Cardiomyocyte EV activity depends on both donor status and exposure duration. In some situations, the protective function of cardiomyocyte-derived EVs can also shift to a detrimental role. In hiPSC-CM models of DMD, 2 h of exposure to either healthy or dystrophin-deficient cardiomyocyte-derived sEVs before deoxy-glucose stress protected recipient cardiomyocytes through extracellular signal-regulated kinase 1/2 (ERK1/2) and P38 signaling (57). A subsequent time-course study comparing 2, 24 and 48 h of exposure found that dystrophin-deficient sEVs lost their protective effect at 24 and 48 h, whereas healthy-donor sEVs remained protective (58). In a further study using 48 h of sEV exposure before stress, depletion of miR-339-5p in dystrophin-deficient cardiomyocyte sEVs preserved mitochondrial membrane potential and reduced stress-induced cell death without reducing reactive oxygen species (ROS) under stress (70). These findings indicate that donor genotype and exposure duration jointly shape EV activity, and that loss of protection should be distinguished from cytotoxicity directly induced by EVs. Another study identified a specific miRNA that EVs use to mediate cardiomyocyte injury. During hypoxic injury, the sEVs derived

from H9c2 cardiomyocytes were enriched with miR-30a and taken up by recipient H9c2 cells. Inhibition of miR-30a or treatment with the EV-release inhibitor dimethyl amiloride maintained Beclin-1 and ATG12 expression and reduced apoptosis, indicating that miR-30a-enriched sEVs may contribute to the hypoxia-induced apoptosis pathway (71). However, these functional experiments did not fully distinguish the contribution of sEV-transferred miR-30a from its intracellular effects.

Endothelial cells are another recipient population through which cardiomyocyte-derived EVs may contribute to cardiac injury. Using an intermittent hypoxia (IH) model, researchers identified 63 dysregulated miRNAs (such as increased miR-125b-5p and decreased miR-101a-5p) in primary adult cardiomyocyte-derived sEVs compared with the control group. sEVs from IH-exposed cardiomyocytes impaired endothelial function, suppressing protein kinase B (AKT)/eNOS activation in human umbilical vein endothelial cells (HUVECs). Consequently, endothelium-dependent vasodilation was blunted in isolated mouse aortic rings, although the contribution of specific miRNA cargoes remained unverified (72). More direct evidence for a cargo-dependent effect comes from diabetic Goto-Kakizaki rats. The sEVs from primary Goto-Kakizaki rat cardiomyocytes inhibited mouse cardiac endothelial proliferation, migration and tube formation. Reducing miR-320 in donor cardiomyocyte-derived sEVs attenuated these inhibitory effects, while reporter assays identified HSP20 as a direct target of miR-320, supporting a contribution of miR-320 to EV-mediated endothelial dysfunction (33).

Cardiomyocyte-derived EVs can promote fibroblast activation. sEVs collected from cardiomyocytes isolated after MI increased myofibroblast markers in primary mouse cardiac fibroblasts by delivering miR-92a. miR-92a targeted SMAD7, decreasing its expression and promoting α-smooth muscle actin (α-SMA) expression and fibroblast activation (73). Hypoxia- or Ang II-conditioned primary adult rat cardiomyocytes released sEVs containing miR-208a, which promoted primary adult rat cardiac fibroblast proliferation and differentiation by suppressing DYRK2; miR-208a antagomir ameliorated this effect. Intramyocardial delivery of these sEVs also increased fibrosis and impaired cardiac function in rats (74). Mechanical stretch increased PELI1 expression in neonatal mouse cardiomyocytes and elevated miR-494-3p levels in their released sEVs. These sEVs increased miR-494-3p in recipient cardiac fibroblasts to promote proliferation, α-SMA expression and collagen I/III transcription by downregulating PTEN expression. sEVs from similarly stretched cardiomyocytes isolated from *PELI1*-knockout mice contained less miR-494-3p and elicited weaker fibroblast responses (75).

Protein-associated communication can also sustain fibrotic signaling caused by cardiomyocyte-derived EVs. Ang II-stimulated NRVMs increased the heat shock protein 90 (HSP90) and interleukin-6 (IL-6) content in the sEVs. This conditioned culture medium containing cardiomyocyte sEVs promoted collagen I/III expression in primary adult rat cardiac fibroblasts, while HSP90 knockdown in donor cardiomyocytes reduced sEV-related IL-6 and weakened the ability to activate fibroblast signal transducer and activator of transcription 3 (STAT3) and increase collagen expression (76).

Fibroblast-derived EVs. Cardiac fibroblasts are a vital compartment in the heart, mainly responsible for depositing and maintaining the extracellular matrix (ECM) under physiological conditions (77). In disease states, the resident cardiac fibroblasts are transformed into activated myofibroblasts, which are more highly specialized in the generation and secretion of fibrillar collagens and matricellular proteins (78). The changes in the ECM further influence the cardiac microenvironment and other types of cells (79,80). In addition, fibroblasts can send paracrine signals to communicate with other cells (81). Previous studies have shown that EV release is one mechanism by which cardiac fibroblasts maintain homeostasis in the cardiac microenvironment. For example, cardiac fibroblast-derived sEVs play a critical role in regulating the autophagy of other fibroblasts (55). Therefore, understanding the role of cardiac fibroblast-derived EVs in the cardiac microenvironment is necessary.

Beneficial roles of fibroblast-derived EVs in cardiac repair. Fibroblast-derived EVs can exert protective functions by acting on cardiomyocytes. Wang *et al* (82) found that sEVs isolated from the cardiac fibroblasts of patients shortened the Ca^{2+} transient time to peak and time to 50% decay of hiPSC-CMs, suggesting that cardiac fibroblast-derived sEVs can influence the activity of cardiomyocytes. sEVs isolated from neonatal mouse cardiac fibroblasts reduced hypoxia/reoxygenation injury in rat H9c2 cells by delivering miR-133a. In recipient cells, miR-133a targeted *ELAVL1* mRNA, decreasing ELAV-like RNA-binding protein 1 (ELAVL1, also known as HUR) expression and downregulating NLR family pyrin domain-containing 3 (NLRP3) expression. Intramyocardial administration of these fibroblast sEVs reduced injury-associated findings in a rat I/R model, while donor miR-133a overexpression enhanced these therapeutic effects (83).

Activated fibroblasts can also produce EVs to limit fibroblast activation. After Ang II treatment, sEVs from NRCFs showed increased PIWIL2 protein and PIWI-interacting RNA 62788 (piRNA-62788) (84). These sEVs reduced TGF-β-induced activation of recipient fibroblasts by mediating *SRF* silencing, while *PIWIL2* knockdown in recipient fibroblasts weakened this response. In mice, the amelioration of remodeling by a piRNA-62788 agomir, and the loss of therapeutic function of these sEVs after loading with a piRNA-62788 inhibitor, also indicated that the therapeutic role of sEVs depends on their specific cargo (84).

Detrimental roles of fibroblast-derived EVs in cardiac disease. Nevertheless, considerable evidence indicates that fibroblast-derived EVs exacerbate cardiac injury. After stimulation by tumor necrosis factor-α (TNF-α), the content of miR-27a, miR-28-3p and miR-34a was upregulated in the rat cardiac fibroblast-derived sEVs, which were all predicted to target *NFE2L2* mRNA, which encodes nuclear factor erythroid 2-related factor 2 (NRF2). These sEVs further boosted the TNF-α-induced oxidative stress in recipient H9c2 cardiomyocytes, as indicated by decreased NRF2 protein expression and upregulated hypertrophic gene expression (85). Neonatal rat fibroblast sEVs promoted renin and angiotensinogen expression and Ang II release in recipient NRVMs, causing a hypertrophic reaction (38). Meanwhile, exogenous Ang II increased sEV release from NRCFs, but equal protein doses of control and Ang II-associated sEVs produced similar

hypertrophic effects. However, another study found that the sEVs from Ang II-treated NRCFs induced more severe hypertrophic responses in NRVMs. These differences may reflect differences in experimental conditions. This research also found that increased miR-27a* in fibroblast sEVs caused the hypertrophic response, as verified by miRNA gain- and loss-of-function experiments in recipient cells (40). In addition to miR-27a*, miR-21* was also enriched in the sEVs derived from NRCFs after Ang II treatment. sEVs from precursor-transfected donors increased miR-21* content in recipient NRVMs and caused a more severe hypertrophic response by targeting and downregulating SORBS2 expression (39).

Fibroblast sEVs also influence cardiac pacing changes by affecting cardiomyocytes. sEVs from rapidly paced neonatal rat ventricular fibroblasts contained more miR-23a-3p. These sEVs caused ferroptosis-associated changes in H9c2 cells by regulating SLC7A11. Inhibiting sEV production by GW4869 reduced susceptibility to AF and attenuated atrial remodeling in a canine model of rapid atrial pacing (86). Furthermore, Ang II treatment enriched miR-21-3p in sEVs from neonatal rat atrial fibroblasts, which further downregulated CAV1.2 expression in atrial cardiomyocytes (87).

Cardiac fibroblast-derived EVs also regulate endothelial cell function. sEVs isolated from TGF-β-treated NRCFs were enriched in miR-200a-3p. When these sEVs were directly applied to adult mouse cardiac endothelial cells, tube formation and migration were impaired and apoptosis increased (88). Furthermore, inhibition of miR-200a-3p in donor fibroblasts reduced its abundance in the sEVs and attenuated sEV-induced changes in ETS1, placental growth factor and vascular endothelial growth factor A (VEGFA) expression in recipient cells. These findings support a contribution of EV-associated miR-200a-3p to the observed molecular changes.

Endothelial cell-derived EVs. Endothelial cells play a central role in cardiac physiology and pathology. They are critical cellular components of the heart, forming the coronary vasculature essential for delivering oxygen and other nutrients to cardiomyocytes. Endothelial cells coordinate compensatory angiogenesis to match vascular density with cardiomyocyte growth, maintaining tissue oxygenation via paracrine signaling with other cardiac cells. Under pathological stimuli, endothelial signaling becomes dysregulated. This leads to microvascular dysfunction, resulting in insufficient oxygen supply relative to increased cardiomyocyte metabolic demand. Consequently, tissue hypoxia triggers cardiomyocyte death, interstitial fibrosis, diastolic dysfunction and ultimately progression to heart failure (89). In addition, endothelial cells themselves can undergo the endothelial-to-mesenchymal transition, losing their characteristic function and acquiring profibrotic properties, further contributing to myocardial stiffness (89).

Beyond these classic functions, researchers found that EV production is also a critical regulatory mechanism through which endothelial cells influence cardiac function. A more in-depth study revealed that the cargo and function of endothelial cell-derived EVs are determined by the states of the endothelial cells (90). sEVs from both unstimulated and pro-inflammatory cytokine-treated human coronary artery endothelial cells (HCAECs) impaired angiogenic responses and carried miRNAs potentially involved in these effects (such as miR-451, let-7 and miR-23a) (90). The following section discusses the functions of endothelial cell-derived EVs based on their diverse roles in cardiac health.

