



# Advances in Cardioprotective Strategies: Bridging Traditional Pharmacotherapy and Regenerative Medicine

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## Abstract

Cardiovascular diseases (CVDs) remain the leading global cause of death and disability, underscoring the need for improved therapies. Key emerging strategies include pharmacological cardioprotection, cell-based therapies and the use of exosomes as therapeutic agents and biomarkers. Aim of this study was to characterise contemporary approaches to cardioprotection in CVDs, including pharmacological agents, cell-based therapies and exosome-based strategies, based on an analysis of evidence-based data. A systematic literature search was performed using databases including PubMed, Clinical Key (Elsevier), Cochrane Library, eBook Business Collection and Google Scholar. Keywords included cardioprotection, exosomes, cell-based therapies and pharmacological approaches. Article selection followed evidence-based medicine principles and the PRISMA guidelines. Current cardioprotective strategies include both traditional pharmacological agents, such as  $\beta$ -blockers, calcium channel blockers, ACE inhibitors, statins and nitrates, as well as innovative approaches like cell-based therapies and the use of exosomes. The advantages and limitations of cell therapy were analysed, including challenges related to low cell survival, failure of differentiation and the risk of arrhythmias. The role of exosomes and microvesicles as promising markers of cardiovascular injury and potential therapeutic agents was also explored. Combining pharmacological, cell-based and exosome-based strategies offers new prospects for cardioprotection in CVDs. Further research is required to optimise the clinical use of cell therapies and to confirm the efficacy of exosome-based interventions.

**Key words:** Cardiovascular diseases; Prevention and control, cardiac; Exosomes; Cell and tissue-based therapy; Therapeutics; Ischaemia; Ischaemic injury; Regenerative medicine; Adrenergic beta-antagonists; Calcium channel blockers; Microvesicles.

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## Introduction

Cardiovascular diseases (CVDs) are the leading cause of global mortality and disability. They include a range of conditions, with atherosclerosis, ischaemic heart disease (IHD), acute cerebro-vascular accidents, cardiomyopathies and heart failure (HF) being particularly significant.<sup>1</sup> These

conditions often coexist and their pathogenesis is closely interconnected, substantially complicating diagnosis and treatment.<sup>2</sup>

The primary risk factors for CVD development include cardiometabolic, behavioural, environmen-

tal and social determinants, which interact to promote the onset and progression of cardiovascular pathology. Globally, the prevalence of these risk factors has led to a continuous rise in CVD burden. From 1990 to 2019, the number of people living with CVD grew from 271 million to 523 million, while CVD-related deaths increased from 12.1 million to 18.6 million.<sup>1</sup> These trends highlight the ongoing rise in CVD incidence and mortality globally. The prevalence of CVDs is expected to grow, driven mainly by population growth and aging, particularly in regions like Northern Africa, Latin America, the Caribbean and Southeast Asia. The proportion of elderly individuals in these regions is expected to double by 2050, placing increased pressure on healthcare systems.<sup>3</sup>

The COVID-19 pandemic, which began in 2019, has added another layer of complexity to global health challenges. More than seven million deaths have been reported and millions more individuals have suffered significant health impairments. Although vaccination programs have substantially reduced mortality and hospitalisation rates, the cardiovascular consequences of COVID-19 remain a pressing concern. Many post-infection patients experience persistent cardiac symptoms, often without clear objective findings, complicating both diagnosis and management. Moreover, the lack of adequately matched control groups and insufficient data for developing effective therapeutic strategies further emphasises the urgency of addressing these issues.<sup>4</sup>

Contemporary IHD management includes percutaneous coronary intervention (PCI), coronary artery bypass grafting (CABG), pharmacotherapy and non-pharmacological approaches. PCI and CABG are highly effective in improving myocardial perfusion, alleviating symptoms and enhancing quality of life. Concurrently, conservative treatment strategies, particularly pharmacotherapy, play a critical role in controlling atherosclerosis progression and preventing atherothrombotic events. Combination therapy, including anti-ischaemic agents ( $\beta$ -blockers, calcium channel blockers), antiplatelet therapy (aspirin, clopidogrel) and lipid-lowering agents (statins), manages IHD symptoms, reduces event risk and slows disease progression.<sup>5-7</sup>

