



Reactive oxygen species-based nanomaterials for the treatment of myocardial ischemia reperfusion injuries

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ARTICLE INFO

Keywords:

Reactive oxygen species
Myocardial ischemia reperfusion injury
Nano-enzyme
Nanocarrier
Antioxidant therapy

ABSTRACT

Interventional coronary reperfusion strategies are widely adopted to treat acute myocardial infarction, but morbidity and mortality of acute myocardial infarction are still high. Reperfusion injuries are inevitable due to the generation of reactive oxygen species (ROS) and apoptosis of cardiac muscle cells. However, many antioxidant and anti-inflammatory drugs are largely limited by pharmacokinetics and route of administration, such as short half-life, low stability, low bioavailability, and side effects for treatment myocardial ischemia reperfusion injury. Therefore, it is necessary to develop effective drugs and technologies to address this issue. Fortunately, nanotherapies have demonstrated great opportunities for treating myocardial ischemia reperfusion injury. Compared with traditional drugs, nanodrugs can effectively increase the therapeutic effect and reduce side effects by improving pharmacokinetic and pharmacodynamic properties due to nanodrugs' size, shape, and material characteristics. In this review, the biology of ROS and molecular mechanisms of myocardial ischemia reperfusion injury are discussed. Furthermore, we summarized the applications of ROS-based nanoparticles, highlighting the latest achievements of nanotechnology researches for the treatment of myocardial ischemia reperfusion injury.

1. Introduction

Cardiovascular disease is the general term used to describe heart and blood vessel conditions, including hypertension, stroke, coronary heart diseases, myocardial infarction, etc. [1–4]. Acute myocardial infarction is the most severe ischemic heart disease caused by rupture of coronary atheromatous plaque [4–7]. In myocardial ischemia, large areas of myocardium tissue are impaired, and cardiomyocytes undergo necrosis and apoptosis. The damaged areas of myocardium tissue are incapable of effective regeneration and restoration due to the low proliferation capacity of myocardial cells [8,9]. Timely reperfusion can effectively

avoid or delay the disease progression and cell death through restoring coronary artery blood flow in the ischemic tissue [10]. However, accumulating evidences demonstrated that the death of cardiomyocytes induced by ischemia reperfusion injury may paradoxically impair cardiac function due to oxidative stress damage from overproduction of reactive oxygen species (ROS), rapid pH correction, and an increase in intracellular Ca²⁺ [11,12]. Meanwhile, the excessive ROS can cause cardiac inflammation, which in turn aggravates myocardial damage by inducing the production of ROS. Therefore, antioxidant therapy and alleviating cardiac inflammation are considered effective methods to improve myocardial reperfusion injuries. Many traditional drugs have

Peer review under responsibility of KeAi Communications Co., Ltd.

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<https://doi.org/10.1016/j.bioactmat.2021.06.006>

Received 27 February 2021; Received in revised form 9 May 2021; Accepted 2 June 2021

Available online 20 June 2021

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been developed for the treatment of myocardial reperfusion injuries, such as antioxidant drugs [13–16], inflammatory regulators [17,18], cardioprotective factors [19,20] and stem cells [21,22]. However, the clinical effects of these therapies far are unsatisfactory owing to the poor drug bioavailability and systemic side effects [9]. (see Table 1)

Compared with traditional drugs, nanomedicine not only can improve the solubility and absorption rate of poorly soluble drugs, but also reduce adverse reactions, improve targeting, and accurate control release [23–27]. Previously, we developed melanin NPs to efficiently decrease brain injury of ischemic stroke via scavenging ROS [26]. Nanotechnology has been applied to the treatment of myocardial ischemia reperfusion injury by direct or indirect reduction of ROS in the infarcted area. Particularly, ROS-based nanomaterials can be divided into two categories based on their functions, including nanomaterials with intrinsic antioxidative activities (also termed nano-enzymes) and drug-loaded NPs [28]. These ROS-based nanomaterials have shown excellently therapeutic effects and are very promising in the treatment of myocardial infarction reperfusion. To the best of our knowledge, there is no review specifically regarding the ROS-based nanomaterials for the treatment of myocardial ischemia reperfusion injury [9,28–34]. In this review, we first summarize in detailed the pathological mechanism of myocardial ischemia reperfusion injury, especially the effects and source of ROS under pathological condition. Meanwhile, we provide a comprehensive description about the applications of nanomaterials in the treatment of myocardial ischemia reperfusion injury (Fig. 1).

2. ROS: biology and physiological effects

ROS are the natural by-products of the aerobic metabolism initially generated by oxygen reduction, mainly including superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radicals ($OH\cdot$) [9, 35–37]. One electron transfer to oxygen to form O_2^- in the process of oxygen metabolism, and can be converted into H_2O_2 either spontaneously or catalytically by superoxide dismutase (SOD). H_2O_2 can be decomposed into water and oxygen by cytoplasmic catalase and glutathione peroxidase antioxidant enzymes. Alternatively, the unstable oxygen bond of H_2O_2 can react with Fe^{2+} to form the highly active $OH\cdot$ [38–40].

ROS are a class of highly reactive molecules [41,42]. ROS operate at low, but measurable concentrations in the cells under normal physiological conditions. Their stable concentrations depend largely on the balance between their rates of production and their rates of removal, a condition called redox homeostasis [43,44]. The redox homeostasis of a cell is kept within a narrow range under normal conditions, while the redox state can be altered to lower or higher values under pathological conditions [43]. Undoubtedly, ROS play important roles in the physiological activity of cells [45–48]. At moderate concentrations, ROS participate in various biological and physiological processes by controlling the redox-responsive signaling pathways, including the cytokines and growth factor signaling pathways, non-receptor tyrosine kinases, protein tyrosine phosphatases, serine/threonine kinases, and various nuclear transcription factors [43,49]. One of the most significant examples has been observed in mitogen-activated protein kinase (MAPK) pathways. It is generally believed that ROS production as a result of MAPK pathway can act as a second messenger and regulate important cellular functions such as proliferation and programmed cell death [50]. Eventually, ROS play important roles in the physiological activity of cells involving defense against environmental pathogens, regulating vascular tone, acting as a sensor for changes of oxygen concentrations, participating in the process of oxidant-mediated adhesion, modulating the immunological functions of immune cells, and inducing cell apoptosis [43,49,51,52].

Paradoxically, overproduction of ROS induces oxidative stress, a deleterious process that can cause severe oxidative damage to cell structures, including lipids, biological membranes, proteins, and DNA [4,43,53]. A major feature of ROS-mediated cellular injury is lipid

peroxidation due to the oxidation of ROS on polyunsaturated fatty acids. Lipid peroxidation is a physiological process in which free radicals transfer electrons from lipids, followed by the production of active intermediates with high biological activity [54–56]. Eventually, the lipid bilayer arrangement of biological membrane is perturbed, thus affecting its functional properties. Moreover, lipid peroxidation also generates unsaturated aldehydes, malondialdehyde and other metabolites, which has cytotoxic and mutagenic effects [53]. ROS can damage both nucleobases and the deoxyribose backbone, especially guanine, resulting in the formation of 8-oxoguanine, with the potential of mutagenic and carcinogenic consequence. ROS also can cause DNA strand breakage by separating the hydrogen atoms from the sugar phosphate backbone of the DNA [57]. Besides, it is also worth to mention the roles of ROS in the oxidation of protein, in which the side chain of all amino acid residues of proteins, especially cysteine and methionine, are sensitive to oxidation reaction of ROS [44]. A characteristic example is the H_2O_2 -induced oxidative modification of cysteine residues in proteins. Cysteine residues are converted into thiolate anion (Cys-S⁻) formed under a normal physiological pH condition. These residues are more easily oxidized than protonated cysteine thiol (Cys-SH). Within the nanomolar range, H_2O_2 oxidizes the thiolate anion to the reversible sulfenic form (Cys-SOH), leading to changes in the conformation and function of proteins [58,59]. Of note, cysteine residues are critical for protein-substrate binding, such as glyceraldehyde-3-phosphate dehydrogenase, p38 kinase, tropomyosin, and numerous other tyrosine phosphatases. It has been demonstrated that H_2O_2 produced by stimulation of epidermal growth factor (EGF) oxidates the catalytic cysteine of protein tyrosine phosphatase 1B (PTP1B) into a thionyl form, resulting in the inactivation of the phosphatase. PTP1B inactivation by H_2O_2 increases the levels of EGF receptor tyrosine phosphorylation, thereby affecting the execution of the downstream growth signaling pathway [59]. However, the elevated levels of H_2O_2 can further oxidize thiolate to irreversible sulfinic (SO_2H) or sulfonic (SO_3H) forms, causing permanent damage to proteins. Oxidized proteins are prone to proteolysis and loss of function, having serious cytotoxic effects and damaging the conduction of downstream signal pathways. For instance, oxidation of mitochondria may accelerate apoptotic and necrotic cell death by triggering the opening of mitochondrial permeability transition pore (mPTP) and energy metabolism disorders [43,44]. Consequently, appropriate levels of ROS are indispensable for the physiological activities of cells.

Most of cells have been found to elicit a small oxidative burst when they are stimulated by certain factors such as cytokines, hormone, etc [43]. But this redox imbalance can be quickly restored to the normal state with the assist of various antioxidant enzymes (e.g., SOD and catalase) that are produced in organisms and work in conjunction to balance the redox homeostasis by scavenging excessive ROS [50,60]. However, oxidative stress occurs when ROS levels greatly exceed the body's ability to remove them. Oxidative stress has been implicated in various pathological conditions such as cardiovascular disease, cancer, and diabetes [50]. In the pathological process of myocardial ischemia reperfusion injury, ROS is the core factors and this section will be described in detail later. Likewise, a persistent lack of ROS, caused by persistent mitochondrial inactivity or antioxidant signaling, also form a state of reductive stress [61]. Reductive stress reverses oxidation of proteins, impede insulin signaling and glucose metabolism, blunts positive effects of exercise on insulin sensitivity, eventually leading to diabetes, obesity and cardiomyopathy [61,62]. Therefore, strictly controlling the redox balance of cells is of great significance for the prevention and treatment of various diseases.

3. Pathological mechanism of myocardial ischemia reperfusion injury

Rapid and early recovery of blood flow is necessary for ischemic myocardial repair and the prevention of further damage after

Table 1
Nanocomposites for myocardial ischemia reperfusion injury therapy.

Category	Nanomaterials	Classification of nanomaterials	Cargo	Biological target	Efficacy	Advantages of nanomaterials	The model of administration	The response to ischemic myocardium	Refs
NPs for regulating NOXs or ROS-scavenging enzymes	HPOX/PVAX	Copolyoxalate polymer	HBA/VA	NOX	Exerting antiinflammatory and antioxidant activity	Increasing targeting and protecting cargo from degradation	Inject directly into the ligation site	H ₂ O ₂ -responsive	[52]
	NPs loaded with Rg3	PEG-b-PPS	Rg3	SOD	Exerting antiinflammatory, antioxidant, and antifibrotic activity	Achieving responsive releasing and increasing solubility of the cargo	Intramyocardial injection	ROS-responsive	[24]
	NPs loaded with baicalin	PEG-modified solid lipid	Baicalin	SOD	Decreasing infarct size of acute myocardial ischemia injury	Improving bioavailability and increasing targeting	Intraperitoneal injection	/	[173]
	NPs loaded with schisandrin B	PEG-modified solid lipid	schisandrin B	SOD/glutathione peroxidases	Decreasing infarct size of acute myocardial ischemia injury	Increased targeting by modifying MMP-sensitive peptide on the surface of NPs; Improving bioavailability and pharmacokinetic parameters	Tail vein injection	MMP-sensitive	[15]
	NPs loaded with GLP	poly(L-lysine)-PEG-poly(L-lysine)	GLP	SOD	Exerting antioxidant effect	Improving bioavailability of the cargo	Intraperitoneal subcutaneous injection	/	[175]
NPs for regulating mitochondrial ETC	NPs loaded with quercetin	PLGA	Quercetin	Mitochondrial ETC complex	Exerting antioxidant effect	Protecting cargo from degradation and increasing bioavailability	/	/	[101]
	NPs loaded with CoQ10	Liposomes	CoQ10	mitochondrial ETC	Reducing infarct size of acute myocardial ischemia injury	Improving bioavailability and solubility of the cargo, as well as contributing to selective accumulation of the cargo in the damaged cardiac tissue	Intracoronary injection	/	[178]
NPs for regulating Ca ²⁺ channel or mitochondrial membrane	NPs loaded with AID peptide	PGMA	AID peptide	L-type Ca ²⁺ channel	Attenuating cardiac dysfunction	PGMA delivery is more diffuse and rapid, without inducing toxicity and immunogenicity	Coronary artery perfusion	/	[180]
	NPs loaded with AID peptide and curcumin	PGMA	AID peptide and curcumin	L-type Ca ²⁺ channel	Exerting antioxidant activity	Improving bioavailability and solubility of the cargo	Coronary artery perfusion	/	[183]
	NPs loaded with resveratrol	PLGA	Resveratrol	mPTP	Increasing cell viability and improving cardiac dysfunction	Improving bioavailability and targeting of the cargo	Tail vein injection	/	[103]
	Mdivi1-NPs	PLGA	Mdivi1	MOMP	Protecting cardiomyocytes from oxidative stress damage	Increasing local drug concentration and promoting intracellular uptake	Intravenous injection	/	[27]
NPs loaded with inflammatory regulator	NPs loaded with pioglitazone	PLGA	Pioglitazone	PPAR γ	Exerting antiinflammatory activity	Rapid delivery of the cargo to damaged myocardium, and making intravenous injection possible	Intravenous injection	/	[188]
	NPs loaded with irbesartan	PLGA	Irbesartan	PPAR γ	Exerting antiinflammatory activity	Improving bioavailability, and promoting the accumulation of the cargo in damaged myocardium	Intravenous injection	/	[163]
	TAK-242-NPs	PLGA	TLR4 inhibitor	TLR4	Exerting antiinflammatory activity	PLGA delivery is more effective and rapid	Intravenous injection	/	[190]
	PUTK/MP	Polyurethane	MP	/	Improving structural and functional damage of myocardium	Achieving ROS-triggered drug release, reducing the toxicity of MP, and increasing the stability of MP	Epicardial cardiac patch placement	ROS-responsive	[23]
							MMP-sensitive	[198]	