Beneficial roles of endothelial-derived EVs in cardiac repair. Accumulating evidence suggests that endothelial cell-derived EVs can also exert cardioprotective effects. Directly administered HUVEC-sEVs improved survival and contractile recovery after I/R in a human heart-on-chip model. Antioxidant, metabolic and stress-associated proteins identified by proteomics were enriched in the HUVEC-sEVs, which may mediate protective functions. However, normoxic and hypoxic donor sEVs were similarly protective at the tested doses despite increased sEV production under hypoxia (91). Isolated HUVEC-sEVs protected adult rat cardiomyocytes against simulated I/R. Inhibition of mitogen-activated protein kinase kinase (MEK) in recipient cells abolished these protective effects (29). Another study confirmed these results. The sEVs from unstimulated HUVECs also protected H9c2 cardiomyocytes against simulated I/R injury and reduced infarct size in isolated perfused rat hearts. Pharmacological inhibition of MEK, nitric oxide synthase (NOS) or soluble guanylyl cyclase (SGC) attenuated protection, supporting the involvement of MEK/ERK and nitric oxide (NO)/SGC signaling (32).

Endothelial cell-derived EVs also restore vascular function to ameliorate cardiac injury. The HUVEC-sEVs improved vascular continuity and average vessel length in engineered tissues containing endothelial cells, dental pulp stromal cells and hiPSC-CMs. sEV sequencing detected that let-7b-5p and miR-126-3p were enriched in the HUVEC-sEVs. Direct mimic transfection into this engineered tissue reproduced aspects of the vascular phenotype (92). In a separate endothelial model, sEVs from unstimulated HCAECs improved recovery of wounded recipient HCAEC monolayers, indicating a beneficial effect on this specific repair endpoint (90).

An immunoregulatory interaction was shown using sEVs from primary mouse cardiac endothelial cells. sEV-associated integrin-β6 was detected by immunoblotting. Donor *ITGB6* knockdown reduced its abundance in sEVs and impaired the induction of mature TGF-β in B cells cultured with lipopolysaccharide (LPS) and anti-CD40. B cells induced with cardiac endothelial sEVs, LPS and anti-CD40 suppressed $CD4^+$ T-cell proliferation. This suppressive effect was enhanced by LPS and reversed by TGF-β neutralization. Notably, β6 from sEVs was important during B-cell induction but was not essential for TGF-β release from already induced B cells. These results establish a conditional endothelial-B-cell-T-cell interaction *in vitro* (93).

Detrimental roles of endothelial-derived EVs in cardiac disease. Endothelial cell-derived EVs can harm myocardial health under pathological stimuli. sEVs from IL-3-stimulated HUVECs lost their protective functions in directly treated H9c2 cells and isolated perfused rat hearts. Comparative proteomic analysis showed that IL-3 stimulation altered sEV protein composition, including increased caveolin-1 and decreased MEK1/2 and HSP90 abundance (32). Similarly, primary mouse cardiac microvascular endothelial cells (CMECs) released sEVs with increased MST1 protein

(encoded by *STK4*) under high glucose. Donor *STK4* overexpression further increased sEV-associated MST1. When applied to high-glucose-treated cardiomyocytes, these sEVs with enriched MST1 caused greater inhibition of autophagy, increased apoptosis and lower glucose uptake than sEVs from control endothelial cells. Endothelial-lineage *STK4* overexpression or deletion also altered cardiac dysfunction in streptozotocin-treated mice. Direct STK4 overexpression in cardiomyocytes increased MST1-DAXX interaction and reduced DAXX-GLUT4 interaction, providing a mechanistic explanation (34). In an epinephrine-induced cardiac hypertrophy model, HCMEC-derived sEVs contained increased miR-126-3p. These miR-126-3p-enriched sEVs induced electrophysiological abnormalities in hiPSC-CMs by directly downregulating RGS3 expression. Transfecting donor endothelial cells with a miR-126-3p mimic or inhibitor respectively increased or decreased its contents in isolated sEVs. Mimic-derived sEVs aggravated selected electrophysiological abnormalities, whereas inhibitor-derived sEVs attenuated several pathological responses (94). These results differ from previous findings on the beneficial cardiac role of miR-126-3p in sEVs, which may reflect differences in recipients and interventions, suggesting that sEV function should not be interpreted solely on the basis of cargo (92,94).

Endothelial EVs participate in fibroblast activation. The *TGFB1* mRNA in sEVs from primary mouse CMECs was increased after exposure to high glucose. These sEVs increased TGF-β1, α-SMA and collagen I expression in the recipient neonatal mouse cardiac fibroblasts. *TGFB1* knockdown in donor endothelial cells resulted in reduced profibrotic activity of sEVs. In streptozotocin-treated mice, intramyocardial injection of high-glucose donor sEVs increased interstitial and perivascular fibrosis, with weaker effects from sEVs collected after donor *TGFB1* knockdown (35).

Endothelial EVs can also impair vascular network formation. sEVs from both unstimulated and cytokine-treated (TNF-α, interferon-γ and TGF-β) HCAECs reduced mesh formation in a Matrigel tube-formation assay. sEVs from cytokine-treated endothelial cells also lost their wound-recovery effects and caused greater impairment (90).

Macrophage-derived EVs. Macrophages are one of the most abundant immune cell types in the heart. In a healthy heart, the heart-resident macrophages include both M1 and M2 types (95). In a diseased state, the heart recruits bone marrow-derived M1 macrophages, which produce high levels of pro-inflammatory cytokines to activate the immune-inflammatory reaction and further damage cardiac function (95,96). In addition to secreting cytokines and performing phagocytosis, macrophages can also regulate cardiac function by directly interacting with cardiomyocytes (97,98). Researchers have also found that macrophage-derived EVs play critical roles in regulating cardiac homeostasis. Similar to other cells, the function of these EVs is determined by the states of the macrophages.

Beneficial roles of macrophage-derived EVs in cardiac repair. Some studies reported the protective function of macrophage-derived EVs in cardiac diseases, especially those derived from IL-4-polarized M2 macrophages. Administration of sEVs from IL-4-polarized THP-1 macrophages improved left ventricular function after MI caused by diet-induced occlusive coronary atherosclerosis (99). In particular, various studies identified cardiomyocytes as critical recipient cells in the sEV-mediated recovery. sEVs from IL-4-polarized rat peritoneal macrophages reduced injury in neonatal rat cardiomyocytes exposed to hypoxia/reoxygenation and in a rat I/R model by decreasing thioredoxin-interacting protein (TXNIP) expression and downregulating Toll-like receptor 4 (TLR4)/NF-κB/NLRP3 signaling. Donor miR-148a inhibition to lower its abundance in isolated sEVs, or recipient *TXNIP* overexpression, weakened these protective effects (100). In another study, IL-4 promoted the miR-378a-3p content in mouse bone-marrow macrophage-derived sEVs. These miR-378a-3p-enriched sEVs ameliorated hypoxia-induced injury in HL-1 cardiomyocytes and MI in mice. miR-378a-3p targeted *ELAVL1* mRNA to decrease HUR expression in recipient cells, which was associated with HUR-NLRP3 signaling. HUR re-expression in recipient cells restored *NLRP3* mRNA stability and weakened the protective effects of M2 macrophage-derived sEVs (101). sEVs from IL-4-polarized human monocyte-derived macrophages also protected hypoxia/reoxygenation-exposed AC16 cardiomyocytes. sEV transfer of miR-145-5p markedly decreased TLR4 expression, while *TLR4* overexpression in recipient cardiomyocytes reversed the function of the sEVs (102). In another study using the same donor cells, miR-1271-5p-containing sEVs reduced AC16 apoptosis and improved cardiac function after mouse MI by targeted suppression of SOX6 expression. Recipient miRNA inhibition weakened protection, whereas *SOX6* knockdown restored it (19). In addition, miR-146a-5p has also been reported in sEVs from IL-4-polarized mouse bone marrow macrophages. These miR-146a-5p-containing sEVs attenuated KCA3.1 upregulation and action-potential shortening in rapidly paced HL-1 cells. Recipient miR-146a-5p mimics and NF-κB inhibition produced similar effects, implicating the participation of NF-κB/STAT3 signaling (103). In Ang II-induced hypertrophy, sEVs released by mouse peritoneal macrophages polarized with IL-4 and IL-13 reduced hypertrophic changes in neonatal rat cardiomyocytes and attenuated cardiac remodeling in mice. Further experiments implicated sEV-associated miR-124-3p in these responses by directly targeting *CAPN1*. Inhibiting miR-124-3p in recipient cardiomyocytes weakened sEV protection (104).

Cardiomyocyte-protective functions of macrophage-derived EVs have also been reported in septic cardiac injury. IL-4-polarized mouse peritoneal macrophage sEVs improved LPS-induced cardiac injury in mice. Reporter assays supported miR-24-3p targeting of TNFSF10 in HL-1 cells, although function was not established *in vitro* (47). sEV preparations from IL-1β-stimulated patient-blood-derived macrophages were enriched with miR-146a and protected LPS-treated rat H9c2 cells and endotoxin-challenged mice. Evidence from the miR-146a-MAPK4 reporter and the MAPK4-DRP1 association supported a proposed mitochondrial mechanism, but this requires further exploration (105).

Proteins incorporated into macrophage EVs also provide protective effects in recipient cardiomyocytes. RAW264.7-derived sEVs carried surface transferrin receptor (TFR), captured transferrin-associated iron and reduced hypoxia-induced injury in neonatal rat ventricular

cardiomyocytes. TFR knockdown in macrophages reduced TFR content in sEVs and abolished protection from intramyocardial sEV administration after mouse MI. Notably, intravenous administration did not improve cardiac function, including at the higher dose tested, showing a route-dependent limitation within the same study (106).

Protective effects of macrophage EVs also involve immune recipient cells. In mouse CVB3 myocarditis, sEVs from IL-4-polarized mouse bone-marrow-derived macrophages promoted macrophage M2-associated markers and reduced cardiac inflammation. sEVs containing the long non-coding RNA (lncRNA) AK083884 decreased PKM2 expression in recipient cells, mediating their inflammation-regulatory functions. Donor AK083884 knockdown decreased this lncRNA in sEVs and attenuated protective functions (107). M2 bone-marrow macrophage sEVs also reduced glucose uptake and mitochondrial ROS levels in CCR2-positive macrophages. These changes were accompanied by reduced inflammatory cytokine production and improved cardiac outcomes after I/R injury in mice, rats and pigs. The inflammatory and metabolic regulatory functions of sEVs were linked to miR-181b-5p; blocking miR-181b-5p in donor cells weakened these effects (31).