Given the limitations of conventional therapies, there is growing interest in regenerative approaches for myocardial repair, particularly in advanced cardiovascular disease. Stem cell-based therapies, owing to their differentiation, regenerative and immunomodulatory properties, offer promising prospects for cardiology. Studies have shown that stem cells can promote myocardial regeneration after infarction and improve outcomes in heart failure patients.<sup>2</sup> These innovative strategies can potentially radically reshape treatment approaches and significantly improve prognosis in individuals with severe cardiac pathology.

Aim of this study was to characterise contemporary approaches to cardioprotection in cardiovascular diseases, with particular emphasis on pharmacological agents, cell-based therapies and exosome-based strategies, based on the analysis of evidence-based data.

## Data collection

A comprehensive search of the literature was carried out across multiple databases, including PubMed, *Clinical Key Elsevier*, the *Cochrane Library*, the *eBook Business Collection* and *Google Scholar*, using keywords like "cardioprotection," "exosomes," "cell-based therapies," and "pharmacological approaches." Studies were selected following established guidelines for systematic reviews. The process was divided into three stages: First, relevant literature was identified using keywords such as Cardioprotection, Pharmacotherapy, Stem Cells, Exosomes, Cardiovascular Diseases and Regenerative Medicine. Second, abstracts were reviewed and studies that did not meet the inclusion criteria were excluded. Finally, full-text articles were examined to assess their relevance and compliance with the inclusion criteria. Inclusion criteria comprised the recency of data (preferably within the last five years), open access to full-text articles and scientific relevance to the topic of cardioprotection in cardiovascular diseases.

## Cardioprotective agents: classification, mechanisms of action and their role in clinical practice

Cardioprotective agents are specialised pharmacological and biologically active substances capable of reducing the risk of myocardial injury, improving metabolic processes within cardiomyocytes and facilitating cardiac adaptation to stressful conditions.<sup>2, 5, 6, 8</sup> The modern approach to cardioprotection relies on the established understanding of underlying molecular mechanisms of action of cardioprotective agents (Table 1) and includes the application of innovative technologies that contribute to quality of life and increasing long-term survival.<sup>8</sup>

Cardioprotection research is advancing rapidly. Gene therapy and nanotechnology are considered promising directions in this area. Genetic approaches enhance cardiac cell stress resistance through targeted modulation of intracellular signalling. Nanotechnologies provide targeted delivery of cardioprotective agents to damaged myocardial areas, significantly improving therapeutic efficacy and minimising side effects. This opens new opportunities for treating cardiovascular diseases and developing therapies based on innovative technologies.

### β-adrenergic receptor blockers

β-blockers exert key cardioprotective effects by reducing myocardial oxygen and energy substrate demand through negative inotropic and chronotropic actions, prolonging diastolic

perfusion and lowering intracellular calcium concentrations. Additionally, they restore β-adrenergic receptor affinity to catecholamines in states of hypercatecholaminaemia common in cardiovascular diseases.<sup>9</sup>

Randomised controlled trials have shown that β-blockers reduce mortality and improve cardiac function. Recent research focuses on developing more selective agents with reduced pulmonary and metabolic side effects, thereby broadening their clinical use.<sup>10-12</sup>

In the perioperative setting, β-blocker continuation is recommended for patients with existing cardiovascular disease. However, a large cohort study (11,875 patients, 2018) questioned their preoperative initiation, showing increased risks of mortality, stroke and myocardial infarction. Initiating β-blocker therapy in low-risk patients may impair compensatory hemodynamic responses to blood loss, highlighting the need for further studies to define optimal perioperative indications.<sup>10-12</sup>

### Calcium channel blockers

Calcium channel blockers (CCBs) improve myocardial perfusion, reduce cardiac energy expenditure and optimise oxygen consumption by limiting calcium influx. They decrease vascular smooth muscle tone, lower peripheral resistance