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Table 1 (continued)

Category	Nanomaterials	Classification of nanomaterials	Cargo	Biological target	Efficacy	Advantages of nanomaterials	The model of administration	The response to ischemic myocardium	Refs
NPs loaded with cardioprotective factors and nucleic acid drugs	GST-TIMP-bFGF/collagen-glutathione hydrogels	Glutathione-modified collagen hydrogel	GST-TIMP-bFGF	MMP/angiogenic factor	Improving structural and functional damage of myocardium	Overcoming the defects of conventional drug delivery including systemic toxicity and repeated dosing	Intramyocardial injection		
	PINC	PGE2-modified Platelet membrane	Cardiac stromal cell-secreted factors	Recruiting CPCs/increasing cycling cardiomyocytes	Improving structural and functional damage of myocardium	Increasing targeting; overcoming the defects of traditional cell therapy including difficult preparation, low viability and retention	Intravenous injection	PGE2-sensitive/the natural infarct-homing ability of platelet membrane	[199]
	ONO-1301-NPs	PEG-modified solid lipid	ONO-1301	Prostacyclin IP receptor/thromboxane A2 synthase	Improving structural and functional damage of myocardium, exerting antiinflammatory activity	Achieving selectively accumulate in cardiomyocytes; reducing adverse reaction; prevention of drug resistance	Intravenous injection	/	[20]
	NPs loaded with miRNAs	PK3 NPs	miRNA-106b/miRNA-148b/miRNA-204	NOX2	Reducing NOX2 expression, improving structural and functional damage of myocardium	Increasing targeting	Intramyocardial injection	pH-responsive	[207]
	NPs loaded with AMO1	Dendrimer-based nano vector	AMO1	Bcl ₂	Upregulating Bcl-2 expression, improving structural and functional damage of myocardium	Providing effective way of gene delivery; increasing targeting and bioavailability	Intravenous injection	/	[206]
Metal and metal-oxide nano-zymes	Ceria nanozymes	Ceria	CPCs	Antioxidant enzymes	Exerting antioxidant activity	/	/	/	[223]
	Ceria nanozymes	Ceria	/	mast cells	/	/	Oropharyngeal instillation	/	[228]
	Iron oxide nanozyme	Iron oxide	/	Antioxidant enzymes	Improving structural and functional damage of myocardium	/	Intravenous injection	/	[229]
	Superparamagnetic iron oxide nanozymes	Iron oxide	/	Cardiomyocytes	/	/	Intramyocardial injection	/	[230]
	Magnetic mesoporous silica coated with N-acetylcysteine and Fe ₃ O ₄ nanozyme	magnetic mesoporous silica/Fe ₃ O ₄	N-acetylcysteine	Antioxidant enzymes	Exerting antioxidant activity	N-acetylcysteine modifying suppresses the toxic effects of Fe ₃ O ₄ nanozyme	/	/	[234]
	Chitosan/graphene functionalized superparamagnetic iron oxide nanozyme	Iron oxide	/	Antioxidant enzymes	Exerting antioxidant activity	Increasing antioxidant efficacy	/	/	[235]
	Iron oxide nanozyme	Iron oxide	/	/	Exerting antioxidant activity	/	/	/	[236]
	Gold nanozyme	Gold	/	Antioxidant enzymes	Exerting antioxidant activity	/	Intraperitoneal injection	/	[238]
	Gold nanozyme	Gold	/	Antioxidant enzymes	Exerting antiinflammatory and antioxidant activity	/	Orally	/	[241]
Copper nanozymes	Copper	/	GSK-3 β	Exerting antioxidant activity	/	Orally	/	[242]	
Carbon-based nano-zymes	MSC-GO/microgel	GO/microgel	MSC	/	Improving structural and functional damage of myocardium	GO microgel encapsulation increased the viability of MSCs	Intramyocardial injection	/	[161]
	MGC/IL-4 pDNA	MGC	IL-4 pDNA	/	/	Overcoming the difficulties in protein delivery, such as	Intramyocardial injection	/	[255]

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Table 1 (continued)

Category	Nanomaterials	Classification of nanomaterials	Cargo	Biological target	Efficacy	Advantages of nanomaterials	The model of administration	The response to ischemic myocardium	Refs
					Exerting antiinflammatory and antioxidant activity	maintaining the biological activity in the body			
DNA nanozymes	Fullerenol/alginate hydrogel encapsulated BADSCs	Fullerenol/alginate hydrogel	BADSCs	/	Exerting antioxidant activity	Promoting the viability of BADSCs	Intramyocardial injection	/	[258]
	TDNs	Single stranded DNA	/	/	Exerting antioxidant activity	Possessing excellent biological safety and biocompatibility; ease for cell uptake and imaging	/	/	[63]
Biomimetic NPs for myocardial ischemia reperfusion injury	MSCs-derived Exosome	Exosome	/	/	Exerting antioxidant activity	No risk of aneuploidy, a lower rate of immune rejection	Intramyocardial injection	/	[267]
	miRNA-19a/19b-loaded exosomes	Exosome	miRNA-19a/19b	/	Improving structural and functional damage of myocardium	Acting as a delivery vehicle for miRNA; enhancing the efficacy of transplanting MSCs	Injection	/	[271]
	MSCs-derived Exosome	Exosome	/	/	Exerting antiinflammatory activity, as well as improving structural and functional damage of myocardium	Long-term stability, easily absorbed by cells, minimal immune rejection and convenient administration	Intramyocardial injection	/	[268]
	MTSNP	PLGA/PLA	Melatonin/circular DNA	ROS/melatonin receptor/VEGF	Exerting antioxidant activity	Increased targeting to the ischemic microenvironment; achieving on-demand drug delivery, improving biocompatibility	Intramyocardial injection	Mitochondrial simulation and microenvironmental targeting	[261]

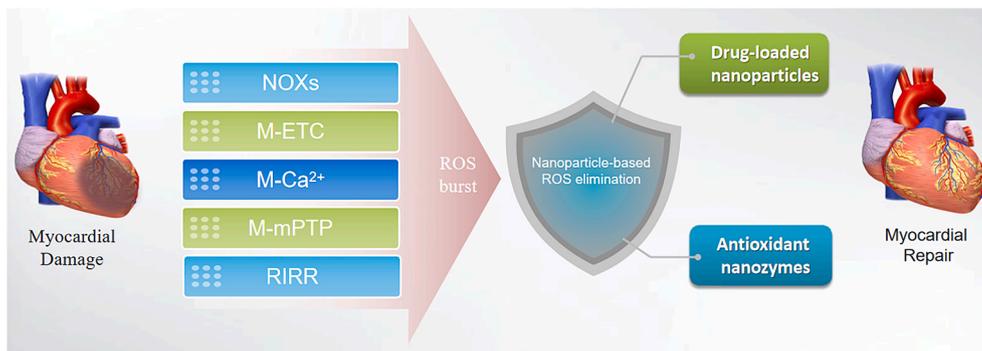


Fig. 1. The scope and focus of this article. Under myocardial ischemia reperfusion, various sources ROS together induced myocardial damage due to redox imbalance: NOXs family enzymes, mitochondrial electron transport chain (M-ETC), mitochondrial Ca^{2+} (M- Ca^{2+}), mitochondrial permeability transition pore (M-mPTP) and ROS-induced ROS release (RIRR). The application of nanoparticles can scavenge excessive ROS through delivery antioxidant drugs or directly antioxidant effects, thus contributing myocardial repair.

myocardial ischemia [63,64]. Ironically, the reperfusion process can induce additional myocardial injuries, arrhythmias and contractile dysfunction [8]. Myocardial ischemia reperfusion injury is characterized by the death of cardiomyocytes which are alive at the beginning of reperfusion and died due to ROS accumulation, calcium overload and inflammation during the restoration of blood flow [65,66]. Therefore, a growing number of studies have investigated changes in ROS and the related molecules mechanisms of reperfusion injury, aiming to identify therapeutic strategies for reducing the infarct size and severity [8]. Excessive ROS can damage cardiac muscle tissues by reacting with various biological molecules (like lipids, proteins, and nucleic acids) under myocardial ischemia reperfusion conditions [67]. The mechanism of ROS and inflammation formation is very complicated in myocardial ischemia reperfusion injury. Inflammation and ROS form a vicious circle. Usually, ROS and inflammation are causal to each other, parallel and strengthen each other. Therefore, it is very necessary to understand the origins of ROS in order to find more effective treatments. ROS overproduction mainly originates from the following aspects in reperfusion of myocardial infarction: 1) nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX) family enzymes; 2) mitochondrial electron transport chain (ETC); 3) mitochondrial Ca^{2+} ; and 4) mPTP and ROS-induced ROS release (RIRR) [68,69]. Reperfusion of myocardial infarction has similar pathological features with many inflammatory diseases, such as increased ROS and inflammatory factors. There are still some differences between them and myocardial infarction reperfusion injury. Take osteoarthritis as an example. Osteoarthritis is a disease of immunodeficiency [70]. Immune deficiency causes chronic inflammation, which lead to abnormal synovial proliferation and abnormal blood vessel proliferation in osteoarthritis. Myocardial infarction reperfusion is an acute inflammation that originates from damage to myocardial cells. In addition, the number of mitochondria contained in myocardial cells ranks first among all human cells. Therefore, the myocardium is easily damaged by ROS. Antioxidants that target mitochondrial electronic chains, calcium ion channels, and RIRR will have a better therapeutic effect for the treatment of myocardial infarction reperfusion than osteoarthritis.