In recipient endothelial cells, IL-4-polarized THP-1 sEVs enhanced proliferation, migration and tube formation, which further promoted cardiac repair after infarction. Inhibition of donor miR-132-3p reduced sEV miRNA abundance and weakened these effects, indicating that miR-132-3p played a critical role in sEV-mediated recovery. Further studies showed that the miR-132-3p target thrombospondin 1 (THBS1) decreased in endothelial cells, while THBS1 re-expression reversed the protective effects of direct miR-132-3p transfection (20). sEVs from human embryonic-stem-cell-derived primitive macrophages also improved early endothelial organisation in engineered human cardiac tissues containing induced-pluripotent-stem-cell cardiomyocytes, HUVECs and dental-pulp stromal cells. sEV-miRNA sequencing showed that let-7-family miRNAs and miR-126-3p could be candidate effectors (108).

Detrimental roles of macrophage-derived EVs in cardiac disease. Several studies found that macrophages can also generate detrimental EVs to promote diverse types of cardiomyocyte injury. sEVs from TNF-α/interferon-γ (IFN-γ)-polarized M1 rat bone-marrow macrophages increased cell death in neonatal rat cardiomyocytes, whereas IL-4/IL-10/IL-13-polarized M2 macrophages did not cause similar injury. Pre-treatment with a TLR4 inhibitor ameliorated the M1 macrophage-induced injury, suggesting the involvement of the TLR4 signaling pathway (109). Another study also reported that changes in cargo could reshape sEV functions. Compared with IL-4/IL-13-polarized M2 RAW264.7 macrophages, IFN-γ/LPS-stimulated M1 macrophages increased Snhg14 and decreased miR-181a-5p contents in the sEVs. sEVs from M1 macrophages aggravated LPS-induced H9c2 injury, whereas sEVs from M2 macrophages improved several injury markers. Direct miR-181a-5p supplementation or Snhg14 knockdown in recipient cardiomyocytes attenuated injury. In addition, sh-Snhg14 also ameliorated sepsis-induced cardiac injury in mice (110). These comparisons suggested that cargo plays a pivotal role in determining EV function. Different stimuli can alter cargo, thereby changing the roles of EVs in the cardiac microenvironment.

Other research also showed that stressed macrophages could produce EVs to promote cardiomyocyte injury. In HL-1 cardiomyocytes, LPS-stimulated RAW264.7 sEVs increased apoptotic signaling and mitochondrial depolarization. These sEVs carried miR-122, which may contribute to their pro-apoptotic effects by targeting the anti-apoptotic protein XIAP (111). Hypoxia/reoxygenation-conditioned mouse bone marrow macrophage sEVs were enriched with miR-29a. These sEVs further reduced MCL1 expression and increased pyroptotic responses in neonatal mouse cardiomyocytes. MCL1 re-expression rescued the effects of direct miRNA transfection, indicating the critical role of MCL1 (112). In addition to pre-treatment, native RAW264.7 cells also produced sEVs that aggravated pyroptosis in neonatal rat cardiomyocytes exposed to uremic serum and worsened cardiac injury in uremic mice. By targeting and decreasing FOXO3A expression, miR-155 was critical for sEV-induced injury, while miR-155-deficient recipient cells ameliorated FOXO3A suppression and pyroptosis (48). Similarly, another study also supported the detrimental roles of miR-155-positive sEVs using granulocyte-macrophage colony-stimulating factor (GM-CSF)/IFN-γ-polarized RAW264.7 cells. These sEVs arrested the cell cycle in neonatal mouse cardiomyocytes by directly decreasing IL-6R expression and downregulating the downstream JAK2/STAT3 signaling pathway (24). sEVs from LPS-stimulated THP-1 macrophages suppressed autophagic flux in AC16 cells and contained less miR-29b-3p. Reduced miR-29b-3p content contributed to impaired cardiomyocyte autophagic flux by relieving repression of GSK3β (113). Similarly, the enriched piR-50971 was involved in LPS-treated THP-1 macrophage sEVs mediating autophagy suppression in H9c2 and AC16 cells. Direct piR-50971 transfection increased WTAP expression in AC16 cells, whereas *WTAP* knockdown reduced piR-50971-induced N^6-methyl-adenosine modification of *MTOR* mRNA and restored autophagic flux. Systemic piR-50971 inhibition also improved cardiac function in septic rats (114). Drug exposure can also generate harmful sEV signaling. sEVs from PD-1-antibody-treated mouse cardiac macrophages induced senescence-associated changes in mouse cardiomyocytes. These sEVs delivered miR-34a-5p to cardiomyocytes, directly decreasing PNUTS expression. Either donor miR-34a-5p inhibition to reduce its sEV abundance or recipient PNUTS re-expression attenuated sEV-induced senescence in cardiomyocytes. Similarly, use of a miR-34a-5p antagomir in mice also ameliorated PD-1 antibody-induced cardiac injury (115). In addition to these sEVs, other types of EVs also participate in the crosstalk between macrophages and cardiomyocytes. EVs (0.1-1 μm) from actinomycin D-induced apoptotic RAW264.7 cells carried TNF-α and rapidly impaired isolated adult mouse cardiomyocyte contraction. TNF-α blockade or NOS inhibition prevented this EV-induced acute response, supporting extracellular receptor signaling (116).

Macrophage-derived EVs also act on endothelial cells to cause cardiac dysfunction. sEVs from GM-CSF/IFN-γ-polarized THP-1 or RAW264.7 macrophages suppressed endothelial angiogenic activity and worsened post-infarction outcomes in mice. This sEV function was associated with miR-155

enrichment, and lentivirus-mediated miR-155 inhibition in the recipient endothelial cells partially rescued these functions. Further reporter assays implicated RAC1, PAK2, SIRT1 and AMPKα2 as potential targets of miR-155 (117). In parallel, sEVs from LPS/IFN-γ-polarized mouse bone-marrow macrophages impaired mouse CMECs and worsened post-MI outcomes in mice. Further experiments showed that MALAT1 contributed to the endothelial toxicity of sEVs. Silencing it in donor macrophages reduced its abundance in sEVs and attenuated their harmful effects. In addition, supplementing endothelial cells with downstream miR-25-3p partially counteracted the effects of sEVs (118). sEVs from LPS-stimulated RAW264.7 and THP-1 macrophages also induced endothelial-to-mesenchymal changes. After these sEV treatments, the decreased CD31 expression and increased α-SMA expression in mouse aortic endothelial cells and HCAECs indicated promotion of endothelial-to-mesenchymal transformation. These responses coincided with reduced miR-23b-3p abundance in RAW264.7 sEVs and increased SMAD3 phosphorylation in HCAECs (119).

Macrophages may also aggravate cardiac injury by promoting fibroblast activation through sEV-mediated communication. sEVs from hypoxic human THP-1 cultures were enriched in miR-210-3p and activated NRCFs. Inhibiting miR-210-3p in recipient fibroblasts attenuated this response. In addition, anti-miR-210-3p improved cardiac function in infarcted rats, indicating the critical role of miR-210-3p (120). High-glucose exposure also enhanced the inflammatory and profibrotic effects of RAW264.7 sEVs on NIH/3T3 fibroblasts. HUR was detected in these sEVs, and donor *ELAVL1* knockdown attenuated the fibroblast response. In macrophage-depleted and Ang II-treated non-diabetic mice, sEVs from *ELAVL1*-knockdown db/db bone-marrow macrophages caused less cardiac fibrosis than control-donor sEVs. However, the study did not establish HUR abundance in sEVs after donor knockdown (121). Direct inflammatory stimulation also caused macrophages to generate profibrotic sEVs. LPS-stimulated RAW264.7 sEVs were enriched in miR-155-5p and increased α-SMA and collagen I/III expression in mouse cardiac fibroblasts while reducing SOCS1. miR-155-5p directly targeted *SOCS1* mRNA to inhibit its expression, and SOCS1 re-expression attenuated fibroblast activation induced by direct miRNA transfection (122).

In all of the aforementioned studies, the detrimental EVs were mainly derived from pathological stress-stimulated M1 macrophages, whereas the beneficial EVs were generated by IL-4-polarized M2 macrophages. However, it should be noted that EV effects cannot be simply predicted from polarization or stimulation alone, especially for fibroblast activation and proliferation. sEVs from IL-4-polarized mouse bone-marrow macrophages activated primary mouse cardiac fibroblasts, promoted their proliferation and aggravated post-MI fibrosis. These fibroblast activation effects of sEVs were due to the circular RNA (circRNA) circUbe3a, and manipulating this circRNA in donor cells altered its abundance in sEVs and their effects on cardiac fibroblasts. Interaction and reporter assays supported a mechanism in which circUbe3a bound miR-138-5p, relieving its repression of RHOC. Supplementing recipient fibroblasts with miR-138-5p reduced sEV-induced RHOC upregulation and partially reversed increases in collagen I, collagen III and α-SMA (26). Conversely, miR-155-enriched sEVs from Ang II-treated mouse bone-marrow macrophages suppressed primary cardiac fibroblast proliferation (123). Even though these sEVs ultimately increased early post-MI cardiac rupture due to fibroblast dysfunction, the contrasting results regarding fibroblast proliferation challenge the prediction of EV effects on recipient fibroblasts solely from the presumed reparative or inflammatory properties associated with donor-cell pre-treatment.

T cell-derived EVs. Emerging evidence has shown the critical function of T cells in cardiac diseases. The positive association between T cells and cardiac dysfunction in patients has been reported in several studies (124,125). Further exploration, especially with advances in single-cell sequencing, revealed that T cells are involved in the pathogenesis of various cardiac diseases by regulating the inflammatory microenvironment. Previous studies have shown that T cells can also secrete EVs to influence cardiac function. Similar to macrophages, the detrimental or beneficial function of T cell-derived EVs depends on the state of the T cells.

Beneficial roles of T cell-derived EVs in cardiac repair. EVs derived from primary regulatory T cells (Tregs) can support repair by modifying inflammatory responses. Isolated EVs from murine splenic Tregs reduced M1-associated and increased M2-associated marker expression in LPS-treated RAW264.7 macrophages. Administering these sEVs via myocardial injection reduced infarct area and apoptosis in mice after MI (23). Similarly, the transfer of wild-type Tregs improved cardiac function and attenuated adverse remodeling in mice after MI in another study. Meanwhile, the splenic Tregs from *NT5E*-knockout (CD73-deficient) mice provided less protection. Complementary *in vitro* experiments showed that sEVs from wild-type, but not CD73-deficient, splenic Tregs suppressed TNF-α and IL-17 release by primary effector T cells. However, CD73 was not directly measured in the isolated sEVs (126). These findings suggest an EV-associated immunoregulatory component of Treg activity in post-MI repair.