*Table 1: Key pharmacological groups of cardioprotective agents<sup>8</sup>*

Agents predominantly targeting energy processes	Antioxidant agents and electron acceptors	Inhibitors of free fatty acid oxidation	ATP-sensitive K <sup>+</sup> channel openers
Phosphocreatine	Quercetin	L-carnitine (Levocarnitine)	
Magnesium gluconate	Lecithin	Trimethylhydrizinium propionate	Nicorandil
Potassium gluconate	Thiotriazoline	Trimetazidine hydrochloride	
Carbonate	Niacin	Ranolazine	
Taurine	L-arginine monohydrate		
Meldonium	Ceruloplasmin		
Sodium adenosine triphosphate	Methylethylpyridinol		
	Ethylmethylhydroxypyridine		
	succinate		
	Ubiquinone		
	Cytochrome oxidase		

and enhance blood flow, benefitting patients with hypertension and ischaemic heart disease.<sup>9</sup>

While CCBs show potential for reducing ischaemia-reperfusion myocardial injury (IRMI), clinical evidence remains inconclusive, necessitating further investigation. These agents also lower heart rate, decreasing myocardial workload and improving cardiac function, which supports their role in managing heart failure. Future studies are essential to clarify their impact on IRMI and long-term cardiovascular outcomes.<sup>12-14</sup>

### Angiotensin-converting enzyme inhibitors (ACEIs) and angiotensin II receptor blockers (ARBs)

ACEIs and ARBs are key therapies for hypertension and chronic heart failure, improving ventricular filling, reducing arrhythmias and mitigating reperfusion injury.<sup>9</sup> Their cardioprotective effects stem from preventing left ventricular hypertrophy and remodelling, enhancing myocardial function and exerting vascular protective actions by inhibiting smooth muscle proliferation. In the perioperative period, ACE-Is and ARBs lower peripheral resistance and improve left ventricular function, although intraoperative use may provoke hemodynamic instability, warranting careful timing of discontinuation.

Levosimendan, a novel calcium sensitiser, offers potent inotropic support and moderate vasodilation, improving hemodynamics and reducing myocardial oxygen demand. However, it requires careful monitoring due to risks of hypotension and tachycardia.<sup>11, 15-17</sup>

### Diuretics

Diuretics remain essential for managing cardiovascular diseases, particularly CHF. Thiazide diuretics effectively reduce blood volume and blood pressure in early hypertension, while loop diuretics like furosemide address more severe heart failure and oedema.<sup>9</sup>

Appropriate diuretic use prevents complications such as pulmonary oedema and heart failure exacerbations, improving quality of life and reducing hospitalisations. Continuous monitoring is necessary to minimise adverse effects and ensure safe treatment.<sup>18, 19</sup>

### Statins

Statins are pivotal in modern cardiology for reducing the risk of major cardiovascular events. Beyond their lipid-lowering properties, they exert anti-inflammatory and antioxidant effects, stabilise atherosclerotic plaques, enhance endothelial function and promote nitric oxide synthesis, contributing to vasodilation and blood pressure reduction.<sup>9</sup> Their multifaceted actions underscore their role in preventing myocardial infarction, stroke and atherosclerotic disease progression.

### Nitrates

Nitrates play a critical role in alleviating acute myocardial ischaemia symptoms. Nitroglycerin reduces preload and afterload, lowers myocardial energy demand and improves subendocardial perfusion by decreasing left ventricular diastolic pressure.

Additionally, nitrates relieve coronary artery spasms, a key trigger of acute ischaemic events and reduce platelet aggregation via nitric oxide-mediated pathways, enhancing endothelial function.<sup>20, 21</sup> The combination of these pharmacological agents provides a comprehensive approach to managing cardiovascular diseases. Therapy should be tailored to individual patient factors and comorbidities. Ongoing refinement of existing therapies and development of new agents promise improved cardiovascular outcomes.