3.1. The source of ROS: NOXs

NOXs are the only enzymes that produce ROS [71–73]. To date, seven members of the NOX family have been identified (i.e., NOX1–5, dual oxidase 1 and 2 [DUOX1 and DUOX2]) [74–76]. Among them, NOX1, NOX2, and NOX4 are mainly localized in myocardial cells [73, 74]. NOXs, as the multiprotein complexes, usually require different subunits for their activation and induce the production of different types of ROS [71,77]. The activation of NOX2 requires the assembly of a membrane-bound domain (p22phox) and cytosolic domains (p47phox, p67phox, and p40phox). Furthermore, the binding of the small G protein Rac to the NOX2 complex is also required. After its activation, NOX2 can be regarded as an electron transfer chain carrying electron across the cell membrane to catalyze the following reaction:

($\text{NADPH} + 2\text{O}_2 \rightarrow \text{NADPH}^+ + \text{H}^+ + 2\text{O}_2^-$) [77,78]. Similar to NOX2, the binding of p22phox and Rac is also required for the activation of NOX1. NADPH oxidase organizer 1 (NOXO1), and NADPH oxidase activator 1 (NOXA1) is also necessary to interaction with the p47phox and p67phox analogs. Subsequently, NOX1 transfers electrons to produce O_2^- through a series of steps. NOX1 and NOX2 are mainly located in the plasma membranes and intracellular vesicles [78,79]. However, NOX4 only interacts with p22phox and its activation is independent of other cytosolic domains. Distinctively, NOX4 predominantly produces H_2O_2 , and is distributed in cell membrane, endoplasmic reticulum, and mitochondria [71,74,75]. NOXs play an important role in the progression of ischemia reperfusion. NOX1^{-/-}, NOX2^{-/-}, or NOX4^{-/-} mice showed a reduction in ROS generation and infarct size after ischemia reperfusion [80,81]. Nonetheless, myocardial ischemia injury was aggravated in NOX2/-NOX4 double-knockout mice. A possible explanation is that a certain amount of ROS produced by NOX2 or NOX4 is essential for the activation of hypoxia-inducible factor-1 α (HIF-1 α) and inhibition of peroxisome proliferator activated receptor alpha (PPAR α) during ischemia reperfusion [78]. HIF-1 α regulate the metabolic adaptation to low oxygen and oxidative stress. Meanwhile, HIF-1 α activation prevents the oxidation of fatty acids through inhibition of PPAR α in the heart [78]. Taken together, appropriate levels of ROS produced by NOX2 and NOX4 can prevent ischemia reperfusion injury. The modulation of NOX

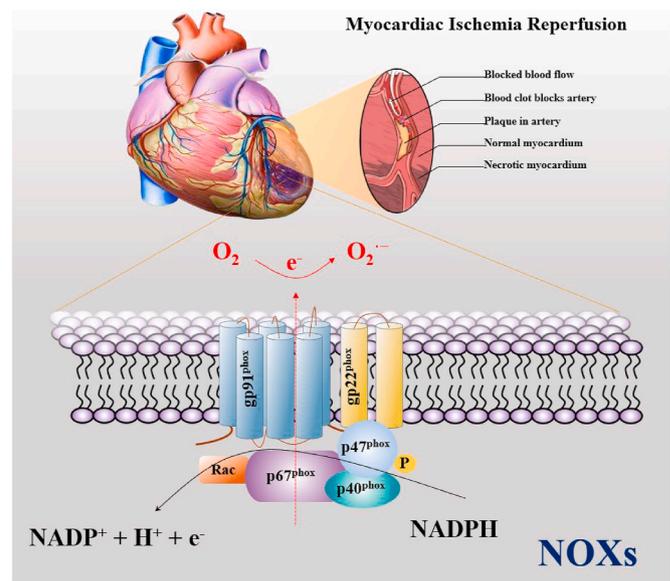


Fig. 2. NOX isoforms need different subunits for their activation, including p22phox, p67phox, p40phox, p47phox and the small G protein Rac. After being activated, NOX can transfer electron to catalyze this reaction: $\text{NADPH} + 2\text{O}_2 \rightarrow \text{NADPH}^+ + \text{H}^+ + 2\text{O}_2^-$. Under myocardial ischemia reperfusion, the overactivation of NOX induces O_2^- overproduction.

activity is particularly attractive for therapeutic exploitation against myocardial ischemia reperfusion injury (Fig. 2) [74,78,82].

3.1.1. The source of ROS: mitochondrial ETC

The mitochondrial ETC, located in the inner mitochondrial membrane, is composed of four multi-subunit complexes (complexes I–IV) coupled with mobile carriers (coenzyme Q [CoQ] and cytochrome *c* [Cyt *c*]) [83–87]. Under normal metabolic activity, these complexes and mobile carriers facilitate the transfer of electrons released from oxidative substrates along the components of ETC, ultimately converging on complex IV (Cyt oxidase) in which oxygen are reduced to water [87]. The ETC can be divided into two circuits: 1) complex I → complex III → complex IV; 2) complex II → complex III → complex IV [87]. However, the decrease of the activity of complexes I and III is closely related to ROS overproduction during myocardial ischemia reperfusion [88–91]. Complex I, as the entrance of electrons from nicotinamide adenine dinucleotide into the ETC, is composed of a hydrophobic arm and a matrix-protruding hydrophilic arm, which involves flavin mononucleotide (FMN) and eight iron-sulfur (Fe–S) clusters. The FMN cofactor acquires the electrons released by nicotinamide adenine diphosphate hydride (NADH), and allows their transfer along the Fe–S cluster to the CoQ reduction site. Subsequently, electrons from complex I are transferred between complexes III and IV, eventually catalyzing the following redox reaction: ($\frac{1}{2}\text{O}_2 + 2\text{H}^+ \rightarrow \text{H}_2\text{O}$) [82,92]. After myocardial reperfusion, oxidative damage to cardiolipin (a phospholipid located in the mitochondrial inner membrane) results in the dissociation of complexes I and III from the supercomplex, which lead to electron leakage from ETC and ROS generation [93]. In addition, the generation of O_2^- can also be caused by reverse electron transport (RET) in complex I when the pool of CoQ becomes over-reduced with electrons from complex II. In RET process, electrons first enter complex II through succinate oxidation and reach CoQ, and then are directed back to complex I instead of proceeding forward to complex III [47,82,94]. Succinates from ischemic accumulation are rapidly re-oxidized by the succinate dehydrogenase of complex II, which transfers electrons from complex II

to complex I, and produces O_2^- [90,95]. Complex III also triggers O_2^- production through the redox reactions between oxygen and ubiquinone [38,47,96]. In summary, complexes I and III are the major sources of ROS during myocardial ischemia reperfusion [97,98]. Antioxidants capable of protecting mitochondrial ETC may be an effective means to eliminate excessive ROS (Fig. 3).

3.1.2. The source of ROS: mitochondrial Ca^{2+}

Mitochondrial Ca^{2+} overload is a key contributor to myocardial ischemia reperfusion injury, which indirectly induces ROS overproduction by impairing mitochondrial function, and eventually leads to the opening of mPTP, Cyt *c* release, and apoptosis/necrosis [88,91,99–102].

Under normal physiological conditions, the Na^+ and H^+ balance between extracellular and intracellular is maintained by the Na^+/H^+ exchangers (NHE) of the plasma membrane, and the cytoplasmic Ca^{2+} is maintained at a low level by the $\text{Na}^+/\text{Ca}^{2+}$ exchangers of the plasma membrane. During myocardial ischemia, the continuous deficiency of oxygen impairs mitochondrial oxidative phosphorylation [101,103]. As a result, the metabolism of ischemic myocardium changes from aerobic metabolism toward anaerobic glycolysis, accompanied by the generation of hydrogen ions and lactic acid [9]. The accumulation of these by-products decreases the intracellular pH, which forces the NHE to export internal H^+ and import external Na^+ , which increase intracellular concentrations of Na^+ [104]. Sequentially, the plasma membrane $\text{Na}^+/\text{Ca}^{2+}$ exchanger is activated to export excessive Na^+ and pump extracellular Ca^{2+} into the cytoplasm (Ca^{2+} overload). Following the occurrence of reperfusion, the restoration of oxygenated blood triggers further activation of NHE to rapidly export internal H^+ and correct the intracellular pH. Thus, the cytoplasmic Ca^{2+} is further increased by the effect of the $\text{Na}^+/\text{Ca}^{2+}$ exchanger [9,104]. Subsequently, Ca^{2+} is transported to the mitochondria, and the excessive mitochondrial Ca^{2+} induces ROS overproduction mainly through the tricarboxylic acid cycle (TCA), decreasing the activity of mitochondrial complexes and opening the mPTP [91,101,102,105]. TCA is the final metabolic pathway of the

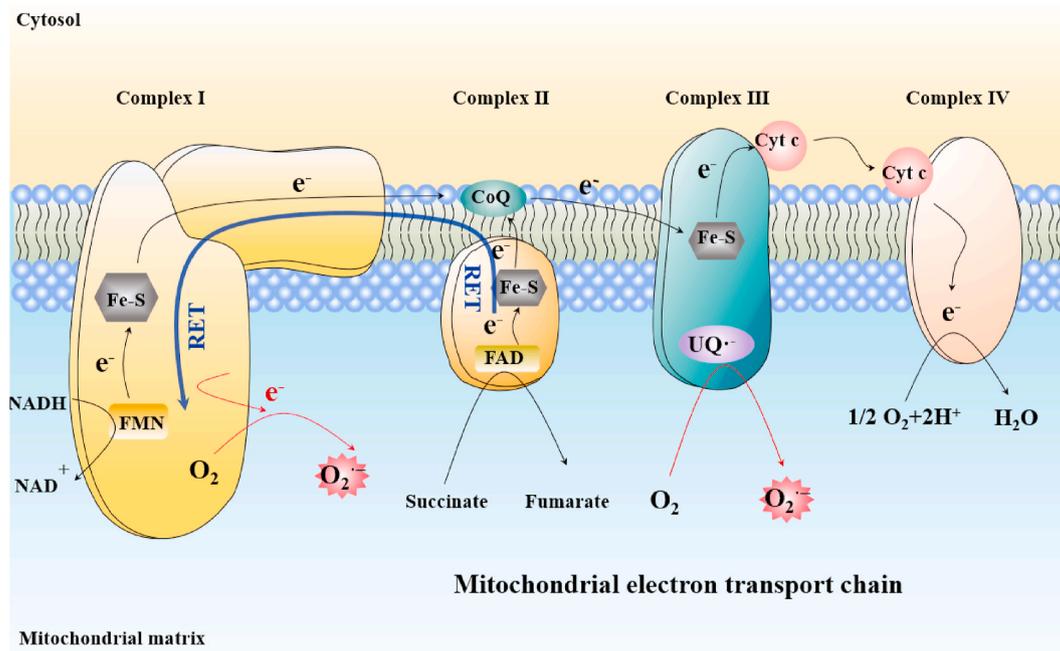


Fig. 3. ROS production by the mitochondrial ETC, which are composed of complex I–IV. Under normal physiological condition, the electrons released from NADH and FAD are shuttled through complex III and IV, eventually catalyzing the reaction: ($\frac{1}{2}\text{O}_2 + 2\text{H}^+ \rightarrow \text{H}_2\text{O}$). During myocardial ischemia reperfusion injury, the production of O_2^- in complex I occurs by RET, where the pool of CoQ becomes over-reduced with electrons from complex II. Subsequently, the electrons leaking from complex I react with oxygen to form O_2^- . Meanwhile, complex III also produces large amounts of O_2^- from the reaction of oxygen with a UQ. In short, the mitochondrial ETC is the major source of ROS. FAD: flavin adenine dinucleotide; UQ: ubiquinone.

three major nutrients (*i.e.*, proteins, glucose and lipids) where these substrates are oxidized to release electrons into the ETC [90,106]. Mitochondrial Ca^{2+} stimulate the TCA cycles, and more electrons flow into the mitochondrial ETC to increase the possibility of ETC electron leakage to generate O_2^- [107]. In addition, mitochondrial Ca^{2+} can dislocate the Cyt *c* of complex III from the mitochondrial inner membrane, either by occupying cardiolipin binding sites or by triggering the opening of mPTP. These effects block the mitochondrial ETC at complex III and induce ROS overproduction [108]. Meanwhile, Ca^{2+} stimulates nitric oxide synthase (NOS) to generate NO , which inhibits the activity of complexes I and IV and promotes the production of more ROS [88, 91]. Furthermore, mitochondrial Ca^{2+} induces the opening of mPTP to produce a large amount of ROS through the mechanism of RIRR (described in part 3.1.4) [9,86,103,104,109,110]. Together, mitochondrial Ca^{2+} -mediated cellular events are highly implicated in ROS production (Fig. 4) [111,112].

3.1.3. The source of ROS: mitochondrial mPTP and RIRR

In cardiomyocytes, the RIRR is also a fundamental mechanism for enhancing ROS production (Fig. 5) [113–115]. During RIRR, the increase in ROS reaches a threshold level that triggers the opening of one of the requisite mitochondrial channels (*i.e.*, mPTP), which in turn causes the simultaneous collapse of the mitochondrial potential and a transient increase in ROS generation by the ETC [116]. Subsequently, these ROS are released into the cytosol and induce the opening of mPTP in neighboring mitochondria to further increase ROS [114]. This mitochondrion-to-mitochondrion mechanism forms a positive feedback mechanism for ROS generation, and eventually leads to severe cellular

damage [115,117].

The opening of mPTP is the core factor in the process of RIRR [80, 115,117]. mPTP is a non-specific channel that spans the inner and outer membranes of mitochondria. Currently, the composition of mPTP remains unclear. Initially, it was thought to comprise a voltage-dependent anion channel, adenine nucleotide translocator, and cyclophilin D. However, a dimer of mitochondrial ATP synthase subunits is necessary for the core of the mPTP, and the *c* domain of mitochondrial ATP synthase is essential for mPTP-dependent mitochondrial signaling transduction [80]. The opening of mPTP can directly release mitochondrial ROS into the cytoplasm, and transmits local mitochondrial perturbations to cardiomyocytes [80,118]. In addition, mPTP opening can cause depolarization of the mitochondrial membrane potential, efflux of Cyt *c*, breakdown of ATP, and ultimately cardiomyocyte death [80,118].