These cardiac regulatory functions of Treg EVs also extend to cardiomyocytes. sEVs isolated from splenic Tregs of mice reduced DOX-induced senescence and improved mitochondrial function in HL-1 cells. In addition, these sEVs increased cardiac mitochondrial and vascular markers and reduced inflammatory markers in aged mice (127). Similarly, a previous study by the authors showed that Jurkat T cells can deliver forkhead box P3 (FOXP3) protein to neonatal rat ventricular cardiomyocytes via sEVs, thereby downregulating ferroptosis and ameliorating pathological hypertrophy (41). Critically, the overexpression of FOXP3 in donor T cells markedly increased FOXP3 protein content in the sEVs and enhanced therapeutic efficacy, indicating that Tregs could deliver FOXP3-enriched sEVs to suppress hypertrophic responses in cardiomyocytes. However, the difference between Jurkat T cells and primary cardiac-resident T cells cannot be ignored, indicating the necessity of further exploration (41).

Sonic hedgehog (SHH)-bearing microparticles from stimulated CEM T cells, isolated by centrifugation at 14,000 x g for 45 min, provide another example of a protective preparation derived from a T-cell line. These particles were related to

increased endothelial nitric oxide availability and preserved coronary endothelial function when administered 24 h before I/R in mice. In cultured human EA.hy926 endothelial cells, cyclopamine treatment or Patched knockdown attenuated microparticle-induced NO production, supporting the involvement of Hedgehog signaling (128). In addition to endothelial cells, cardiomyocytes could also be influenced by these SHH-bearing microparticles produced by CEM T cells. These microparticles increased NO production and reduced Ca^{2+} transient amplitude and electrically evoked shortening in isolated adult rat ventricular cardiomyocytes, with these effects blocked by cyclopamine. The particles also shortened the heart-rate-corrected QT interval in healthy rats, and treatment with recombinant SHH protein limited infarct size and protected against reperfusion arrhythmia (129). The beneficial functions of SHH-bearing microparticles were also verified in pigs, with reduced infarct size after I/R (130).

Detrimental roles of T cell-derived EVs in cardiac disease. Conversely, EVs derived from pro-inflammatory T cell subtypes were often associated with poor outcomes. EVs (100-1,000 nm) from activated human peripheral-blood $CD4^+$ T cells disrupted barrier integrity and impaired wound closure in HCMECs and increased mitochondrial oxidative stress and apoptosis (131). Further study showed that MEK1/2 and ERK1/2 were enriched in sEVs from primary murine splenic T cells after anti-CD3/CD28 activation. In recipient cells, U0126 (MEK inhibitor) or FR180204 (ERK inhibitor) reduced sEV-induced NOX4 up-regulation, attenuated total ROS accumulation and restored NO availability. However, *NOX4* silencing in recipient cells did not reverse the mitochondrial ROS response but only attenuated the ROS/NO imbalance (132).

Activated T cell-derived EVs also induced cardiac fibroblast activation. sEVs from anti-CD3/CD28-activated mouse splenic $CD4^+$ T cells promoted proliferation, migration and α-SMA expression in primary neonatal mouse cardiac fibroblasts. Administration of these sEVs also aggravated post-MI remodeling in mice (133). These sEVs from activated T cells were enriched with miR-142-3p, which directly decreased APC expression and promoted β-catenin signaling in recipient fibroblasts. A miR-142-3p inhibitor in fibroblasts reversed the sEV-induced activation of fibrosis (25).

However, as in macrophages, a regulatory T-cell phenotype does not guarantee an anti-fibrotic and pro-repair EV effect. sEVs from activated rat splenic $CD4^+CD25^+$ cells designated as Tregs increased NRCF proliferation and TGF-β expression. KV1.3-related interventions altered donor-cell activity and sEV-related effects, but neither KV1.3 nor TGF-β was established as the functional sEV cargo (134).

In addition to these cardiac diseases, T cell-derived EVs also have functions in a further field, cardiac allograft rejection. miR-142-3p was detected in the sEVs from primary T cells isolated from healthy human peripheral blood and activated with phytohemagglutinin/interleukin-2 or anti-CD3/CD28. These sEVs delivered miR-142-3p to HUVECs, thereby directly reducing RAB11FIP2 expression and increasing endothelial permeability. *RAB11FIP2* silencing reproduced the permeability increase, whereas pre-treating recipient HUVECs with anti-miR-142 restored RAB11FIP2 expression and reversed sEV-induced hyperpermeability. Importantly, serum sEVs from heart transplant recipients with acute cellular rejection contained more miR-142-3p than those from recipients without rejection (51). Another study focused on circulating T-cell-associated sEVs in C57BL/6 mice during rejection of BALB/c cardiac allografts. By using centrifugation and antibody-based immunocapture, they isolated a preparation of sEVs enriched with CD3. When directly applied to primary mouse cardiomyocytes, $CD3^+$ sEVs collected on post-transplant day 7 increased terminal deoxynucleotidyl transferase dUTP nick-end labelling-positive apoptosis in donor-strain BALB/c cells compared with CD3-unbound sEVs, whereas recipient-strain C57BL/6 cells were largely unaffected. FASL, granzyme B, perforin and T-cell receptor proteins were identified in the $CD3^+$ sEV fraction, with higher levels during rejection. In addition, miRNA sequencing revealed a time-dependent cargo shift profile in the T cell-derived sEVs after heart transplantation in mice. At day 4 after transplantation, the enriched miRNAs were associated with immune-regulatory processes. This profile of miRNAs shifted to apoptosis-related processes at day 7, such as those predicted to target B-cell lymphoma 2/PTEN. Notably, circulating CD3 sEVs from patients with grade 2 acute cellular rejection showed increased levels of FASL, granzyme B and perforin, along with increased miR-let7i, miR-21a and miR-101b (50).

Dendritic cell (DC)-derived EVs

Beneficial roles of DC-derived EVs in cardiac repair. Previous studies revealed the protective roles of DC-derived EVs in regulating cardiac function. Stimulation of bone marrow-derived DCs (BMDCs) with the supernatant from necrotic HL-1 cardiomyocytes produced a special population of sEVs with cardiac repair functions. Splenic $CD4^+$ T cells could take up these sEVs to alter the expression of several cytokines. In addition, these sEVs also improved ejection fraction and fractional shortening in infarcted mice via intravenous administration (21). Another study used a similar approach and found that the sEVs from BMDCs treated with necrotic HL-1 cardiomyocyte culture supernatant were enriched with CCR7. These CCR7-enriched sEVs improved left ventricular ejection fraction and fractional shortening, whereas donor *CCR7* knockdown weakened both effects. In addition, CCR7-enriched sEVs were more abundant in the spleen and increased the $CD4^+$ T-cell proportion alongside increased *IL4* and *IL10* transcripts, suggesting these sEVs could improve the overall immune environment to enhance cardiac function (22). This hypothesis was verified by subsequent research. The authors found that supernatant-pre-treated BMDC sEVs increased $FOXP3^+$ and $CD206^+$ cells and decreased $INOS^+$ cells in the infarct border zone, suggesting an immune-regulatory function of these sEVs. Notably, these sEVs polarized the macrophages to a pro-inflammatory M1 subtype, whereas Treg exposure caused an anti-inflammatory M2 subtype, suggesting that the sEVs may influence the inflammatory environment through indirect cellular communication. However, this BMDC sEV-Treg-macrophage pathway was not established by this research (135). More recent work identified increased indoleamine 2,3-dioxygenase 1 (IDO1) protein in sEVs from necrotic-cardiomyocyte-conditioned BMDCs. In cultures of purified murine splenic $CD4^+$ T cells, increasing IDO1 content in BMDC sEVs via donor *IDO1*

overexpression increased the kynurenine-to-tryptophan ratio, aryl hydrocarbon receptor (AHR) protein abundance and the proportion of $CD4^+FOXP3^+$ cells. Conversely, sEVs from *IDO1*-knockdown BMDCs reduced all three readouts relative to their corresponding control sEVs. In addition, the AHR inhibitor CH-223191 attenuated the enhanced Treg response to IDO1-enriched sEVs and reduced their cardiac protection in infarcted mice. This pharmacological reversal supports the involvement of an IDO1-associated AHR pathway in BMDC sEV-mediated cardiac immune regulatory function (136).

Necrotic cardiomyocyte-conditioned BMDC-derived sEVs also influenced endothelial cells to promote angiogenesis. sEV treatment enhanced endothelial tube formation and VEGF expression in primary CMECs. Meanwhile, myocardial delivery increased vascular reconstruction after MI in mice (137). These beneficial effects on endothelial cells were attributed to the enrichment of miR-494-3p in the sEVs. Using a mimic or inhibitor to alter miRNA expression in the donor BMDCs enhanced or suppressed tube formation ability in the recipient endothelial cells, respectively. Following intramyocardial administration in mice with MI, these sEV preparations increased or decreased myocardial VEGF and CD31 expression and vessel counts, respectively (137).

DC-derived EVs have also been highlighted in cardiac transplantation and in the suppression of rejection reactions. Predominantly immature BMDCs generated from donor-strain LEW.1W rats produced sEVs that were characterized by enriched major histocompatibility complex class II (MHC-II), with low CD86 and undetectable CD80. Administering these sEVs to LEW.1A recipients 14 and 7 days before heart transplantation prolonged graft survival. Splenic T cells collected from recipients on post-transplant day 5 showed reduced $CD4^+$ T-cell proliferation after 3 days of restimulation with irradiated donor-strain splenic DCs. *IFNG* mRNA expression was also reduced in the cardiac allografts (138). Furthermore, the combination of LF15-0195, a type of immunosuppressant, with these BMDC-sEVs achieved long-term graft survival in all treated recipient rats. These recipients accepted a second donor-strain heart but rejected third-party hearts from BN lines, and transfer of their splenocytes conferred donor-specific protection in irradiated recipients. The combination also reduced graft vascular lesions compared with LF15-0195 alone. *Ex vivo*, donor-reactive proliferation was reduced in whole splenocyte cultures but remained intact in purified T-cell cultures, leaving the cellular mediator of tolerance unresolved (139). Another study similarly found these immune regulatory functions using immature BMDC-sEVs combined with rapamycin. This combination produced substantially longer graft survival than either treatment alone, with evidence of donor specificity and transfer of protection by recipient splenocytes (53). These sEVs from immature BMDCs showed lower major histocompatibility complex class I (MHC-I), MHC-II, CD80 and CD86 expression compared with those from mature BMDCs, but no particular component was assigned responsibility for these protective effects.

DC-to-DC communication offers another possible route of immune regulation during cardiac allograft rejection. sEVs from vitamin-D3-treated immature BMDCs from donor mice, unlike those from LPS-conditioned mature BMDCs, prolonged mouse cardiac allograft survival after pre-transplant administration (140). Further experiments showed that the miRNA content differed between sEVs from immature and mature BMDCs. Meanwhile, the miRNA reporter assay indicated that DC2.4 cells could take up these sEVs, indicating that this sEV-mediated DC-to-DC cellular communication may mediate cardiac allograft rejection. However, the contribution of both this communication profile and miRNA cargo to immune regulation needs further exploration (140).