### Specific aspects of cardioprotection in the perioperative period

Myocardial ischaemia-reperfusion injury (IRI) in the postoperative period is a complex, multifactorial process, involving disturbances in oxygen supply-demand balance even in patients with anatomically normal coronary arteries.<sup>22, 23</sup>

Factors contributing to ischaemic imbalance include significant coronary stenoses, coronary artery spasms, microvascular dysfunction and embolisation, as well as increased myocardial oxygen consumption.<sup>9</sup> Additional perioperative triggers include tachyarrhythmias, hypertension or hypotension, bradyarrhythmias impairing

cardiac output and hypoxaemia, all exacerbating myocardial ischaemia.<sup>24-26</sup>

Other contributors such as severe anaemia, coronary vasospasm, electrolyte imbalances, acidosis and shock further elevate the risk of type 2 myocardial infarction. Therefore, meticulous monitoring and management of these variables are critical for optimising myocardial perfusion, facilitating recovery and preventing serious post-operative complications.

### Halogenated inhalational anaesthetics

Modern inhalational anaesthetics, notably sevoflurane and desflurane, offer cardioprotection through anaesthetic preconditioning, attenuating apoptotic pathways during reperfusion. Their dose-dependent haemodynamic effects — reduced afterload with preserved contractility and improved diastolic function — facilitate myocardial adaptation to surgical stress. However, clinical evidence remains mixed, influenced

by patient and procedural factors.<sup>27, 28</sup> Current guidelines support their use in hemodynamically stable patients with IHD and preserved ejection fraction.

### $\alpha$ 2-adrenergic receptor agonists

$\alpha$ 2-adrenergic receptor agonists may provide cardioprotection by decreasing heart rate, lowering blood pressure and reducing oxygen consumption, while promoting coronary vasodilation via nitric oxide and adenosine. They also possess anti-inflammatory, antioxidant and antiapoptotic properties. Dexmedetomidine, a newer agent, shows promise but requires further validation. While early studies suggested a reduction in perioperative myocardial infarctions, later meta-analyses found no decrease in mortality or ischaemic events and confirmed increased risks of bradycardia and hypotension.<sup>29-31</sup> Thus,  $\alpha$ 2-agonists are currently not recommended for perioperative cardioprotection.

## Non-pharmacological cardioprotection and limitations of cell therapy

Decades ago, it was found that cardiomyocytes have signalling pathways that protect against ischaemia-reperfusion injury, a mechanism known as "cardioprotection".<sup>32-34</sup> Cardioprotection can be induced by ischaemic preconditioning and postconditioning of the myocardium.<sup>32</sup> While highly protective, these methods involve

ischaemia-reperfusion, which carries risks like atherosclerotic damage and microembolisation, especially during postconditioning. Alternatively, cardioprotection can be induced through brief ischaemia-reperfusion at remote non-cardiac sites (remote ischaemic conditioning).<sup>32, 35</sup>

*Table 2: Characteristics of cell sources for therapeutic cardiac regeneration (adapted from<sup>37</sup>)*

Generation	Cell type	Source	Markers	Advantages	Limitations	References
First generation	Bone marrow and peripheral blood progenitor cells	Bone marrow, Peripheral blood	CD117 <sup>+</sup> , CD34 <sup>+</sup>	Limited regenerative potential; Phase 3 trials; Minimal cardiac function improvement, limited engraftment; Easy cryopreservation; Genetically modifiable; Safe; Accessible; No ethical or immunological issues; Extensive clinical experience	Limited differentiation potential; Limited yield	38-52