3.2. Calcium overload in myocardial ischemia reperfusion injury

Ca^{2+} , as a secondary messenger, plays an essential role in maintaining excitation-contraction coupling in cardiomyocytes. Therefore, Ca^{2+} overload will further exacerbate the degree of myocardial ischemia reperfusion injury in cardiac muscle cells [119]. So far, it has been well recognized that the dysfunction of calcium relevant proteins, including L-type voltage-dependent calcium channel (L-VDCC), sphospholamban (PLB), $\text{Na}^+/\text{Ca}^{2+}$ exchanger, Na^+/H^+ exchanger, *etc.* together contributes to calcium overload in cardiomyocytes during ischemia-reperfusion injury, of which L-VDCC and $\text{Na}^+/\text{Ca}^{2+}$ exchanger are two key factors in regulating Ca^{2+} homeostasis. The calcium increase can be generally divided into two phases: the early phase is partially mediated calcium

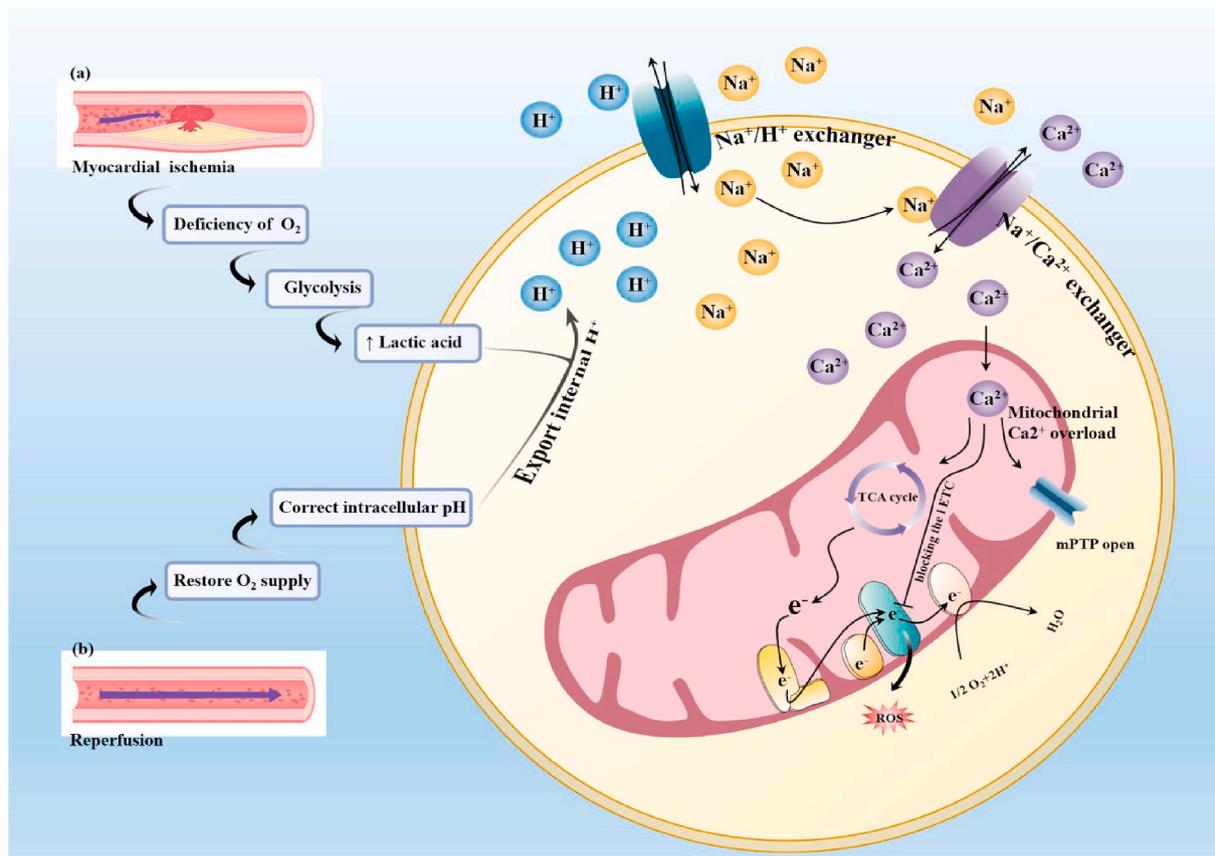


Fig. 4. The overload of mitochondrial calcium induce ROS overproduction. After myocardial ischemia, the ischemic myocardium is forced to from aerobic metabolism toward anaerobic glycolysis due to the oxygen deficiency, which can cause the cytoplasmic Ca^{2+} overload by the effects of NHE and $\text{Na}^+/\text{Ca}^{2+}$ exchanger. When reperfusion, the restoration of oxygenated blood can activate the NHE, and then further increasing cytoplasmic Ca^{2+} . Subsequently, the cytoplasmic Ca^{2+} transfer into mitochondria, resulting in mitochondrial Ca^{2+} overload. The overload mitochondrial Ca^{2+} induce ROS production by impairing ETC, inducing mPTP opening and stimulating TCA cycle.

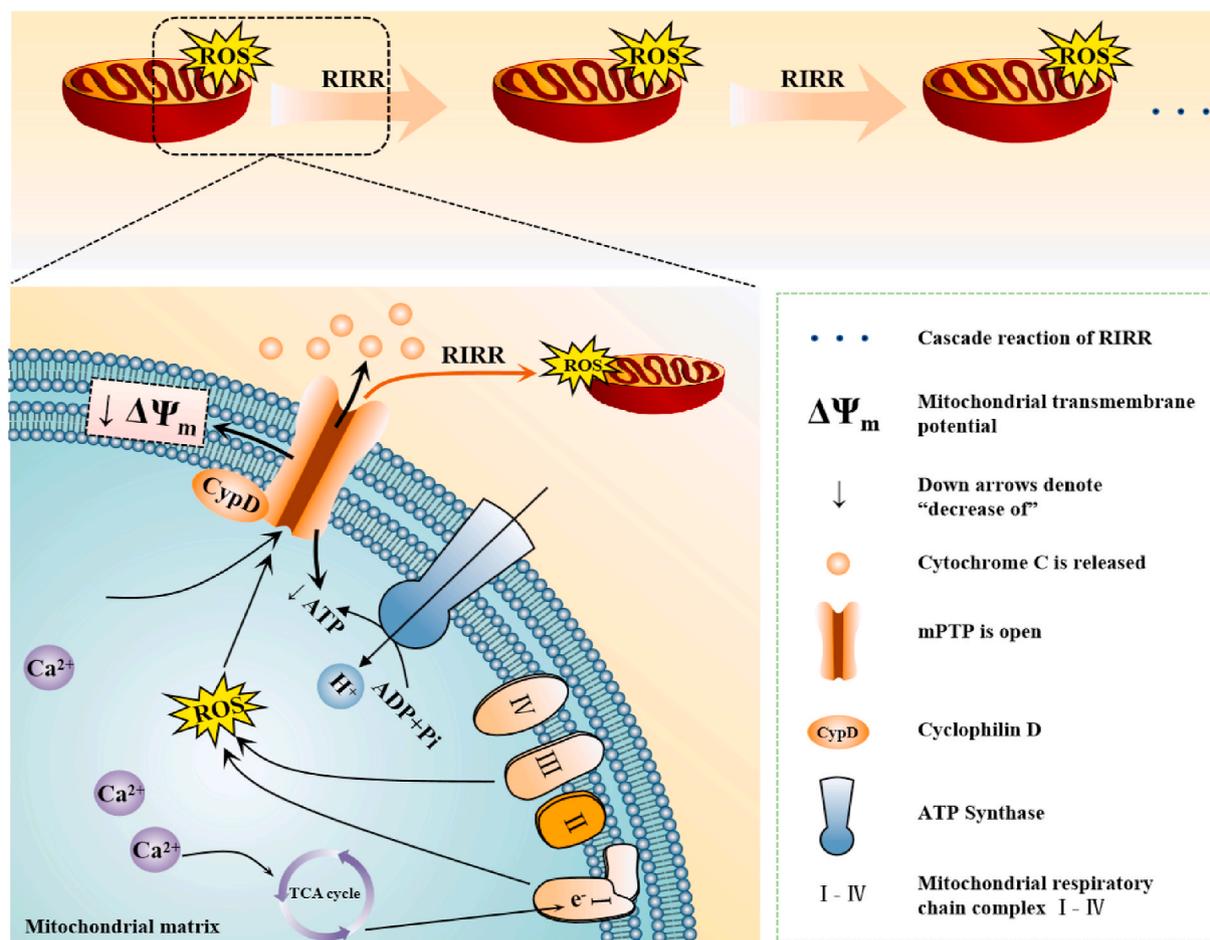


Fig. 5. The RIRR phenomenon in cardiomyocytes. Under myocardial ischemia reperfusion, the ETC-produced ROS and calcium together induce mPTP opening, which in turn causes the simultaneous collapse of mitochondrial potential and a transient increased ROS generation by the ETC. Then, these ROS induce the opening of mPTP in neighboring mitochondria, thus further increasing ROS production. Meanwhile, mPTP opening also can impair mitochondrial membrane potential, release Cyt c, inhibit ATP production, ultimately causing cardiomyocytes death.

channels (such as L-VDCC); the latter one is mainly mediated by $\text{Na}^+/\text{Ca}^{2+}$ exchanger [120].

Ischemia induces the depolarization of membrane potential in the early stage of myocardial ischemia reperfusion, L-VDCC opens and then leads to a larger amount of Ca^{2+} influx into the cytoplasm. However, L-VDCC blockers have not shown benefit effects on myocardial ischemia reperfusion injury in many clinical trials [121,122]. This is because Ca^{2+} influx from L-VDCC is the main trigger for Ca^{2+} release from the sarcoplasmic reticulum. In the late stage of myocardial ischemia reperfusion injury, $\text{Na}^+/\text{Ca}^{2+}$ exchangers are responsible for Ca^{2+} influx into cells, and the specific mechanism are described above (section 3.13). Notably, a selective $\text{Na}^+/\text{Ca}^{2+}$ exchanger inhibitor, SEA400, has been shown to protect myocardium by reducing Ca^{2+} overload [121]. Additionally, excessive ROS accumulation also further aggravate Ca^{2+} overload in the myocardial cells. ROS could decompose cell membrane phospholipid components and impair membrane structure, which cause increased membrane permeability and excessive extracellular Ca^{2+} influx. ROS also damages to the sarcoplasmic reticulum membrane, ultimately impairing the ability of sarcoplasmic reticulum to uptake Ca^{2+} and further increasing intracellular calcium levels [111,123].

Excessive intracellular Ca^{2+} can seriously damage cell structure and function. First, intracellular Ca^{2+} can activate some phospholipases, which can decompose and destroy the cell membrane skeleton [124]. Second, excessive intracellular Ca^{2+} can be transferred into mitochondria, resulting mitochondrial Ca^{2+} overload. This change will not only directly affect the energy metabolism in mitochondria, but also impede

signal transmission between cells, eventually causing the apoptosis of cardiomyocytes [125]. Third, Ca^{2+} overload also activate caspase, endonuclease and phospholipases, which can induce the breakdown of important substance within the cells (such as proteins and lipid) and impact the normal physiological activity [90,126]. In addition, myocardial calcium overload can also damage the structure and function of coronary blood vessels and microvascular endothelial cells, which promotes adhesion, accumulation, and infiltration of inflammatory cells to aggravating inflammatory response [123].

3.3. The inflammation in myocardial ischemia reperfusion injury

The excessive inflammation also plays a central role during the pathogenesis of myocardial ischemia reperfusion injury [18,119]. The immune response is overactivated during ischemia reperfusion injury [8,17]. After myocardial ischemia, cardiomyocytes release arachidonate and complement-derived chemotactic factors such as leukotriene B4 and C5a, which recruits and activates neutrophils, splenic monocytes, and macrophages [127,128]. These innate inflammatory cells widely express pattern recognition receptors, such as Toll-like receptors (TLRs) and NOD-like receptors (NLRs). These receptors will be activated by the ischemia reperfusion injury due to ROS accumulation and Ca^{2+} overload, leading to the activation of proinflammatory signaling, which is followed by the transcriptionally-regulated production of proinflammatory mediators including cytokines, chemokines and adhesion molecules. Eventually, these proinflammatory mediators can cause

severe tissue inflammation and exacerbate ischemia reperfusion injury [18,129].