All of the aforementioned findings highlight the potential of DC-derived EVs in suppressing cardiac allograft rejection. Further modification can boost this therapeutic effect. *METTL3* knockdown in LPS-matured mouse BMDCs reduced *METTL3* mRNA and METTL3 protein, together with ICAM-1 and MHC molecules, in isolated sEVs (54). These sEVs showed weaker responses when directly added to LPS-stimulated recipient splenic T cells and prolonged cardiac allograft survival after prophylactic administration, whereas sEVs from unmodified mature DCs aggravated rejection (54).

A different strategy increased programmed death ligand-1 (PD-L1) in the sEVs from the DC2.4 cell line through direct overexpression. Western blotting and nanoflow cytometry confirmed increased PD-L1 on the resulting sEVs, while donor MHC-I ($H2^b$) expression was retained. These sEVs suppressed proliferation and promoted apoptosis in activated T cells isolated from BALB/c recipients 6 days after B6 heart transplantation. In addition, these sEVs also prolonged the survival of B6 cardiac grafts, which shared the $H2^b$ background of the sEV-producing cells, but not third-party C3H grafts with an $H2^k$ background (141).

Detrimental roles of DC-derived EVs in cardiac disease. Because DCs can activate T cells, DC-derived EVs are also vital in activating allograft reactions. Montecalvo *et al* (142) recovered DCs from heart allografts, showed sEV release *ex vivo* and traced vesicle transfer to splenic DCs after adoptive DC transfer. Purified allopeptide-bearing sEVs were also directly incubated with DCs, which subsequently stimulated antigen-specific $CD4^+$ T cells. Donor and recipient MHC-II genetic controls established requirements for antigen presentation, while mature-DC sEVs showed increased MHC-II abundance. These were antigen-presentation experiments rather than validation of an intercellular cargo-target pathway or a rescue. Notably, serum sEVs collected from transplant recipients did not reproduce the T-cell response, and the tracking experiments involved isolated, transduced and reinfused DCs. The aforementioned study supports a short-range DC-to-DC antigen relay; it does not directly show that physiological circulating sEVs worsen heart-graft survival or cardiac function.

Using BMDCs from recipient mice after cardiac allograft exposure to a donor-derived antigenic peptide, Montecalvo *et al* (142) found that these sEVs could stimulate other unexposed BMDCs and induce peptide-specific primary $CD4^+$ T-cell proliferation. In addition, sEVs from LPS-matured BMDCs contained more MHC-II and induced stronger T-cell responses than those from immature BMDCs in mice. This antigen-presenting function of sEVs was verified in another study. The purified sEVs from IL-1β/TNF-α-matured donor BMDCs enhanced recipient BMDC maturation and allo-stimulatory activity. Immature-DC sEVs and disrupted vesicles were less active. In DC co-cultures, *RAB27A* knockdown in

donor cells reduced sEV release and donor MHC transfer to recipient DCs. Depletion of recipient conventional DCs *in vivo* also reduced alloreactive T-cell responses and prolonged cardiac allograft survival, supporting their role as antigen-presenting intermediaries (52). Previous research has explored this function of sEVs *in vivo* in depth. DC-specific deletion of either *RAB27A* or *RAB27B* prolonged mouse cardiac allograft survival. *RAB27A* deletion also reduced recipient DC acquisition of donor MHC and alloimmune activation (143). Proteomics and immunoblotting identified chloride intracellular channel 1 (CLIC1) in purified DC sEVs, which can interact with surface TMEM16F in recipient DCs. This interaction facilitated sEV binding and the acquisition of donor MHC molecules by recipient DCs. Niclosamide, an inhibitor of this interaction, reduced donor MHC acquisition in recipient BMDCs exposed to sEV-containing donor-cell supernatants. Niclosamide decreased splenic recipient DC cross-decoration and prolonged cardiac allograft survival *in vivo* (143).

Neutrophil-derived EVs. Elevated blood neutrophil-derived EVs have been observed in cardiac diseases including atherosclerosis, MI and infective endocarditis (144-146). However, few studies have investigated the regulatory function of neutrophil-derived EVs in cardiac diseases. Gomez *et al* (144) found that neutrophil-derived EVs accumulated at atheroprone regions exposed to disturbed flow. These EVs delivered miR-155 to endothelial cells, suppressing the negative regulator BCL6 and enhancing NF-κB-mediated inflammatory activation. In *APOE*$^{-/-}$ mice on a Western diet, administration of wild-type neutrophil EVs increased atherosclerotic plaque formation and macrophage accumulation, whereas miR-155 deficiency abolished such effects. El Habhab *et al* (147) showed that EVs released by activated rat splenocytes induced oxidative stress, inflammatory signaling and premature senescence in primary porcine coronary endothelial cells. Immuno-depletion of ~80% of neutrophil-derived EVs reduced endothelial senescence by 55%, supporting the contribution of neutrophil-derived EVs to the harmful effects. However, solid evidence with purified neutrophil-derived EVs is still required, and the key cargo remains to be elucidated.

Platelet-derived EVs. Platelets are anucleate blood cells that contribute to hemostasis and thrombosis. Platelets also participate in inflammatory and tissue repair responses by releasing EVs that carry proteins, lipids and nucleic acids. The composition and biological activity of platelet-derived EVs vary with platelet activation and disease condition.

Beneficial roles of platelet-derived EVs in cardiac repair. Platelet EVs could support the ischemic heart via vascular repair. The purified sEVs from serum-converted human platelet lysates not only reduced apoptotic cell death and promoted tube formation in HCMECs, but also improved ventricular function, reduced scar formation and increased vascularization in mice after I/R (148). sEVs isolated from thrombin-stimulated human platelets promoted angiogenesis and increased the number of capillaries with perfusion in rat myocardium after coronary ligation. Molecular inhibitors targeting VEGF, basic fibroblast growth factor, platelet-derived growth factor, SRC, phosphoinositide 3-kinase and ERK signaling attenuated the response in rat aortic rings *in vitro*, supporting the involvement of several pro-angiogenic signals (149). More recently, fractionation of the release from collagen-related peptide-stimulated mouse platelets separated sEV-enriched (<100 nm) and large-EV-enriched (>100 nm) fractions from EV-depleted soluble material. Intramyocardial injection of the sEV-enriched fraction at the time of permanent coronary ligation preserved ventricular function and increased myocardial α-SMA-positive vascular structures. The large-EV-enriched and EV-depleted soluble fractions did not improve cardiac function (150).

Platelet EVs can also directly act on cardiomyocytes. In a Transwell model containing human platelets with commercially sourced human ventricular cardiomyocytes, platelet exposure altered metabolic activity and injury readouts. Meanwhile, the content of CD61-positive sEVs in the culture medium was positively correlated with cardiomyocyte metabolic activity. Purified sEVs were not independently administered to establish their contribution (151). Another study directly isolated sEVs from serum-converted human platelet lysates. These sEVs protected rat H9c2 and human AC16 cardiomyocytes from hypoxia/reoxygenation injury (148).

Platelet EVs can also alter the inflammatory response to infarction. sEVs isolated from thrombin-treated healthy human platelet-rich plasma reduced inflammatory cytokines and ICAM-1 in oxidized low-density lipoprotein-treated HUVECs and reduced M1-associated markers in LPS-treated THP-1-derived macrophages. These effects were accompanied by lower recipient-cell miR-34a-5p, increased SIRT1 and reduced NF-κB P65 acetylation in both endothelial cells and macrophages. Increasing miR-34a-5p in the recipient cells weakened the anti-inflammatory response (152). EVs (100-400 nm) from human venous blood contained miR-4306 and transferred it to human monocyte-derived macrophages. These miR-4306^{+} EVs further decreased VEGF expression by direct targeting and suppressed migration, along with ERK1/2 and NF-κB activity. Inhibiting miR-4306 in recipient cells reversed microparticle-associated changes, while *VEGFA* silencing opposed the migration increase caused by miR-4306 inhibition (153).

The inflammatory regulatory function and cardiac protective effects of platelet EVs also extend to viral myocarditis. A lyophilized human platelet-derived sEV preparation reduced inflammatory myocardial injury in CVB3-infected mice, indicated by reduced immune cell infiltration, suppressed myocardial mast-cell degranulation and improved cardiac function (154). A subsequent study compared platelet sEV preparations pooled from 10 male and female donors of various ages with those pooled from 10 women younger than 50 years. Both sEV preparations markedly attenuated CVB3-induced myocarditis compared with vehicle. Even though there was no statistically significant difference in myocarditis severity between the two sEV-treated groups, the sEVs from male and female donors showed improved suppression of inflammation and perivascular fibrosis. Proteomic profiling and miRNA sequencing revealed distinct molecular compositions, including 31 differentially abundant miRNAs. These compositional differences could explain the different patterns of cardiac immune and fibrosis regulation (155).

Detrimental roles of platelet-derived EVs in cardiac disease. Patients with sepsis provide an example of cardiac injury associated with circulating platelet-related EVs. Azevedo *et al* (156) used sEVs from the plasma of patients with septic shock to treat isolated rabbit hearts. They found that these sEVs reduced contractile performance relative to baseline in isolated rabbit hearts. This contractile suppression may relate to the NO production potential of the sEVs, which needs further verification. Notably, this research isolated sEVs only from plasma, not from platelets, because most plasma sEVs were found to be released by platelets.

A distinct relationship between platelet-derived EVs and impaired cardiac function was reported for EVs (25,000 x g, 60 min) generated from adenosine diphosphate-stimulated platelets of patients with acute MI. Exposure of AC16 cells to these microvesicles carrying ACSL1 protein increased iron accumulation, upregulated lipid oxidation and reduced viability, with ferrostatin-1 attenuating microvesicle-induced injury (157).

Environmental stimulation can similarly change the biological activity of platelet sEVs. Direct exposure of rat platelet-rich plasma to fine particulate matter *ex vivo* altered the miRNA profiles in the sEVs. When transferred into recipient rats, these sEVs caused cardiac histological injury, accompanied by reduced cardiac VEGF and increased caspase-3 (158).

Adipose-derived EVs. Epicardial adipose tissue (EAT) lies in close proximity to the heart and could influence cardiac function through EV-mediated communication. However, the cardiac environment has relatively few adipocytes, prompting studies using primary adipocytes from other adipose depots to investigate how adipocyte-derived EVs affect cardiac function. Whether these effects also occur with EVs from pericardial adipocytes remains to be established, given potential differences in adipocyte properties and EV composition. Nevertheless, these studies show that adipocytes can participate in cardiac regulation through EV release and provide a basis for further investigation of EV-mediated communication between pericardial adipose tissue (PAT) and the heart.