First generation	Mesenchymal stem cells	Embryonic, adult tissues, tooth germ	CD105 <sup>+</sup> , CD117 <sup>+</sup>	Limited regenerative potential; Phase 3 trials; Minimal cardiac function improvement, limited engraftment; Easy cryopreservation; Genetically modifiable; Safe; Accessible; Source of paracrine factors	Limited differentiation potential; Yield-dependent	53
	Side population cells	Heart biopsy	Abcg2 <sup>+</sup> , Mdr1 <sup>+</sup>	Limited regenerative potential; No clinical strategy	Limited yield	54–56
	Epicardial progenitor cells	Heart development	Wt1 <sup>+</sup> , Tbx18 <sup>+</sup> , CD90 <sup>+</sup> , CD44 <sup>+</sup>	Limited regenerative potential; No clinical strategy	Limited yield	57–60
	Isl1 <sup>+</sup> progenitor cells	Heart development	Isl1 <sup>+</sup>	Limited regenerative potential; No clinical strategy	Limited yield	61–63
	Cardiosphere-derived cells	Heart biopsy	c-kit <sup>+</sup> , Sca-1 <sup>+</sup> , CD105 <sup>+</sup> , CD29 <sup>+</sup> , CD45 <sup>-</sup>	High regenerative potential; Phase 1–2 trials; Improved cardiac function; Limited engraftment	Limited yield	64
Second generation	c-kit	Heart biopsy	c-kit <sup>+</sup> , CD45 <sup>-</sup>	High regenerative potential; Phase 2 trials; Improved cardiac function; Limited engraftment	Limited yield	65–68
	Sca-1	Heart biopsy	c-kit <sup>+</sup> , CD31 <sup>+</sup> , CD14 <sup>-</sup> , CD34 <sup>-</sup> , CD105 <sup>+</sup> , CD45 <sup>-</sup>	High regenerative potential; Preclinical stage; Limited engraftment	Limited yield	69–71
	Embryonic stem cells	Blastocyst inner mass	Oct4 <sup>+</sup> , Nanog <sup>+</sup> , SSEA4 <sup>+</sup>	High regenerative potential; Preclinical; Phase 1 trials	Ethical concerns; Teratoma formation; Immunogenicity	72
	Induced pluripotent stem cells	Reprogrammed somatic cells	Oct4 <sup>+</sup> , Nanog <sup>+</sup> , SSEA4 <sup>+</sup>	High regenerative potential; Preclinical stage; Improved cardiomyocyte differentiation	Tumorigenicity	73–76

An alternative approach involves attempting to regenerate lost myocardium or improve myocardial function. However, due to the terminal differentiation of mammalian cardiomyocytes, spontaneous myocardial regeneration through cell proliferation is extremely limited. Therefore, efforts have focused on myocardial regeneration via the injection of various stem cell types.<sup>36</sup> Yet, the biological activity of transplanted cells varies significantly depending on cell source, preparation and delivery methods (Table 2).

Cell therapy holds significant potential for treating cardiovascular diseases (CVD), but its clinical implementation faces several important challenges. Key obstacles include poor engraftment, low survival of transplanted cells and inefficient cell differentiation. Additionally, minimising the risk of adverse effects, such as arrhythmias following cell therapy, remains critical. To establish cell therapy as a standard treatment, these challenges must be addressed through further research. A detailed analysis of these aspects is provided below.<sup>37</sup>

## Low engraftment of bone marrow and blood-derived endothelial progenitor cells

Cell therapy is limited by poor engraftment, as few transplanted cells survive and contribute to repair—mainly due to insufficient blood supply in ischaemic tissue.<sup>77-79</sup>

## Poor viability of transplanted cells in ischaemic tissues

Ischaemic areas often lack sufficient blood supply, leading to tissue damage and impaired organ function. Transplanted cells suffer from oxygen and nutrient deprivation, resulting in high rates of cell death. Additionally, insufficient removal of metabolic waste products further compromises cell viability.<sup>80</sup>

## Limited differentiation of adult stem cells into functional heart cells

A key aim of cell therapy is to generate mature cardiomyocytes that repair damaged heart tissue. However, poor differentiation—due to inadequate induction or hostile tissue environments—often limits its effectiveness.<sup>81</sup>

## Limited recruitment of circulating or resident cardiac stem cells

Another key issue is the insufficient recruitment of cardiac stem cells to the injured myocardium. Both circulating and resident stem cell pools are often inadequate, with impaired activation mechanisms limiting their availability for therapy.<sup>82</sup>