Furthermore, excessive inflammation can in turn induce ROS production to form a positive feedback between inflammation and ROS [128,130]. For example, the activated neutrophils induce NOX to produce many ROS, such as O_2^- and H_2O_2 , which further induce the formation of $OH\cdot$ and hypochlorous acid (HOCl). Eventually, these strong oxidants damage endothelial cells and cardiomyocytes [128,131,132]. Resident macrophages and splenic monocytes can polarize into proinflammatory M1 macrophages under ROS stimulation. Notably, macrophages are typically divided into two different subsets according to their phenotype, namely proinflammatory M1 and prohealing M2 [133–135]. M1 macrophages release various proinflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-1 (IL-1). In contrast, M2 macrophages secrete antiinflammatory cytokines, including IL-4 and IL-10 [136]. Importantly, this polarization process also generally enhances NO production (due to high expression of iNOS in M1 macrophages), which reacts with O_2^- to form peroxynitrite (an oxidant with high reactivity) [137–139].

4. The pharmacological therapy for myocardial ischemia reperfusion injury

Recent advances in pathological mechanisms of myocardial ischemia reperfusion injury have promoted to develop more drugs with the ability to alleviate myocardial injury [140]. In the past decades, many clinical trials have been performed to find promising pharmacological intervention to ameliorate the myocardial reperfusion injury [119,141]. These drugs mainly can be divided into the following groups: inhibitors of calcium overload, anti-inflammatory drugs, antiplatelet agents and drugs that regulate other important targets (such as mPTP and apoptotic pathways) [141,142]. Among these drugs, the most promising therapeutic agents is cyclosporin A and exenatide. Cyclosporin A alleviates myocardial injury through inhibiting mPTP. Exenatide, as glucagon-like peptide-1 (GLP-1) receptor agonists, can activate anti-apoptotic pathways to attenuate oxidative stress and reduce infarct size [142]. Despite the pre-clinical result of most targeted therapeutic agents is promising, their clinical applications in reperfusion injury have been disappointing. These drugs are largely limited by pharmacokinetics and route of administration, such as short half-life, low stability, low bioavailability, and side effects for treatment myocardial ischemia reperfusion injury. It has been found that there is a 10-min gap between reperfusion and pharmacological therapy, which results in a significant decrease in the cardioprotective effects of the therapeutic agents [6].

5. Nanotechnology in the treatment of myocardial ischemia reperfusion injury

There are currently four alternative methods to enrich therapeutic drugs in the area of cardiac infarction, such as intramyocardial injection, intracoronary injection, epicardial cardiac patch placement, and intrapericardial injection [23,143–146]. These methods have higher drug retention properties and lower systemic toxicity than traditional intravenous injection [145,147]. Intramyocardial injection can directly reach the area of myocardial infarction, coronary artery injection can irrigate the drug to the area of myocardial infarction, and the heart patch can attach the therapeutic agent to the pericardium to achieve better specific local treatment. However, these three methods generally require thoracotomy to achieve drug delivery [145–147]. Unlike the above three methods, intrapericardial injection can be achieved through minimally invasive surgery [143,144]. For example, Li et al developed a ROS-responsive hydrogel loaded with basic fibroblast growth factor to treat myocardial infarction by intrapericardial injection. The method had good drug retention and safety, and could effectively promote cardiac angiogenesis, protect cardiac function, and reduce cardiac fibrosis [144].

Besides, intravenous administration is also usually applied for delivering a therapeutic agent after myocardial ischemia reperfusion injury owing to advantages such as the simplicity, low risk, and low cost of the procedure, and is regarded as the safest method for delivering a therapeutic agent after myocardial ischemia reperfusion injury. Nevertheless, the efficient delivery of therapeutics to myocardial tissue remains low [145,147–150]. In practical application, it is very promising to load therapeutic agents into nanocarriers in order to overcome these shortcomings [9,30,124,147–149,151–153]. For nanomedicines, they have the following advantages compared with conventional intravenous formulations: increased targeting, high stability, improved pharmacokinetics, and low systemic toxicity. On the one hand, ischemia reperfusion injury increases cardiac vascular permeability. NPs can reach the cardiac tissues through the “enhanced permeation and retention” (EPR)-like effect [154,155], and effectively be retained within the injury site after intravenous injection (Fig. 6A) [156]. In addition, the surface-specific modification of NPs can further promote the accumulation of NP in the infarct area. For example, Nguyen et al. prepared peptide-polymer NPs that could specifically recognize MMP2/MMP4 upregulated in heart tissue post myocardial infarction [156]. In general, the EPR effect is the dominant mechanism for the passive targeting of NPs, and specific modification can further improve the retention efficiency of NPs. The peptide-polymer NPs undergo a morphological transition from discrete micellar NPs to network-like scaffolds in response to upregulated MMPs compared with non-modified particles, resulting in long-term retention (up to 28 days) of the NPs at the infarction site (Fig. 6B) [157]. On the other hand, nanocarrier-based therapies can protect therapeutic agents from degradation in the circulatory system after injection, thus altering the pharmacokinetic properties of the drugs including the high dosing and frequent administration. More importantly, injectable nanomedicines significantly reduce the toxicity of conventional injections through increasing targeting and reducing the use of toxic solubilizing agents. Especially, the combination of siRNA with NPs can reduce the toxicity issues of siRNA, successfully achieving the intravenous delivery of siRNA [158]. Overall, injectable nanomedicines have been considered an attractive option to deliver therapeutic agents, considering the high delivery efficiency and bioavailability. More importantly, some nano-enzymes with intrinsic antioxidative activities can directly scavenge excessive ROS [159,160]. Therefore, we will describe the characteristics of nanotherapy for myocardial ischemia reperfusion injury in this section.

5.1. Nanocarrier for myocardial ischemia reperfusion injury

Thus far, a wide range of therapeutic drugs have been formulated in NPs to improve treatment efficiency for myocardial ischemia reperfusion injury [25]. These nanocarriers are mainly divided into five categories based on the type of drug loaded, namely enzyme-regulated antioxidants, mitochondrial ETC-protected antioxidants, mPTP-inhibited antioxidants, Ca^{2+} channel-regulated antioxidants, inflammatory regulators, cardioprotective factors and nucleic acid drugs [9,20,24,103,161–164]. These ROS-based nanocarriers can reduce excessive ROS either directly or indirectly, and reduce oxidative damage of myocardium, and maintain myocardial function.

5.1.1. NPs for regulating NOXs or ROS-scavenging enzymes

NOXs is in the cytoplasm of cardiomyocytes. The redox balance of cardiomyocytes is maintained by ROS-generating enzymes (such as NOXs) and ROS-scavenging enzymes (such as SOD) under physiological conditions. However, this redox balance is disrupted, excessive ROS cannot be removed after myocardial ischemia reperfusion [159]. On the one hand, NOXs are an important source of ROS during myocardial ischemia reperfusion, especially for NOX1, NOX2 and NOX4 [78]. As a result, it is a promising strategy for NPs to regulate the NOXs function for treating myocardial ischemia reperfusion injury [52,165–168]. On the other hand, NPs has also been gaining increasing interest by

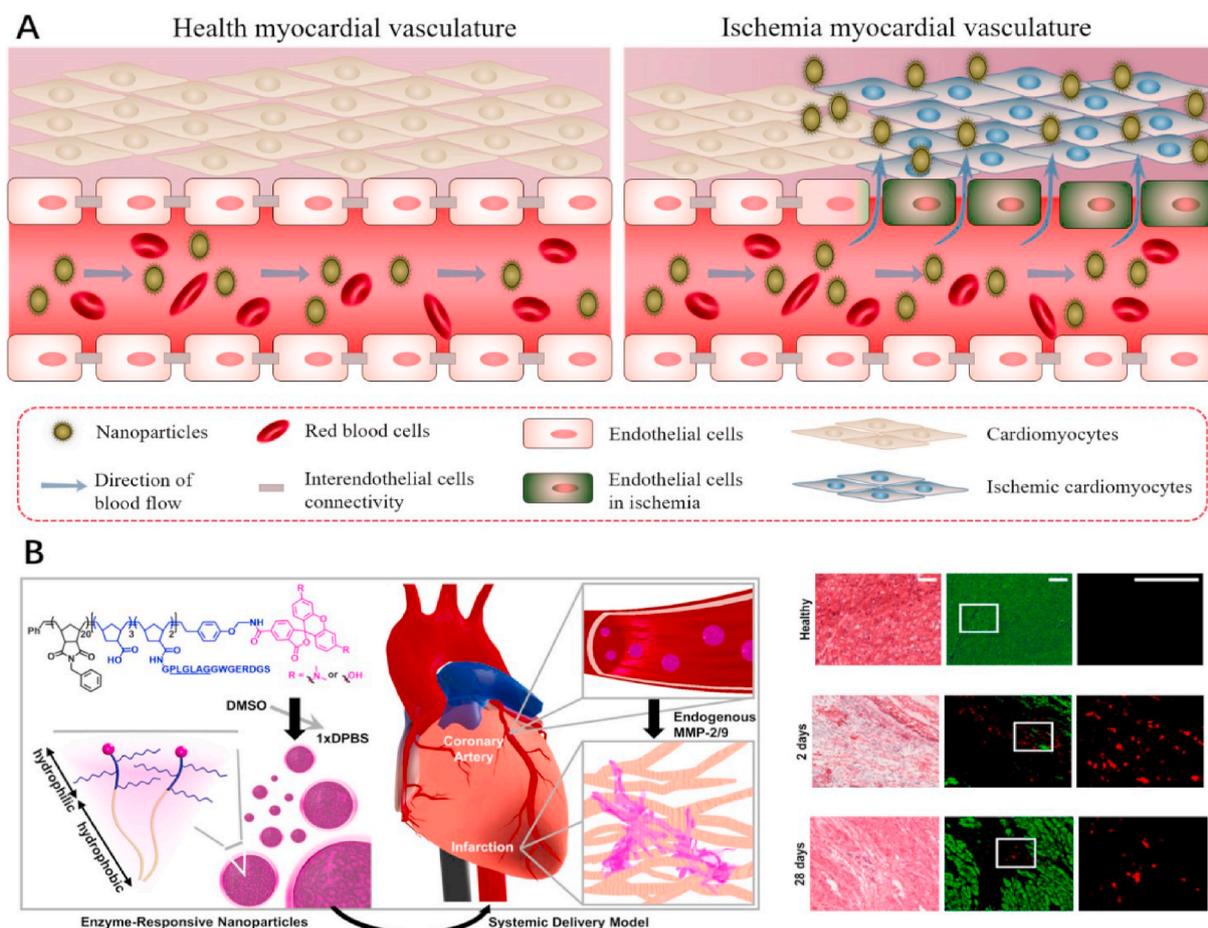


Fig. 6. The intravenous administration of nanoparticle can selectively accumulate in ischemic cardiomyocytes by the EPR effect and the surface modification or targeted fragment customization of nanoparticles. (A) The EPR effect is mediated by the high permeability of damaged endothelial cells, which contributes to nanoparticles enter the infarct tissue through the leaky ischemia myocardial vasculature. (B) The nanoparticles containing MMP-2/9 specific recognition sequence can be retained in myocardial tissue for a long time through undergoing a morphological transition in response to up-regulated MMP within the infarct. However, the intravenous injected responsive particles do not accumulate in healthy myocardium, and very few non-responsive nanoparticles accumulate in infarct tissue. Reprinted with permission from Ref. [156].

upregulating the endogenous ROS-scavenging enzymes activity for the treatment of myocardial perfusion injury (Fig. 7) [159,160].

Some bioactive natural drugs have been found to decrease myocardial ischemia reperfusion injury by inhibiting NOX activity [169,170]. However, these drugs are limited by poor bioavailability and low retention rate in myocardial infarction by intravenous injection. NPs loaded with these drugs can be regarded as a good choice for overcoming these problems [52]. For example, Bae et al. developed H_2O_2 -responsive antioxidant polymer NPs (PVAX and HPOX) for treating myocardial ischemia reperfusion injury [52,171,172]. These polymers were chemically engineered to possess H_2O_2 -responsive peroxalate ester linkages and therapeutic agents [hydroxybenzyl alcohol (HBA) or vanillyl alcohol (VA)] in their backbone. In the reperfusion area of myocardial infarction with high H_2O_2 concentration, PVAX and HPOX can be rapidly hydrolyzed to release HBA or VA through H_2O_2 -induced oxidation of peroxalate esters. The HBA and VA decrease NOX-derived H_2O_2 production by reducing expression of NOX2 and NOX4 in cardiomyocyte. As a result, the PVAX and HPOX can synergistically remove H_2O_2 by inhibiting NOXs expression and inducing peroxalate esters oxidation. PVAX and HPOX could significantly improve cardiac output and reduce the infarction area in mice after myocardial ischemia reperfusion (Fig. 8A–B) [52].