Beneficial roles of adipocyte-derived EVs in cardiac repair. sEVs isolated from primary epididymal adipocytes of non-diabetic mice reduced subsequent H_2O_2-induced injury together with increased phosphorylation of AMP-activated protein kinase (AMPK), ERK and acetyl-coenzyme A carboxylase in primary adult mouse cardiomyocytes. In non-diabetic mice, sEVs also protected the heart from I/R-induced cardiac injury. Flow analysis detected adiponectin on the sEV external surface. sEVs from *ADIPOQ*-knockout (adiponectin-deficient) donors and antibody blockade of surface adiponectin reduced protection, while recipient *ADIPOR1* gene deletion or ADIPOR1 receptor inactivation abolished responsiveness (159). Evidence for adipose sEV protection also extends to myocardial iron handling. sEVs collected from PAT of adipsin-overexpressing mice improved survival, ventricular function and remodeling caused by coronary ligation. Increased adipsin in cardiomyocytes further bound to IRP2 and weakened the downstream iron accumulation (160). In addition to these cargoes, mitochondrial cargo, including voltage-dependent anion channel and cytochrome *c* oxidase subunit IV, was also enriched in sEVs from palmitate-treated differentiated mouse adipocytes. Notably, although these sEVs initially induced oxidative stress in the adult mouse heart, they ultimately suppressed I/R-induced cardiac injury. *In vitro*, sEVs from palmitate-treated adipocytes also increased mitochondrial function in adult mouse cardiomyocytes and decreased ROS (161).

Detrimental roles of adipocyte-derived EVs in cardiac disease. Similar to other cells, the functions of adipocyte-derived EVs are partly controlled by the donor state. The PPARγ activator, rosiglitazone, increased miR-200a in sEVs from differentiated 3T3-L1 and primary epididymal adipocyte systems, and PPARγ antagonism reduced that increase. Using a Transwell system, these sEVs from rosiglitazone-treated adipocytes were proposed to induce a hypertrophic response in NRVMs (162). However, the direct relationship between the sEVs and these responses remains to be explored further.

Similarly, diabetic donor conditions can reverse the protective behavior of adipocyte EVs. sEVs from primary epididymal adipocytes of high-fat-diet mice or adipocytes exposed to high glucose/high lipid were enriched with miR-130b-3p. These sEVs further increased neonatal rat cardiomyocyte injury during simulated I/R and worsened acute myocardial I/R outcomes. miR-130b-3p directly decreased AMPKα1/2, BIRC6 and UCP3 expression in recipient cells, while miR-130b inhibition or overexpression of the protective target proteins reduced sEV-associated injury (163). Similarly, another study reported that the sEVs from diabetic primary adipocytes and high-glucose/palmitate-treated 3T3-L1 adipocytes contained miR-130b, which increased infarction and promoted cardiac injury after I/R. These sEVs targeted and suppressed PGC-1α expression in neonatal rat cardiomyocytes. Recipient miRNA inhibition restored PGC-1α-related responses, and PGC-1α overexpression reduced sEV-induced mitochondrial ROS and caspase-9 activation (164). In addition, miR-802-5p was reported in the sEVs from 3T3-L1 adipocytes and was enriched after palmitate-induced hypertrophy. miR-802-5p directly suppressed HSP60 expression, causing insulin resistance and oxidative stress in NRVMs; however, inhibiting miR-802 in the donor before sEV collection weakened these effects and restored HSP60 in recipients (165).

Mitochondrial cargo was associated with cardiomyocyte damage in palmitate-treated adipocytes. After treatment, adipocyte sEVs were enriched in mitochondrial proteins, including TOM20, ACADM and ACSL1, and in protein carbonylation. Direct application of palmitate-adipocyte sEVs to AC16 cells increased mitochondrial ROS and fragmentation, impaired membrane potential and respiratory function, and activated injury-related responses (166). These results differed from another study showing that sEVs from palmitate-treated adipocytes with mitochondrial cargo ameliorated cardiac injury by acting on cardiomyocytes, which was described as adaptive antioxidant signaling after low-level sEV exposure (161). The differences in EV composition, exposure conditions and recipient-cell metabolic state could also explain these differences.

Cardiac fibroblasts are another recipient of pathological adipocyte EVs. Primary epididymal adipocytes exposed to Ang II released sEVs enriched in miR-23a-3p. These sEVs increased fibroblast collagen, upregulated α-SMA expression and promoted functional impairment by directly decreasing

RAP1 expression. Recipient RAP1 restoration attenuated both miRNA-mimic and sEV-related profibrotic responses, indicating the critical role of RAP1 in this process (167).

In addition to adipocytes from other tissues, EAT also releases EVs to regulate cardiac function. In high-fat-diet mice undergoing I/R, EAT removal reduced cardiac inflammation and injury. Using an EAT-macrophage Transwell co-culture system, researchers found that EAT-conditioned medium promoted inflammatory markers in LPS-activated RAW264.7 macrophages, and this effect was weaker after sEV depletion (168). Other studies have identified this promotion of cardiac pathological processes using EAT from patients. sEVs from the EAT of patients undergoing AF surgery increased fibrosis in the rat heart after ventricular-wall injection and shortened action-potential duration in hiPSC-CMs. Further cytokine, protein and miRNA measurements identified disease-associated differences in the sEVs, including increased miR-146b and decreased miR-133a/miR-29a (169). A similar study also identified several miRNAs in the sEVs from the EAT of patients with AF, including miR-1-3p and miR-133a-3p. Direct transfection of miR-1-3p or miR-133a-3p mimics into NRVMs reduced conduction velocity, with changes in *KCNJ2* and *KCNJ12* expression (170).

Epicardial cell and neural cell-derived EVs. The epicardium forms the outermost layer of the heart and provides signals that support cardiac development and injury responses, while autonomic neurons regulate cardiac activity. EV release provides a pathway for these cells to communicate with cardiomyocytes. Although evidence remains limited, studies of EVs derived from epicardial and neural cells suggest effects on cardiomyocyte cell-cycle activity and survival, with responses varying across cellular sources and experimental models.

Beneficial roles of epicardial cell and neural cell-derived EVs in cardiac repair. In 2022, Del Campo *et al* (171) identified epicardial sEVs for the first time. Primary human epicardium-derived cells (EPDCs) were expanded from right atrial appendage biopsies obtained during coronary artery bypass surgery. Mouse embryonic epicardial sEVs promoted the proliferation of primary neonatal murine cardiomyocytes *ex vivo*, whereas sEVs from spindle-shaped human EPDCs stimulated cell-cycle activity in mouse cardiomyocytes and hiPSC-CMs. Acute treatment with human EPDC-sEVs also improved force generation in cryoinjured engineered human myocardium at day 7. miRNA sequencing of mouse and human epicardial sEVs identified several miRNAs reported to enhance proliferation in mouse and human primary cardiomyocytes. Among these miRNAs, adeno-associated virus serotype 9-mediated expression of miR-30a, miR-100, miR-27a or miR-30e enhanced human embryonic stem cell-derived cardiomyocyte proliferation. miR-30a and miR-100 also improved force generation in injured engineered human myocardium (171).

EVs from cardiac parasympathetic ganglia neurons (PGN) have shown protective effects in glucose-exposed cardiomyocytes. Singla *et al* (172) found that PGN-derived sEVs reduced apoptosis and improved cell viability in glucose-exposed H9c2 cardiomyocytes, whereas sympathetic ganglionic neuron-derived sEVs did not show comparable protection. The specific cargo that enables the therapeutic function of PGN-derived EVs has yet to be identified. A previous study suggests that not all neuron-derived EVs share this cardioprotective effect. The sEVs from rat fetal neural stem cells were used to treat oxygen- and glucose-deprived H9c2 cardiomyocytes. This treatment did not markedly alter cell viability and respiratory capacity, indicating no evidence of cardiotoxicity or cardioprotection by the neural stem cell-derived sEVs (173).

Detrimental roles of epicardial cell and neural cell-derived EVs in cardiac disease. Cardiac injury has been widely reported in ischemic stroke models. Supernatant from primary rat neuronal cells exposed to oxygen-glucose deprivation markedly reduced cell viability and mitochondrial activity in rat cardiac myocytes (174). Although the aforementioned study aimed to explore the relationship between brain stroke and cardiac injury, the *in vitro* explorations also suggest potential harmful effects of neuron-derived EVs on cardiomyocytes. Direct evidence on the cargo and molecular mechanisms needs further exploration.

Circulating EVs as diagnostic and prognostic biomarkers. Because EV molecular cargo can reflect changes in donor-cell state, EVs are also being investigated as new diagnostic and prognostic biomarkers in cardiac disease. Clinical studies have identified candidate miRNAs in MI and heart failure (HF). In a study of 150 patients with acute MI and 50 healthy controls, miR-186-5p in serum sEVs was elevated at admission and decreased after percutaneous coronary intervention. Higher levels and slight decreases after treatment were associated with subsequent major adverse cardiac events, suggesting potential value for longitudinal risk assessment (175). Serum sEV-associated miR-92b-5p was also elevated in 28 patients with reduced ejection fraction hospitalized for acute HF compared with 30 controls and was inversely correlated with left ventricular ejection fraction (176).

EV-associated miRNAs have also been explored as indicators of HF development after MI. Matsumoto *et al* (177) reported that serum P53-responsive miRNAs, including miR-192, miR-194 and miR-34a, were elevated during early convalescence in patients who developed HF within one year, particularly in sEVs. More recently, a study of 4,541 patients with acute coronary syndrome associated plasma miR-223-3p and miR-378c with HF hospitalization or cardiovascular death after adjustment for clinical factors and established biomarkers (178). These studies highlight the potential of sEVs as clinical biomarkers. However, further clinical translation requires attention to both analytical reliability and clinical relevance. Blood collection, platelet activation, hemolysis, storage and EV separation can alter measured signatures, while co-isolated lipoproteins and ribonucleoproteins may affect the results. Moreover, circulating EVs originate from multiple cell types, complicating the interpretation of their molecular signatures. The prognostic performance of EVs requires validation in larger, multicenter clinical cohorts.

5. Application of EVs in cardiac therapy

As aforementioned, EVs deliver various cargoes to recipient cells to exert physiological functions. Especially when carrying protective cargo, EVs could help mitigate disease. This characteristic qualifies EVs as a potential therapeutic

Table II. Clinical trials of EV products for cardiac diseases.