## Abnormal electromechanical coupling leading to arrhythmias

One serious risk of cell therapy is the disruption of normal cardiac electrical activity due to abnormal coupling between transplanted and native cells. This can trigger dangerous arrhythmias and represents a major safety concern.<sup>83</sup>

## Limitations of left ventricular ejection fraction (LVEF) as an indirect marker in assessing cell therapy

LVEF is commonly used to assess the effectiveness of cell therapy. However, because it is highly load-dependent, it may not reliably reflect true

myocardial recovery. Changes in preload and afterload can mask actual improvements in contractility.<sup>84</sup>

## Inappropriate selection of patient population

Another limitation is the selection of patients with relatively preserved cardiac function, such as those with baseline LVEF around 50 %, who may not benefit significantly from cell therapy.<sup>84</sup>

## Availability of well-developed alternative therapeutic strategies

Several established interventions, such as percutaneous coronary intervention, fibrinolysis, ACE inhibitors and  $\beta$ -blockers, may offer better or comparable outcomes, reducing the relative need for cell therapy.<sup>85</sup>

## Lack of experimental validation of cell preparations during clinical trials

Many trials lack robust experimental validation of cell preparations, leading to inconsistent results and raising concerns about the reproducibility and standardisation of therapies. Thus, despite some promising results in preserving myocardium and improving cardiac function, cell therapy for CVD has not yet succeeded in increasing the number of cardiomyocytes. Recently, attention has shifted toward factors secreted by stem cells that may mediate paracrine effects. Among these, exosomes have emerged as a promising mediator of therapeutic effects.<sup>36</sup>

## Exosomes and microvesicles as biomarkers in cardiovascular disease

Nearly all cardiovascular cells release small lipid vesicles called exosomes. Though their isolation and analysis remain technically challenging, exosomes from different sources have shown strong cardioprotective properties.<sup>36</sup> Extracellular vesicles (EVs), particularly exosomes, play a paracrine role by releasing into the extracellular space and inducing anti-apoptotic, angiogenic, immunomodulatory and anti-fibrotic responses.<sup>87,88</sup>

**Table 3: Molecular components of exosomes (adapted from<sup>87</sup>)**

Type	Category	Examples	References
Proteins	Tetraspanins	CD9, CD37, CD53, CD55, CD63, CD81, CD82	96, 97
	Cytoskeletal proteins	Actin, tubulin, cofilin-1, moesin, myosin, vimentin, ezrin, radixin, perlecan, fibronectin, THBS1, IQGAP1, keratin	96–98
	Tetraspanins	CD9, CD37, CD53, CD55, CD63, CD81, CD82	96, 97
	Biogenesis proteins	ESCRT-0, I, II, III, Her, Vps4, TSG101, Alix, flotillin, clathrin	98
	Transport and synthesis	RAS-related proteins 5 and 7, annexins I–VI, dynamin, syntaxin-3, RAB4, RAB5, RAB7, RAB11, RAP1B, RABGDI, SLC3A2, CLIC1	96–98
	Heat shock proteins	αB-crystallin, HSP20, HSP22, HSP27, HSP40, HSP60, HSP70, HSP90, HSC70, HSPA5, CCT2	96, 98, 99
	Adhesion molecules	ICAM-1, integrins, lactadherin, MFGE8, P-selectin	96, 97
	Antigen presentation	Human leukocyte antigens class I and II	96
	Signaling proteins	GTPase, HRAS, syntenin-1, Gi2a, 14-3-3 proteins, ARF1, CDC42, NRAS, EHD1, EHD4, RAN, PEBP1, MIF, RRAS2, stomatin, PDCD6	96–98
Enzymes	Transport	Transferrin receptor	96
	Glycosylation and metabolism	Glucose-6-phosphate isomerase, fatty acid synthase, GAPDH, PFKL, peroxiredoxin-1, hexokinase, PGK1, PGAM1, pyruvate kinase M1/M2, ATP-citrate lyase, ATPase, AST, aldehyde reductase, enolase-1, LDH, aldolase-1, DPP-4	96–98, 100
Cytokines	Anti-inflammatory proteins	Tumor necrosis factor-alpha (TNF-α)	96
Anti-apoptotic	Apoptosis regulators	Alix, thioredoxin peroxidase	97
Transcriptional regulators	Transcription factors	EEF1A1, EEF2, LGALS3, EEF1A2	98
Caveolae	Caveolin-related proteins	Caveolin-1, caveolin-3	101
Lipids	Phospholipids	Phosphatidylcholine, phosphatidylserine, lysophosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, lysobisphosphatidic acid	102
	Cholesterol	—	102
	Ceramides	—	102, 103
	Sphingolipids	Sphingomyelin, hexosylceramide, lactosylceramide	97
	Gangliosides	GM1, GM3	104, 105
	Other lipids	Arachidonic acid, prostaglandin E, 15-d-PGJ2, diacylglycerol, triacylglycerol, hexadecylglycerol	102, 106