Ginsenoside Rg3 (Rg3), the active constituent in ginseng, has been shown to prevent myocardial ischemia reperfusion injury via enhancing the SOD activity [24]. Unfortunately, the low solubility of Rg3

significantly hinder its clinical applications [24]. Recently, Li et al. developed ROS-responsive nanomaterials via the self-crosslinking of polyethylene glycol (PEG) and poly (polylene sulfide) (PPS) for Rg3 delivery [24]. PPS could be oxidized by ROS, and changed from hydrophobic to hydrophilic in the reperfusion area of myocardial infarction, which leads to the release of Rg3 and the up-regulation of forkhead box O3 (FoxO3a). FoxO3a could upregulate the expression of SOD to reduce oxidative stress (Fig. 8C–G). Like Rg3, schisandrin B and baicalin also possess high antioxidant activity by upregulating the level and activity of SOD and glutathione peroxidases. Nevertheless, they are limited by first-pass metabolism, low bioavailability, short half-time, and poor solubility. PEG-modified solid lipid NPs loaded with schisandrin B and baicalin significantly improved their pharmacokinetic profiles and bioavailability [15,173]. Schisandrin- or baicalin-loaded NPs significantly ameliorated the infarct size with myocardial ischemia in rats. In addition, exenatide, as the agonist of endogenous incretin GLP-1, has also been shown to exert antioxidant effects by upregulating the activity of SOD [16,174]. Zhang et al. found that pretreatment with exenatide-loaded PEG-poly(L-lysine) NPs significantly attenuated the oxidative stress and improved myocardial function after myocardial ischemia reperfusion [175].

5.1.2. NPs for regulating mitochondrial ETC

Mitochondria continue to produce O_2^- due to the decreased activities of various complexes of ETC when myocardial reperfusion. The

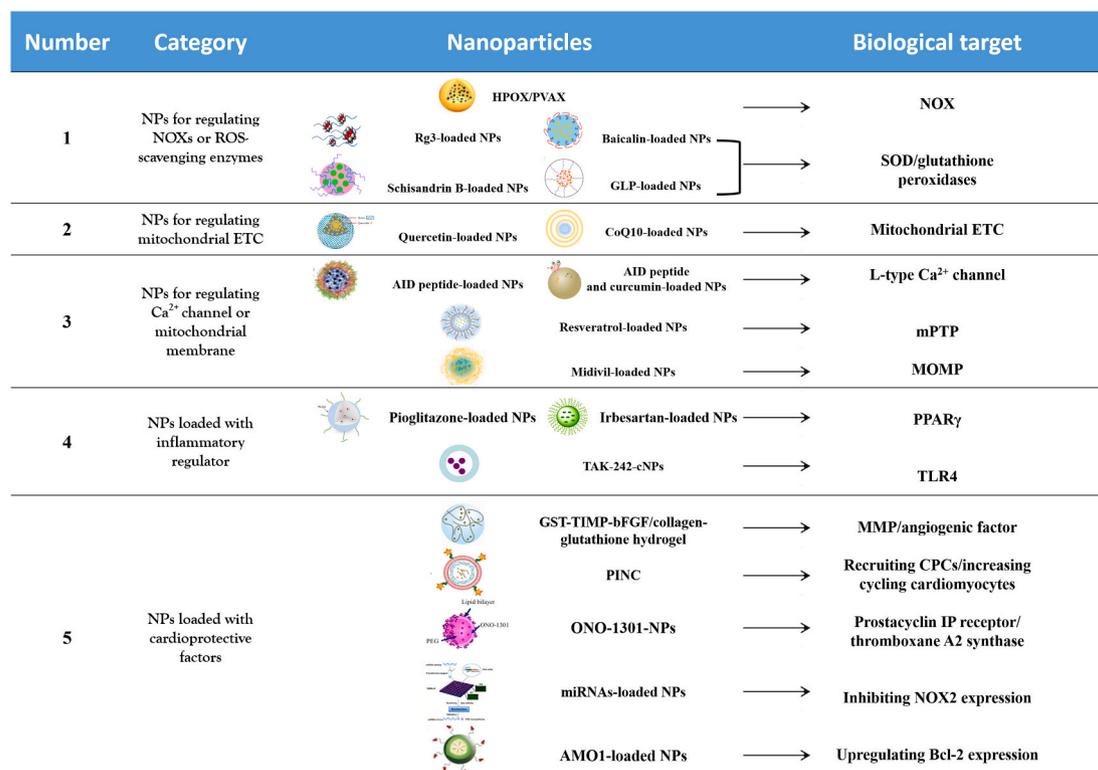


Fig. 7. Schematic illustration of nanocarrier used in drug delivery for myocardial ischemia reperfusion injury. Depending on the mechanisms of the loaded therapeutic agents, nanocarrier can be divided into five categories: 1) NPs for regulating NOXs or ROS-scavenging enzymes, of which the loaded drugs exert therapeutic effects by inhibiting NOXs or upregulating ROS-scavenging enzymes; 2) NPs for regulating mitochondrial ETC, of which the loaded drugs exert therapeutic effects by protecting mitochondrial ETC; 3) NPs for regulating Ca²⁺ channel or mitochondrial membrane, of which the loaded drugs exert antioxidant effects by inhibiting the opening of Ca²⁺ channel, mPTP, or MOMP; 4) NPs loaded with inflammatory regulator, of which the loaded drugs effectively alleviate myocardial inflammation; 5) NPs loaded with cardioprotective factors, of which the loaded drugs can directly improve cardiac dysfunction and structural damage.

development of mitochondrial ETC-protected antioxidants is of great importance for myocardial ischemia reperfusion injury therapy [88, 176]. Quercetin can maintain the integrity and potential of mitochondrial ETC by integration with mitochondrial complex I and III. For example, Lozano et al. developed poly(lactic-co-glycolic acid) (PLGA) NPs loaded quercetin for treatment of myocardial reperfusion. PLGA NPs could significantly improve the bioavailability of quercetin. Especially in the quercetin-PLGA nanocomposites group, the levels of O₂⁻ and H₂O₂ produced by mitochondrial complex I and III were significantly lower than those measured in the free quercetin group. Meanwhile, PLGA-quercetin group also greatly increased the ATP generation rate (approximately 11.9%) (Fig. 9) [101,126]. CoQ10 plays important roles in mitochondrial ETC via serving as a mobile electron transporter between FMN and Cyt c, and can attenuate the mitochondrial structural damage by reducing the depletion of ATP. However, the poor bioavailability of CoQ10 limit this application for myocardial ischemia reperfusion injury therapy. To address this issue, Verma et al. prepared CoQ10-loaded liposomes [177,178]. Liposomes could efficiently deliver CoQ10 into cardiomyocytes to maintain normal energy metabolism in the damaged myocardium, and reduced myocardial ischemia reperfusion injury.

5.1.3. NPs for regulating Ca²⁺ channel or mitochondrial membrane

Maintaining intracellular Ca²⁺ homeostasis and inhibiting mPTP opening also decrease ROS production in the mitochondria [68,179]. For example, Clemons et al. prepared poly(glycidyl methacrylate) (PGMA) NPs to deliver a peptide against the alpha-interacting domain (AID) peptide into cardiac tissue [180]. The L-type Ca²⁺ channel is the main route for Ca²⁺ influx into cardiomyocytes; it is composed of three different subunits, namely the α_{1c} , β_2 , and $\alpha_{2\delta}$ subunits. Notably, the α_{1c} subunit facilitates ion conductance, while the β_2 subunit is involved in

the transportation of the α_{1c} subunit to the plasma membrane through binding to the α_{1c} subunit at the AID [181,182]. The AID peptide can inhibit activation of the L-type Ca²⁺ channel by binding to the its AID region. Therefore, the AID peptide-PGMA NPs significantly decreased the levels of intracellular Ca²⁺ through inhibiting Ca²⁺ influx. In a guinea pig model of myocardial ischemia reperfusion, AID peptide-PGMA NPs attenuated cardiac dysfunction. Similarly, Hardy et al. also developed a strategy to codelivery of the antioxidant curcumin and Ca²⁺ channel blocker in PGMA NPs for attenuating myocardial injury in rat heart after ischemia reperfusion [183]. NPs loaded with both curcumin and Ca²⁺ channel blocker showed stronger ROS-lowering effects than NPs only encapsulating curcumin (Fig. 10A and B).

In addition, Cheng et al. prepared resveratrol-PLGA NPs decorated with ischemic myocardial mitochondria-targeted peptide (IMTP) and Szeto-Schiller 31 (SS31) through nanoprecipitation [103,149,184]. IMTP and SS31 could allow resveratrol-PLGA NPs to accumulate at the inner mitochondrial membrane in ischemic areas. Resveratrol could increase the mitochondrial phosphorylated signal transducer and activator of transcription 3 (STAT3) to decrease mPTP opening and maintain mitochondrial integrity. STAT3 plays important roles in transmitting extracellular signals from the plasma membrane to the mitochondria. Notably, serine-phosphorylated STAT3 interacts with the mediator of mPTP cyclophilin D, thus regulating the opening of mPTP [185]. The resveratrol-PLGA NPs could increase the viability of hypoxia/reoxygenation-injured H9c2 cells, and reduce the infarct size of rats with myocardial ischemia reperfusion injury (Fig. 10 C, D). Similarly, Ishikita et al. developed PLGA NPs containing mitochondrial division inhibitor 1 (Mdivi1) (Mdivi1-NPs) to protect the myocardium from ischemia reperfusion injury [27]. The PLGA nanocarrier increased the distribution of Mdivi1 in the myocardium and intracellular uptake. Mdivi1-NPs could inhibit mitochondrial outer membrane