Study registration number	Title	Origin of EVs	Clinical trial phase
NCT05774509	Treatment of non-ischemic cardiomyopathies by intravenous EV of cardiovascular progenitor cells	Cardiovascular progenitor cells	Phase 1
NCT05669144	Co-transplantation of mesenchymal stem cell-derived exosomes and autologous mitochondria for patients candidate for CABG surgery	Mesenchymal stem cells	Phase 1, Phase 2
NCT04327635	Safety evaluation of intracoronary infusion of EVs in patients following coronary stent implantation	Blood	Phase 1

CABG, coronary artery bypass grafting; EV, extracellular vesicle.

avenue. Indeed, researchers are investigating EV-based therapeutic approaches for a range of diseases. To date, over 100 EV products targeting different disease therapies have been registered for clinical trials (179), but only one has reached a phase III clinical trial, being developed by Direct Biologics for the treatment of adult respiratory distress syndrome (179). For cardiac diseases, only three EV products have been registered in clinical trials, all at phase 1 or 2 (Table II). Most EVs for cardiac disease therapy remain at the laboratory stage. Herein, advances in EV-mediated therapy for cardiac diseases are summarized, which may provide valuable insights for further exploration.

Diverse sources in EV-based cardiac therapy. For EV-based drug therapy, the identification of a suitable source is critical for the larger-scale development of homogeneous, stable and innocuous EVs (180). So far, in addition to these cardiac resident cell-derived EVs, other sources of EVs have also been identified and applied in EV-based cardiac therapy, including blood, mesenchymal stem cells (MSCs), cardiac progenitor cells (CPCs), milk and plants. This section summarizes some sources of EVs used in cardiac therapy and their characteristics.

Blood plasma is a commonly used body fluid for EV harvest. Blood EVs mainly derive from blood cells, including red blood cells, platelets and leukocytes. A previous study found that, in rats treated with ischemic preconditioning, platelet-derived EVs (<1,000 nm) increased markedly, and treatment with blood-derived EVs from ischemic preconditioning rats markedly mitigated cardiac damage and restored cardiac function in rats with I/R injury (181). Meanwhile, Hou *et al* (182) found that circulating EVs harvested from rats that underwent long-term exercise protected the heart from myocardial I/R injury via miR-342-5p. However, blood composition is highly donor-dependent, making it difficult to ensure reproducibility and therapeutic consistency of blood EVs. The immunogenicity of blood EVs is another challenge. To date, only one blood EV-based therapy has been registered for a clinical trial (NCT04327635). The effect of blood EVs on cardiac diseases remains unclear, and further investigation is needed.

MSCs are multipotent stem cells derived from mesoderm that can differentiate into multiple cell types and exert marked immune regulatory functions. Their ease of *ex vivo* culture and proliferation has enabled the extensive development of MSCs for multiple disease therapies. More than 12 types of MSC-based therapies are currently approved for clinical use (183). However, MSC therapeutic effects decrease with each *ex vivo* passage. In addition, their potential oncogenicity also limits MSC applications (184). Previous research has shown that MSC paracrine signaling, especially via MSC-derived EVs, is involved in MSC-mediated function (185,186). Indeed, MSCs are a widely studied cell source for EV-based therapies. Over 50% of the MSC-based therapies progressing through clinical trials use MSC-derived EVs (179). In cardiac therapy, numerous MSC-based therapeutic approaches have been developed. Native MSC-derived EVs have shown cardioprotective effects in preclinical models of cardiac disease (187,188). Meanwhile, MSC-derived EVs can be modified to enrich specific cargo or to carry targeted surface proteins, thereby enhancing their therapeutic functions (189,190). However, only one MSC-derived EV-based therapy has been tested in a clinical trial to date (NCT05669144), indicating that further efforts are needed.

In addition to MSCs, CPCs are also an excellent source of EVs for cardiac therapy. CPCs have been identified as a valuable cell resource for restoring cardiac function. Previous studies have shown that CPCs can produce bioactive factors, including EVs, that can ameliorate cardiac disease (191,192). Indeed, a good manufacturing practice-grade process has been developed for producing CPC-derived EVs, which have entered a phase I clinical trial for cardiomyopathy (NCT05774509) (193). In addition to stem cells, other cell sources of EVs, including 293T cells, macrophages and T cells, are also being explored for cardiac disease therapy. However, all of them remain at the laboratory stage, and their safety and therapeutic effects require further exploration.

Among body fluid-derived EVs, milk-derived EVs represent the most extensively studied and clinically applicable class. Compared with cell-derived EVs, milk-derived EVs are high-yielding and easy to produce at scale at low cost. In addition, milk-derived EVs are highly stable, enabling them to withstand proteases at low pH and high temperatures, suggesting they can be designed for oral administration (179). However, compared with EVs obtained from cultured producer cells, milk-derived EVs are less readily amenable to conventional producer-cell genetic engineering. Current literature indicates

that these vesicles are primarily isolated from raw milk or dairy products across multiple mammalian species, including humans, cattle, pigs, sheep and horses, with bovine-derived sources accounting for the vast majority (194-196). In the cardiac field, only bovine milk-derived EVs have been used as a drug delivery platform for hypertrophic HF. In that study, miR-30d was encapsulated into milk-derived EVs to alleviate cardiac hypertrophy and rescue cardiac function in murine models of hypertrophic HF (197). Other sources of milk-derived EVs remain to be further explored.

Plants are a recently discovered source for developing EV-based therapeutics. Plant-derived nanoparticles contain lipids, nucleic acids, proteins and other biological phytochemicals that exert various pharmacological activities. Plant sources can be divided into edible plants, including fruit and vegetables, and medicinal plants. Using bitter melon (*Momordica charantia L.*) as an example of an edible plant, Ye *et al* (198) found that the *Momordica charantia L.*-derived nanoparticles ameliorated DOX-induced cardiac injury by suppressing the ubiquitination and degradation of P62; the recovered P62 then bound Kelch-like ECH-associated protein 1, promoting NRF2 nuclear translocation and the expression of the downstream protein heme oxygenase 1. Medicinal plants, such as *Salvia miltiorrhiza*, have also been explored for cardiac disease therapy (199). Compared with other EV sources, plant-derived nanoparticles offer greater abundance and contain bioactive phytochemicals. However, similar to milk-derived EVs, plant-derived nanoparticles are also difficult to genetically modify. Meanwhile, producing pure nanoparticles from plants requires a complicated and costly isolation process. More importantly, using medicinal plants as the source may be costly, as most medicinal plants are expensive. In general, although current research on plant-derived nanoparticles has made notable advances, further efforts are needed to enable broader application.

Modification strategies for EV-based therapy. EV engineering strategies can be broadly categorized into two major approaches, depending on whether manipulation is performed at the level of EV-producing cells or on isolated EVs (200). In the former, producer-cell engineering, donor cells are genetically or conditionally programmed to package desired cargoes and display specific membrane elements, thereby enabling the cells to secrete EVs with tailored contents in a scalable and potentially sustained manner. In the latter, the modification is performed directly on isolated EVs to load specific cargo or functionalize the surface using techniques such as incubation, physical permeabilization and chemical conjugation. In the present study, some methods for modifying the EVs are briefly described (Table III).

Producer-cell engineering. Because EVs carry contents from donor cells, modifying donor cells can achieve specific cargo loading in EVs, enabling the development of EV-based therapy (201). Genetic engineering of producer cells is widely used to modify EV cargo and surface composition (202). By delivering the target-gene expression vector to donor cells, this strategy directly increases specific cargo content in EVs (201). Moreover, the target genes can be fused to EV-specific proteins, enabling selective sorting of the desired cargo to the EV membrane or its internalization (203). Although genetic engineering is widely used to prepare EV-based cardiac therapies, it is suitable only for cargo that can be expressed by endogenous cells, such as nucleic acids, proteins or peptides. Synthetic molecules cannot be loaded into EVs using this method. In addition, although various vectors have been developed and applied in EV-based therapy, genetic engineering remains challenging. Establishing a stable engineered donor cell line for EV-based therapy is complex and time-consuming. Moreover, although genetic engineering alters the expression of only one or two genes, the complexity of cellular biological processes affects other molecules, indicating that more than one type of cargo on the EVs would be altered.

In addition to directly engineering gene expression, previous studies have used stimuli to achieve specific cargo loading into EVs (204,205). Compared with genetic engineering, this strategy offers a more conventional, readily modifiable approach to modifying donor cells for EV-based therapies. However, the stimuli altered the gene expression profiles of the donor cells, indicating that the EV cargo would be affected by these stimuli rather than by a single content change.

Direct modification of EVs. Because engineering donor cells cannot acutely alter EV contents, more researchers prefer to engineer EVs directly through various techniques. Co-incubation is a simple method for directly modifying EVs (206). By incubating cargo with EVs, the concentration difference between the surrounding medium and the vesicle lumen allows cargo to diffuse into EVs. The loading efficiency of co-incubation depends on cargo chemistry, EV membrane properties, concentration gradients and incubation conditions (207). Notably, this strategy is not restricted to hydrophobic cargo, although cargo hydrophobicity can influence loading efficiency (208). For example, a study of lipid-conjugated small interfering RNAs found that greater hydrophobicity was associated with higher EV loading efficiency (209).

To overcome these flaws, various physical permeabilization approaches, such as sonication, have been applied to the cargo loading of EVs. This approach applies mechanical shear to transiently perturb the EV membrane, promoting the incorporation of otherwise poorly permeable hydrophilic cargoes, including small-molecule drugs, proteins and nanomaterials (210,211). Electroporation enables cargo encapsulation into EVs by applying an external electric field to transiently form nanoscale pores in the vesicle membrane, thereby increasing membrane permeability and facilitating drug entry (212,213). Even though these physical permeabilization approaches improve cargo loading efficiency, membrane integrity may be compromised during the loading process, which may alter the inherent characteristics of the EVs. Electroporation can induce cargo aggregation, potentially leading to overestimation of loading efficiency. For both sonication and electroporation, experimental parameters need to be calibrated for different cargoes.

Chemical modification, including chemical coupling and transfection, is another type of approach for engineering EVs. Chemical coupling involves both non-covalent and covalent interactions. Non-covalent coupling connects specific cargo to the EV membrane through non-covalent forces, such as electrostatic interactions or hydrophobic insertions (214). Covalent EV-surface functionalization can be achieved through several conjugation strategies, including click chemistry. Native EV functional groups can serve as sites for chemical modification.

Table III. Engineering strategies for EV-based therapy.