Nucleic acids	RNA	miRNAs (Let-7, miR-1, miR-15, miR-16, miR-17, miR-18, miR-19b, miR-20, miR-21, miR-29a, miR-126, miR-143, miR-145, miR-151, miR-155, miR-181, miR-200, miR-214, miR-320, miR-375, miR-382)	96, 102
	Other RNA types	mRNA, circRNA, mitochondrial RNA, tRNA, spliceosomal RNA, precursor RNA	96, 107
	DNA	DNA, viral DNA	107

The term *exosome* was originally introduced in 1981 to describe submicron lipid vesicles secreted by cells.<sup>89</sup> It was later defined more specifically as vesicles 50–150 nm in size, containing transferrin receptors and released by maturing blood reticulocytes.<sup>90</sup> Exosomes carry RNA and soluble proteins and display surface receptors that target specific recipient cells. The RNA cargo includes both mRNA and microRNA (miR), capable of modulating gene expression in recipient cells.<sup>91</sup>

EVs include microvesicles and exosomes. Microvesicles (100–1000 nm) are shed from the plasma membrane and carry bioactive molecules like proteins, lipids and RNA. Exosomes (30–150 nm) originate from intracellular organelles and contain RNA and proteins in a lipid membrane. The plasma of healthy individuals contains about  $10^{10}$  EVs per millilitre.<sup>92,93</sup> All cell types, including platelets, erythrocytes, lymphocytes, endothelial cells and parenchymal cells, contribute to the EV pool.<sup>86</sup>

Although exosomal content shares similarities with other EV types, analytical studies have revealed distinct molecular components (Table 3),<sup>94</sup> which often vary by the originating cell type.<sup>95</sup>

Microvesicles and exosomes are implicated in various cardiovascular conditions, involving endothelial dysfunction and coagulopathies. Elevated levels of endothelial-derived microvesicles with procoagulant properties have been identified in patients with acute coronary syndrome.<sup>108</sup> Assessing endothelial microvesicles may aid in identifying patients at risk for coronary artery disease.<sup>109</sup> Platelet-derived EVs have a dual role, being both prothrombotic and angiogenic. While they can promote clot formation, they also stimulate angiogenesis in ischaemic tissues. In a rat model of chronic myocardial ischaemia, platelet microvesicles increased functional capillaries. Following vascular injury, activated platelets release EVs, including exosomes and microvesicles.<sup>110-113</sup> Platelet-derived EVs interact with angiogenic cells, modulating SDF-1 $\alpha$ /CXCR4 signalling to enhance their maturation and re-endothelialisation.<sup>86,114</sup>

Cardiomyocytes release exosomes and cardiac fibroblasts, which make up most non-myocyte cardiac cells, influence myocardial function through signalling molecules. Exosomes from fibroblasts, containing microRNAs like miR-21, can induce cardiomyocyte hypertrophy by targeting specific genes. Inhibiting miR-21 reduced hypertrophy in a cardiac injury model, emphasising the role of fibroblast-derived exosomes in this process.<sup>115-118</sup> Non-coding RNAs (RNAs), which make up 98 % of RNA in the body, include ribosomal RNA, transfer RNA, microRNA, long non-coding RNA and circular RNA. MicroRNA regulates gene expression by binding to messenger RNA, causing degradation or repression. Long non-coding RNA and circular RNA affect gene expression through epigenetic and translational mechanisms. Given their role in regulating signalling pathways, non-coding RNAs are being explored as potential therapeutic agents with systemic delivery strategies.<sup>119-121</sup>