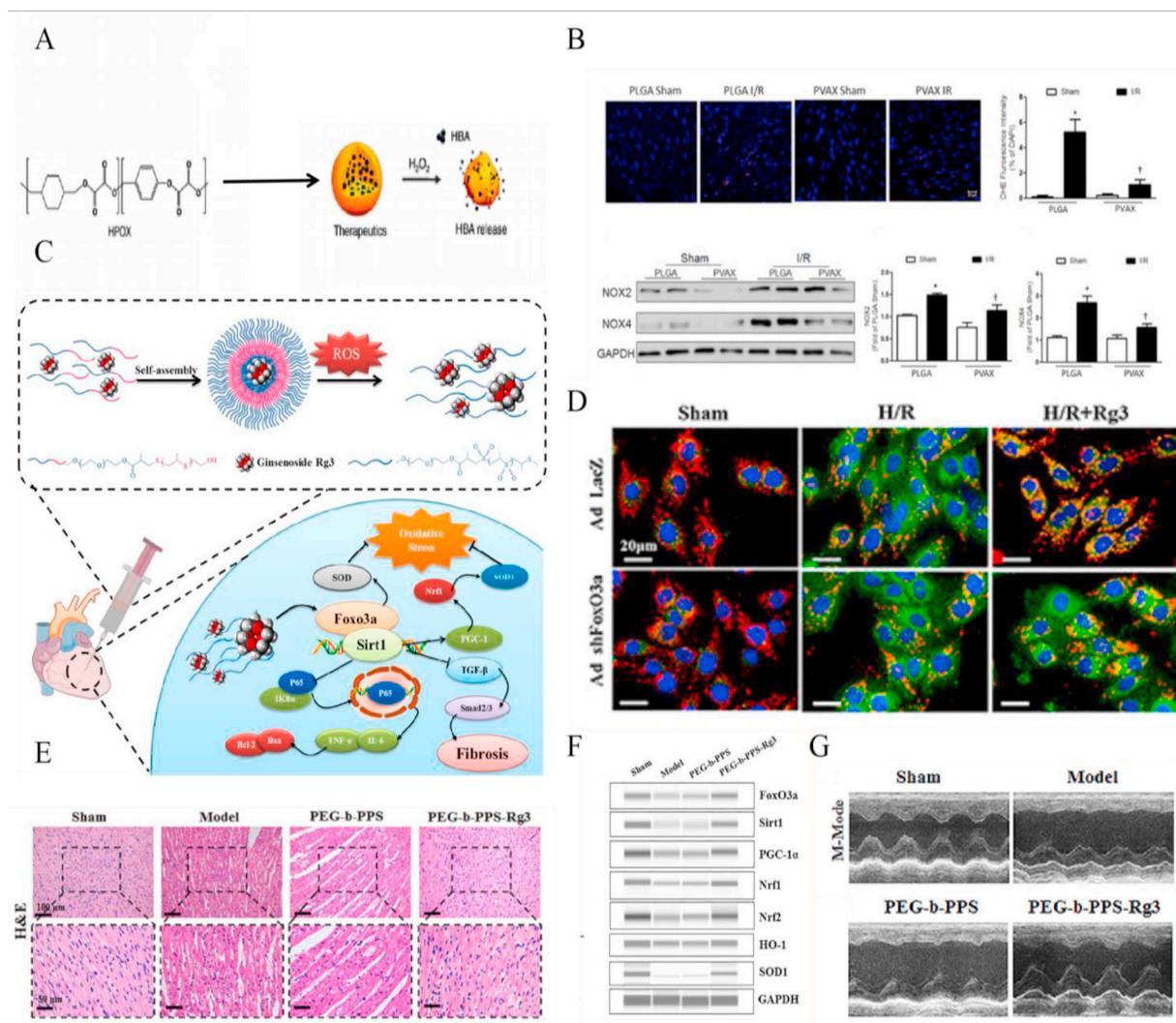


Fig. 8. (A) HPOX and PVAX nanoparticles can be exploited as a carrier to deliver drugs to the injured site after ischemia reperfusion injury, and the process is activated by H_2O_2 stimulation. (B) The antioxidant activities of PVAX nanoparticles after myocardial ischemia reperfusion injury. PVAX effectively reduced the generation of ROS caused by ischemia reperfusion, and the mechanisms are related with decreased NOX2 and NOX4 expression. Reprinted with permission from Ref. [171]. (C) Rg3-loaded PEG-b-PPS nanoparticles responded to ROS, and the released Rg3 alleviated myocardial ischemia reperfusion injury by inducing SOD expression. (D) JC-1 fluorescence staining exhibited that Rg3 recovered mitochondrial damage through targeting FoxO3a. (E) The PEG2-b-PPS-Rg3 attenuated ischemia myocardial injury including inflammatory cell infiltration, edema and architecture disruption. (F) The PEG2-b-PPS-Rg3 upregulated FoxO3a and SOD expression of cardiac tissue. (G) Compared with the model group, the amplitude of the left ventricular wall motion was stronger in the PEG2-b-PPS-Rg3 group, suggesting that PEG2-b-PPS-Rg3 can improve cardiac function. Reprinted with permission from Ref. [24].

permeabilization (MOMP) that induce the release of Cyt *c* from the mitochondrial membrane space into the cytoplasm, followed by the activation of caspases, RIRR, and apoptosis. Particularly, MOMP-mediated cell death is independent of the mPTP opening. Mdivi1-NPs have protective effects on CyD-knockout mice, but have no effect on Bax-knockout mice. Bax is the key component of MOMP while CyD is the core of mPTP [27,186,187].

5.1.4. NPs loaded with inflammatory regulator

As mentioned above, excessive, prolonged and dysregulated inflammation has been implicated in the pathogenesis of ischemia reperfusion injury, and macrophages play an important role in pathological conditions [188–193]. After myocardial ischemia, the oxidative stress and excessive inflammation regulated by activated macrophage damage to the myocardium and enlarge the infarct size [133–135]. Recent studies have successfully combined immunotherapy with nanotechnology to develop new approaches for post-infarction therapy, showing significantly improved efficacy compared with traditional

immunotherapy.

For example, Tokutome et al. developed a pioglitazone-PLGA NPs (pioglitazone-NPs) for treatment of ischemia reperfusion injury [188]. Pioglitazone is a highly effective agonist of PPAR γ , which is a ligand-activated nuclear transcription factor to regulate M1 macrophages toward M2 macrophages [189,194]. Pioglitazone-NPs reduced monocyte chemoattractant protein 1 (MCP-1)-expressing cardiomyocytes and inhibited the recruitment of inflammatory immune cells including MCP-1 and CD11b-positive monocytes/macrophages. In addition, pioglitazone-NPs differentiated M1 macrophages into the M2 phenotype and reduced inflammatory cytokine secretion (Fig. 11A–C). Similarly, Nakano et al. also prepared irbesartan (an agonist of PPAR)-PLGA NPs (irbesartan-NPs) for treatment of ischemia reperfusion injury. Irbesartan-NPs markedly reduced the infarct size and ameliorated left ventricular remodeling by inhibiting monocyte-mediated inflammation [163]. The nanocarrier significantly improved the efficacy of pioglitazone and irbesartan in targeting ischemia reperfusion injury compared with free pioglitazone or irbesartan [188].

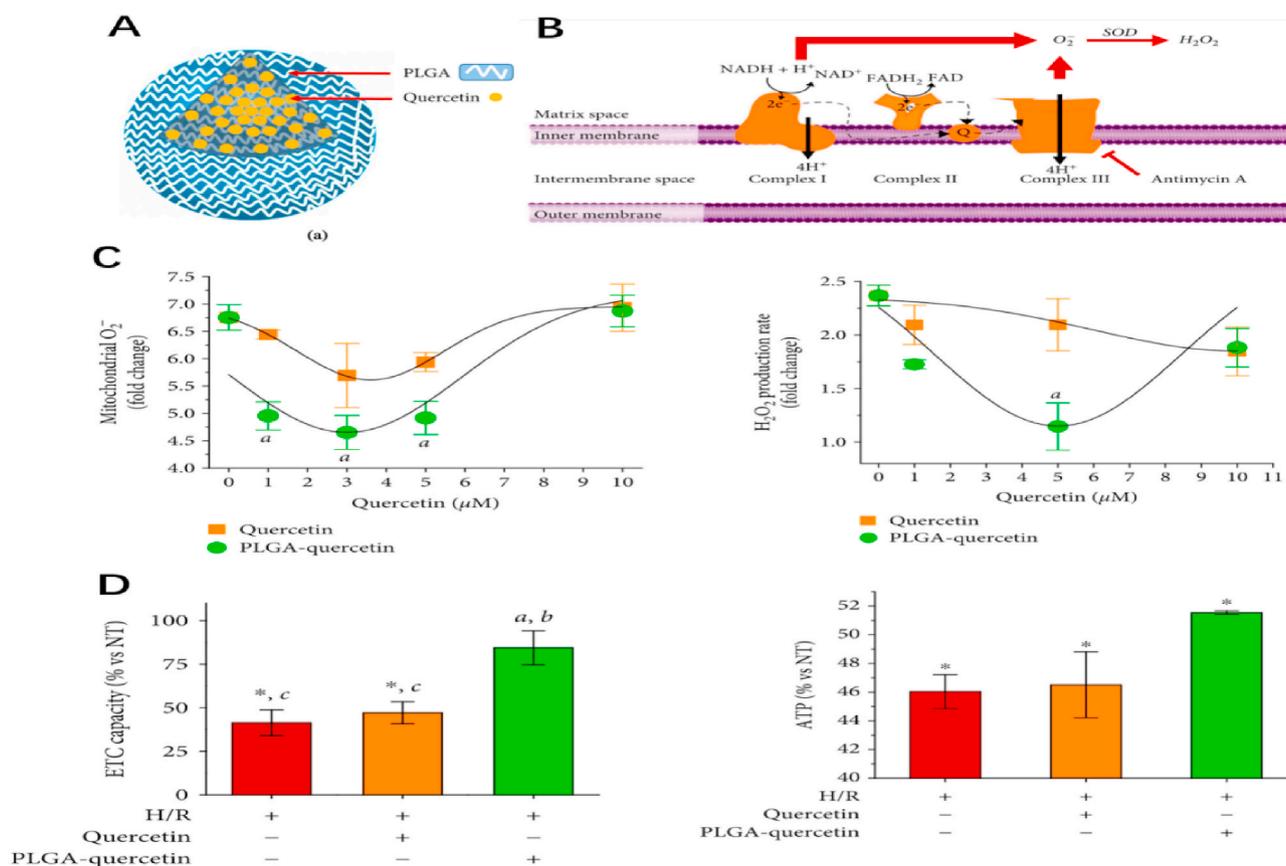


Fig. 9. (A) The schematic representation of PLGA-quercetin. (B) Description of the ROS production in the mitochondria due to antimycin A stimulation, which blocks the complex III of the ETC. (C) Treatment with quercetin or PLGA-quercetin inhibited mitochondrial O_2^- and H_2O_2 production in response to antimycin A stimulation. (D) Protection of PLGA-quercetin against hypoxia-reoxygenation H9c2 cell, of which PLGA-quercetin preserved ETC capacity and increased ATP production. Reprinted with permission from Ref. [101].

In another study, Masaki et al. developed PLGA NPs loaded with TLR4 inhibitor (TAK-242-NPs) for treatment of ischemia reperfusion injury [190]. Under myocardial ischemia reperfusion, damage-associated protein patterns released by damage cardiomyocytes can initiate the innate immune response to accelerate damage to cardiomyocytes by binding to TLR4 receptors of immune cells [195,196]. TLR4 activation can increase the expression of chemokine (C-C motif) receptor 2 (CCR2) to recruit/activate inflammatory M1 monocytes/macrophages by increasing NF- κ B activity [193]. The intravenous administration of TAK-242-NPs significantly decreased the infarct size and attenuated cardiac remodeling after ischemia reperfusion (Fig. 11D).

Specially, the ROS-responsive-nanocarriers have dual functions: nanocarrier itself can eliminate excess ROS, and eliminate inflammation by delivering anti-inflammatory drugs to the damaged cardiomyocytes. Glucocorticoids such as methylprednisolone (MP) and dexamethasone (Dx) are a commonly anti-inflammatory drug in clinical, but the effect are limited by various factors such as administration route, dosage and duration of action time in the treatment of myocardial infarction reperfusion [197]. Recently, Yao et al. developed a ROS-responsive polyurethane nanocarrier to load MP (PUTK/MP) for treatment of myocardial infarction reperfusion [23]. In the presence of excess ROS, the thioether bonds in PUTK were cleaved by ROS and the MP was released at the damaged cardiomyocytes. PUTK/MP treatment could greatly protect the myocardium from oxidative injury, effectively reduce the fibrosis and negative cardiac remodeling, and promote the cardiac function and angiogenesis.

5.1.5. NPs loaded with cardioprotective factors and nucleic acid drugs

Cardioprotective factors can induce angiogenesis or inhibit extracellular matrix (ECM) degradation to alleviate oxidative and inflammatory damage to cardiomyocytes [198]. However, cardioprotective factors [basic fibroblast growth factor (bFGF) [198], cardiac stromal cell secretion factor, prostaglandin E2 (PGE2), etc.] are proteins [199], which are easily degraded by proteases in vivo, and have extremely low bioavailability directly through intravenous injection. NPs loaded with cardioprotective factors can effectively solve these problems [144].

For example, Fan et al. synthesized a glutathione-modified collagen hydrogel to deliver a specially designed protein, which was composed of the following three units: bFGF, glutathione-S-transferase (GST), and MMP2/9 cleavable peptide PLGLAG (tissue inhibitor of metalloproteinase 1 [TIMP]) (bFGF-GST-TIMP) [198]. bFGF serves as an angiogenic factor to promote angiogenesis after myocardial ischemia; TIMP is the inhibitor of MMP. The highly expressed MMP degrades ECM to impaired myocardial mechanical performance after myocardial infarction. GST can be combined with the glutathione peptide of collagen hydrogel, which enables bFGF-GST-TIMP to be effectively loaded into the collagen hydrogel. The bFGF-GST-TIMP hydrogels can overcome the defects of conventional drug delivery including systemic toxicity and low bioavailability. Moreover, effective protection of the drug from degradation and controlled drug release were achieved based on the exquisitely tunable physical properties of these hydrogels. Intravenous administration of bFGF-GST-TIMP hydrogel significantly improve cardiac function by promoting angiogenesis and decreased MMP2/9 activity in rats with myocardial ischemia (Fig. 12A–B).