Classification	Subtypes	Engineering objectives	Advantages	Disadvantages
Producer-cell engineering	Genetic engineering	Endogenous cargo modulation	• Enables enrichment of selected genetically encoded cargo during EV biogenesis	• Complex experimental design • Only suitable for the cargo that can be endogenously expressed • May alter other contents in the EVs
	Stimulus-based producer-cell preconditioning	Endogenous cargo modulation	• Easy to operate	• Achieving precise control over the loading level of the target cargo in EVs is challenging
Co-incubation	-	Direct cargo loading	• Easy to operate	• Loading efficiency depends on cargo chemistry, EV membrane properties, concentration gradients and incubation conditions • Uncontrolled drug loading
Physical permeabilization	Electroporation	Direct cargo loading	• Facilitates cargo entry through transient membrane permeabilization	• May compromise membrane integrity • Difficulties distinguishing encapsulated from externally associated material • Cargo aggregation • Requires parameter optimization
	Sonication	Direct cargo loading	• Enhances cargo incorporation under optimized conditions	• May alter EV membrane structure or cargo bioactivity • Requires optimization of sonication parameters and temperature
Chemical modification	Non-covalent coupling	EV-surface functionalization	• Often compatible with mild reaction conditions • Easy to operate	• Weak connection • Substantial batch-to-batch variability is observed
	Covalent coupling	EV-surface functionalization	• Provides stable surface attachment of functional molecules	• Requires optimization of reaction conditions • May alter native surface molecules or EV biological activity • Unreacted reagents require removal.
	Chemical transfection	Direct cargo loading	• High loading efficiency • Does not disrupt the lipid bilayer structure	• Requires transfection reagents • The residual transfection agents cause side effects
Hybridization with other materials	-	Addressing EV limitations and expanding function	• Combines EV-related properties with additional material functions	• Requires evaluation of material compatibility, structural integrity, stability and biological activity • Increases formulation complexity and reproducibility requirements

EV, extracellular vesicle.

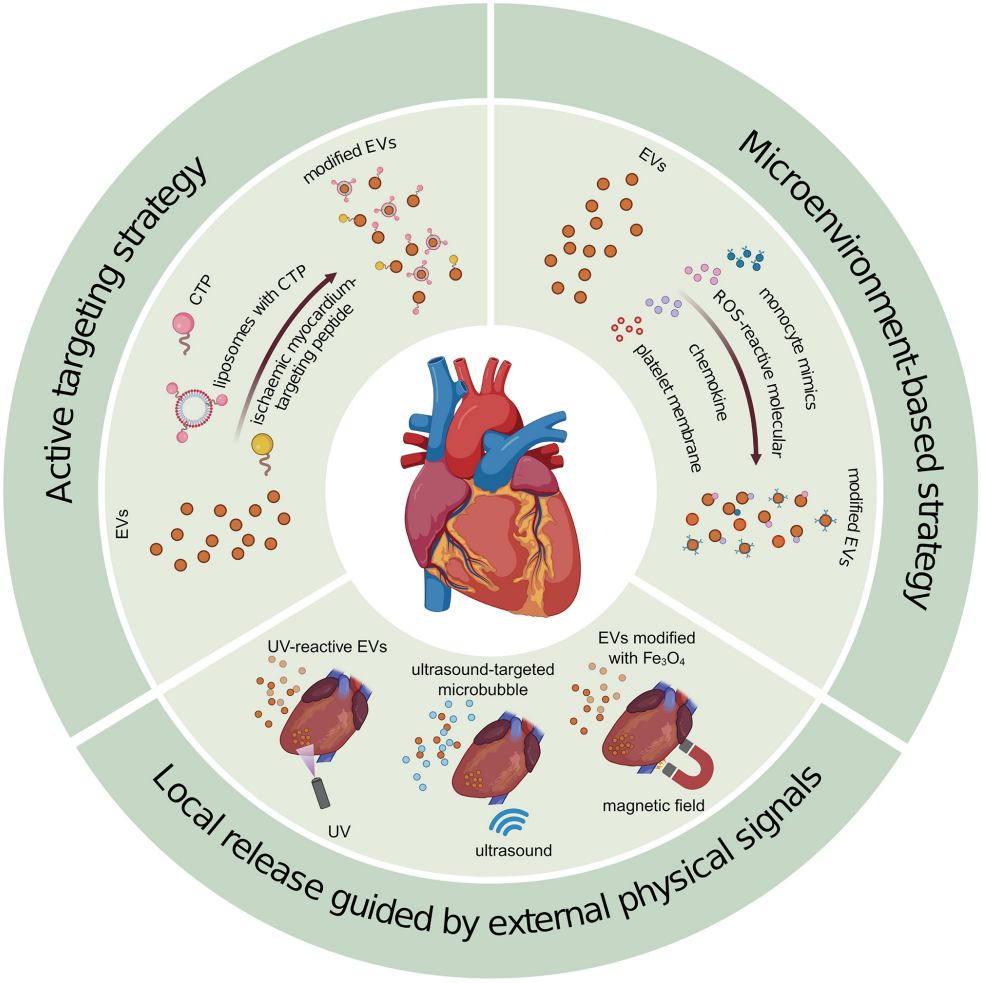


Figure 3. Strategies for EV-based targeting of cardiomyocytes. Current strategies to enhance the myocardial targeting efficiency of EVs are broadly categorized into active targeting strategies, microenvironment-based strategies and local release strategies guided by external physical signals. CTP, cardiac-targeting peptide; EV, extracellular vesicle; ROS, reactive oxygen species. The figure was created using BioRender; https://BioRender.com/g8vlt92.

For example, Lu *et al* (215) generated azide-bearing sEVs through metabolic glycoengineering and conjugated them with a targeting single-chain variable fragment modified with dibenzo-cyclo-octyne via strain-promoted azide-alkyne cycloaddition. This approach enables covalent attachment of targeting ligands to the EV surface.

Separately, transfection reagents have been used to promote cargo loading into EVs. This approach requires transfection reagents, and EV membrane integrity should be assessed after treatment. Residual cargo-reagent complexes may contribute to the observed delivery effects, making it difficult to attribute these effects specifically to EVs (216). Appropriate purification and controls are therefore needed to evaluate cargo loading and EV-mediated delivery.

In addition to modifying EVs by altering donor cells or directly modifying EVs, hybridization with other materials, such as hydrogels, is also employed in the engineering of EV-based therapies (212,217). This strategy can address delivery limitations and expand the functionality of EV-based formulations.

Engineering EVs for cardiac disease therapy. Although EVs have numerous advantages, they do not meet all the requirements of cardiac disease therapy. Therefore, modifying the EVs or hybridizing them with other advanced materials to confer new characteristics on EV-based therapeutics is necessary. So far, numerous advanced technologies have been applied in EV-based therapy, including genetic engineering, surface chemical modification, cargo loading and hybridization with other materials, which have been extensively reviewed in other studies (200,218) (Fig. 3). In the present review, the modification of cardiac targetability in EV-based therapy is the focus.

Cardiac-targeting peptides include cell-penetrating peptides screened by phage display technology that have high affinity for cardiomyocytes. For example, the cardiomyocyte-specific peptide (CMP; WLSEAGPVVTVRALRGTGSW) (219) and the cardiac-targeting peptide (CTP; APWHLSSQYSRT) (203) have been used in EV-based therapy. By fusing the cardiac-targeting peptide to the membrane via the EV lysosome-associated membrane protein 2B, EVs can achieve cardiac targetability, thereby delivering specific cargo to cardiomyocytes to ameliorate cardiac diseases (220,221). In addition to direct connection to the EV membrane, hybridization with CTP-containing advanced materials, such as liposomes (222), is also a strategy to target cardiomyocytes. Although cardiac-targeting peptides have been tested and show high affinity for cardiomyocytes, off-target effects

cannot be ignored, since these peptides do not exclusively target cardiomyocytes. In addition, the detailed mechanism of the cardiac-targeting peptides remains to be further explored.

The homeostasis of the cardiac microenvironment is maintained by various types of cells. During cardiac injury, various pathological changes occur in the cardiac microenvironment, including inflammatory infiltration, ROS accumulation, ECM remodeling and even local pH changes. Various cardiac-targeting strategies have been designed based on these pathological changes and have been applied in EV-based therapy. Taking inflammatory infiltration as an example, Zhang *et al* (223) developed a new strategy that builds on the monocyte recruitment process in the heart after I/R to enable cardiac-targeting EV-based therapy. They modified MSC-derived EVs with monocyte mimics via membrane fusion to achieve cardiac targeting through the macrophage-1 antigen/lymphocyte function-associated antigen 1/ICAM-1 interaction. The ROS-responsive strategy is also applied in EV-based therapy. By connecting the ROS-responsive molecule to the EVs, Lu *et al* (224) successfully delivered rapamycin to the heart to ameliorate I/R damage after heart transplantation. In addition, upregulated chemokines in cardiac injury, such as stromal cell-derived factor 1, can be used for cardiac targeting. One study developed C-X-C motif chemokine receptor 4-high-expressing EVs to achieve cardiac targeting and reverse I/R-induced cardiac injury (225). In addition, modifying the EV surface with the ischemic myocardium peptide CSTSMLKAC can enhance cardiac targetability and support EV-based therapy (226). In the ischemic heart, ECM components, including collagen, fibronectin and von Willebrand factor, promote platelet adhesion. Building on these characteristics, fusing the platelet membrane with the EV membrane markedly promotes EV accumulation in the injury zone after I/R (227).

Using external physical signals to guide local EV release can also achieve cardiac targetability. For example, by modifying EVs with Fe_3O_4, EVs can be guided to the heart when exposed to an external magnetic field, concentrating them in the heart (228). Meanwhile, ultrasound-targeted microbubble destruction technology can facilitate targeted EV release in the heart (229,230). By directly mixing commercial microbubbles with sEVs, Chen *et al* (231) achieved the targeted release of EVs in the heart to ameliorate DOX-induced cardiac injury. In addition to magnetic fields and ultrasound, photo-controlled systems can also be used for targeted therapy (232). However, EV-based therapy for cardiac diseases remains underexplored.

6. Conclusions and future perspectives

With marked advances in methods for isolating and characterizing EVs, research on EVs has expanded greatly. In the present review, various sources of cardiac EVs and their regulatory functions in controlling the cardiac microenvironment were summarized. Through these intensive explorations, the ubiquitous key role of EVs in heart disease has been recognized. However, numerous questions remain unanswered. A systematic, in-depth exploration of EVs and their contributions to pathway-level changes at single-cell resolution is needed. In addition, given the heterogeneity of EVs, the development of new biomarkers to characterize EVs from each route and the drawing of single-vesicle maps are necessary to provide a comprehensive understanding of EVs in cardiac diseases. Advances in methods, such as single-vesicle analysis, nanoflow cytometry and microfluidic platforms, may address these challenges.

The high bioavailability and low immunogenicity of EVs suggest strong potential for developing next-generation therapeutics in cardiovascular medicine. With the development of genetic engineering, the surface biomarkers and inner contents of EVs can be modified and customized, providing great opportunities for biomedical applications. Although researchers have developed various unique and modified EVs to treat a range of heart diseases, and have explained the underlying mechanisms, clinical application remains a distant goal. The administration route, short half-life of EVs and large-scale production remain formidable challenges. Addressing these challenges is necessary to advance the clinical development of EV-based cardiac diagnostics and therapies.

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Authors' contributions

JHH and PT completed the writing of the main text and prepared the figures and tables. YL and HGZ provided professional guidance and critical review. All authors read and approved the final version of the manuscript. Data authentication is not applicable.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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