Current methods for diagnosing acute myocardial infarction (AMI) rely on detecting proteins like cardiac troponin, which are released during cardiomyocyte necrosis. Necrosis can also release protein-bound miRNAs into the bloodstream. However, exosomes, actively secreted by injured cells, offer the potential for earlier and more specific pre-necrotic identification. Released within minutes of infarction, these vesicles carry a complex cargo, including miRNAs, mRNAs, lncRNAs and proteins, offering a potential molecular fingerprint of infarction.<sup>91</sup>

## Therapeutic potential of exosomes as an alternative to stem cell therapy

Exosomes, known for decades, show promise in regenerative medicine, particularly for cardiac diseases. While simpler than cell therapies, exo-

some-based treatments may have limited efficacy and require further clinical research to determine their potential in cardiovascular disease. Exosomes, despite not being living organisms, contain proteins and can be classified as biological medicinal products or advanced therapy products, depending on their source. A regulatory framework for EVs has been proposed by ISEV, with exosomes potentially commercialised as cell manufacturing by-products.<sup>122, 123</sup>

Clinical trials on EVs for cardiac conditions are in early stages. The EV-AMI trial is testing exosome infusion for AMI safety, while another Iranian study explores mesenchymal stem cell (MSC)-derived exosomes and mitochondria in coronary artery bypass grafting (CABG). A French study is assessing the safety and efficacy of EVs from cardiac progenitor cells in severe heart failure. These trials aim to address safety, but challenges remain, especially with repeated EV administrations.<sup>124</sup>

MSC-derived exosomes influence atherosclerosis by regulating macrophage polarisation via the miR-let7/HMGA2/NF- $\kappa$ B pathway, promoting the anti-inflammatory M2 phenotype and reducing inflammation. They also decrease macrophage infiltration into plaques by inhibiting the miR-let7/IGF2BP1/PTEN axis, limiting macrophage survival. Additionally, miR-129-5p in MSC-derived exosomes reduces cardiac inflammation and improves heart function. Exosomes from endothelial progenitor cells (EPC-Exos) prevent myocardial fibrosis. While MSC-derived exosomes show strong cardioprotective effects, challenges remain in standardising production, understanding their interactions with cardiac tissues and ensuring long-term safety in clinical applications.<sup>125-132</sup>

## Conclusion

- While traditional drugs (eg,  $\beta$ -blockers, statins) provide substantial clinical benefits in managing cardiovascular diseases, they are insufficient in fully preventing myocardial remodelling and heart failure progression, especially in high-risk patients.
- Stem cell-based approaches for myocardial regeneration face challenges such as low cell survival, limited engraftment, incomplete differentiation and arrhythmogenic risks.

Clinical outcomes remain variable and require validation in large-scale trials.

- Exosomes offer a promising, cell-free alternative for cardiac repair due to their anti-inflammatory, pro-angiogenic and anti-apoptotic properties. They hold potential as biomarkers for early myocardial injury, though issues such as standardised production and long-term safety need resolution.
- The future of cardioprotection lies in combining pharmacotherapy with regenerative and nanotechnology-based strategies. Personalised medicine, driven by molecular diagnostics and targeted delivery, will further enhance therapeutic outcomes.
- Further multidisciplinary research is required to overcome current challenges and translate exosome-based and regenerative therapies into standard clinical practice, ultimately improving patient outcomes in cardiovascular disease.

## Ethics

This study was a secondary analysis based on the currently existing data and did not directly involve with human participants or experimental animals. Therefore, the ethics approval was not required in this paper.

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The authors declare that there is no conflict of interest.

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## Data access

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