In addition, Su et al. prepared a platelet-inspired nanocell (PINC) by loading PGE2-modified platelet membrane and cardiac stromal cell-

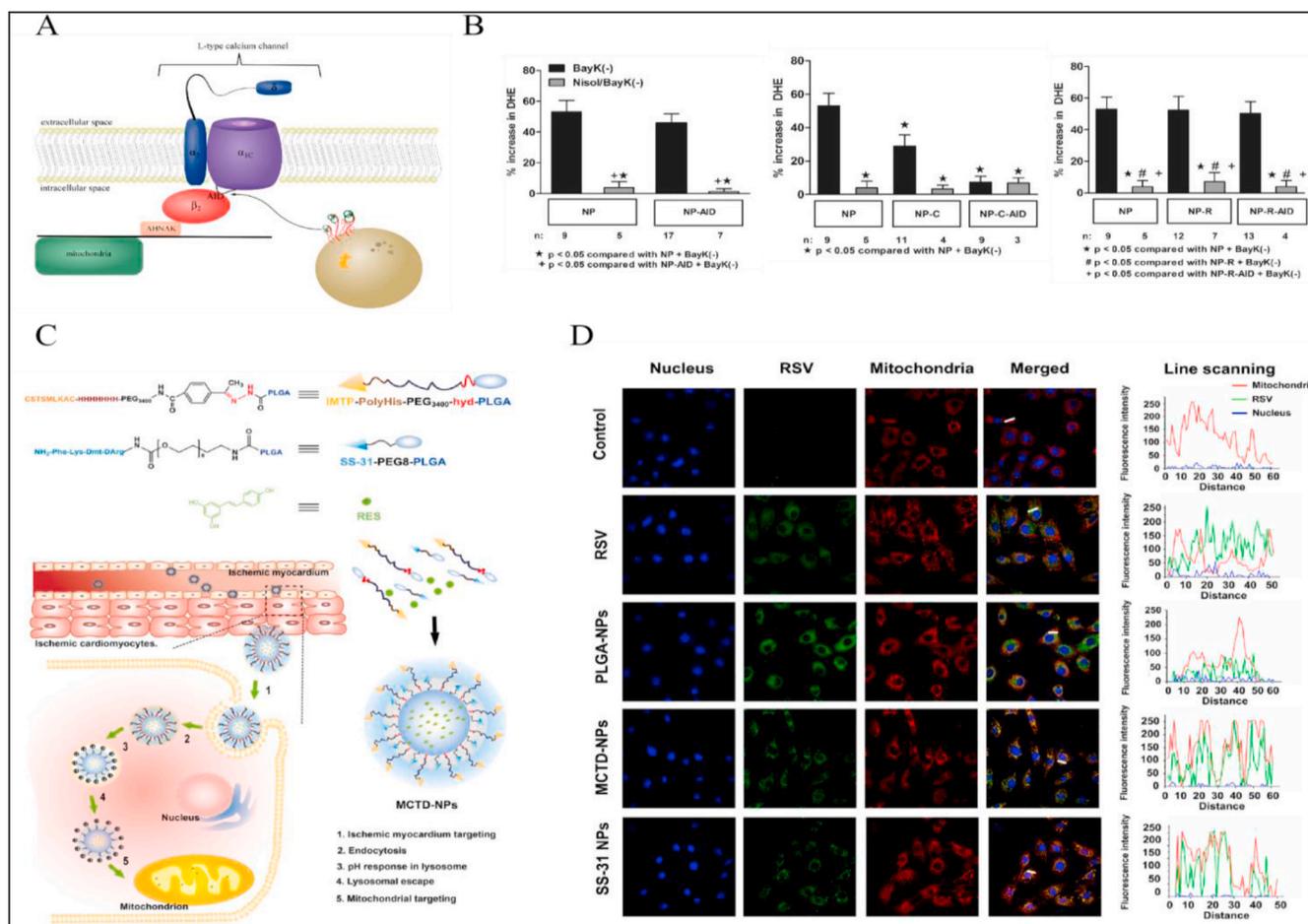


Fig. 10. (A) Schematic representation of the L-type Ca^{2+} channel and polymeric nanoparticles delivering antioxidant and AID peptide. (B) The nanoparticle loaded with antioxidant curcumin and AID peptide has the best effect of decreasing ROS content. Reprinted with permission from Ref. [183]. (C) Schematic image of precise treatment of myocardial ischemia reperfusion injury through multistage mitochondrial delivery of resveratrol. (D) The mitochondrial distribution and the fluorescence intensity after RSV, PLGA-NPs, MCTD-NPs and SS31-NPs were incubated with hypoxia/reoxygenation injured H9c2 cells. The nanoparticles (MCTD-NPs) containing IMTP and SS31 delivered more amount of RSV to mitochondrial of hypoxia/reoxygenation injured H9c2 cells. Reprinted with permission from Ref. [103]. RSV: resveratrol; MCTD: multistage continuous targeted drug delivery carrier.

secreted factors [199]. PGE2 is an important signal transduction molecule that participates in numerous biological processes, such as promoting the differentiation of endogenous stem/progenitor cells. In addition, PGE2 can bind to prostaglandin receptors (EP receptors) overexpressed in the plasma membrane of cardiomyocytes after ischemia reperfusion. The natural infarct-homing ability of the platelet membrane further contributes to PINC accumulation in the infarct area. Cardiac stromal cell-secreted factors (like stromal cell-derived factor-1 [SDF-1], vascular endothelial growth factor [VEGF] and hepatocyte growth factor [HGF] can improve cardiac regeneration and repair by promyogenic and proangiogenic effects [200,201]. PINC promoted mitotic activity of cardiomyocytes, and the recruitment of $\text{Nkx2.5}^{+/+}$ cardiac progenitor cells (CPCs) to the damaged cardiac tissues. These effects were accompanied by a larger amount of CD34-positive endothelial progenitor cells and enhanced von Willibrand Factor-positive vessel density. More importantly, PINC overcomes the limitations of traditional cell therapy, such as difficulty in preparation and low viability and retention (Fig. 12C–D).

ONO-1301 is a synthetic prostacyclin I2 receptor (IP) agonist, which can activate the IP and inhibit thromboxane A2 synthase, which induces the release of cytoprotective factors such as VEGF and inhibits the secretion of proinflammatory cytokines such as IL-6, IL-1 β , and TNF- α in cardiomyocytes. Recently, Yajima et al. developed a PEG-modified solid lipid NPs loaded with ONO-1301 (ONO-1301-NPs) for treatment of myocardial infarction reperfusion [20]. After intravenous injection,

ONO-1301-NPs can be selectively accumulated in cardiomyocytes by the EPR effect, and significantly reduced the infarct size by alleviating cardiac inflammation.

miRNA usually negatively regulates gene expression at the post-transcriptional level by preventing messenger RNA (mRNA) translation or promoting mRNA degradation [202]. Currently, the application of miRNAs or miRNA inhibitors provides a new direction in myocardial ischemia reperfusion injury therapy [203–208]. Unfortunately, the delivery of nucleic acid drugs to the myocardial area continues to face numerous challenges, including degradation by environmental enzymes, low bioavailability, and poor pharmacometrics. Recent studies have showed that NPs loaded with miRNA or miRNA inhibitors significantly decreased the myocardial infarct size and reversed damage to cardiomyocytes by regulating the expression of the antioxidant enzymes expression and apoptosis-related proteins [204, 206,207,209]. For example, Yang et al. prepared a miRNA-targeted screening system consisting of a self-assembled cell microarray [207]. They identified that three miRNAs, namely miRNA-106b, miRNA-148b, and miRNA-204 could inhibit NOX2 expression in both human and mouse macrophages. Subsequently, they used polyketal (PK3) NPs to separately encapsulate these three miRNAs, and observed their effects on myocardial ischemia in mice. PK3 NPs were effectively taken into endosomes/phagosomes and degraded under a low pH microenvironment to transport miRNAs to the cytosol of macrophages. The PK3-miRNA NPs could down-regulate NOX2 expression to decrease ROS

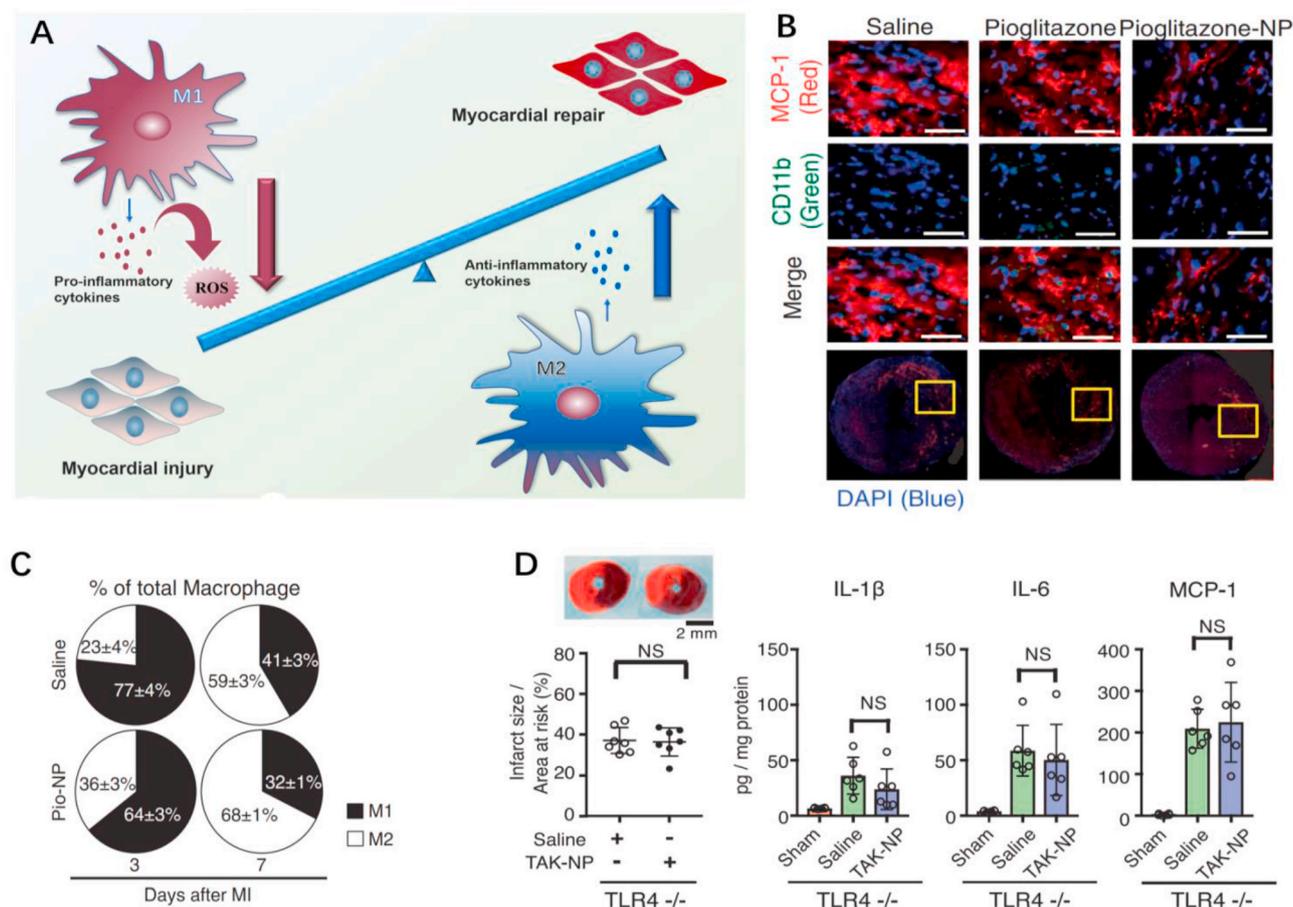


Fig. 11. (A) Macrophages act as double-edged sword in the regulation of myocardial tissue repair. M1 macrophages secrete a large amount of pro-inflammatory cytokines that can induce ROS production to aggravate myocardial injury. M2 macrophages produce anti-inflammatory, contributing to cardiac repair. Under myocardial ischemia reperfusion injury, the balance of M1/M2 macrophage is broken, leaning toward M1 macrophage, thus further aggravating myocardial damage. (B) Fluorescent immunostaining results demonstrated that pioglitazone-NPs decreased the number of MCP-1-expressing cardiomyocytes and inhibited the recruitment of the inflammatory immune cells including leucocytes expressing MCP-1 and CD11b-positive monocyte/macrophage. (C) The effects of pioglitazone-NPs on macrophage polarity. The pioglitazone-NPs promote the polarization of M1 macrophages into M2 macrophages. Reprinted with permission from Ref. [188]. (D) TAK-NP had no effects on the infarct size and the protein levels of IL-1 β , IL-6 and MCP-1 in TLR4-deficient mice, suggesting that the therapeutic effects of TAK-NP are achieved by targeting TLR4. Reprinted with permission from Ref. [190].

levels (Fig. 13).

The B-cell CLL/lymphoma 2 (Bcl-2) plays an antiapoptotic role primarily through isolating the death-driving cysteine proteases termed caspases (a complex of apoptosome) and inhibiting the release of mitochondrial apoptogenic factors (e.g., Cyt c) [210–212]. Antisense oligonucleotide (AMO1) can attenuate cardiomyocyte apoptosis and infarct size by upregulating the expression of Bcl-2. Recently, Xue et al. developed a dendrimer-based nano vector for delivery of antisense AMO1 [206]. The dendrimer-based nanovector is composed of dendri-graft poly-L-lysine (DGL), PEG, and angiotensin II type 1 (AT1). DGL had a strong binding ability to AMO1 thanks to the high density of amine. AT1 could specifically bind to the AT1 receptor, which was over-expressed at the beginning of the ischemia reperfusion. The GDL-PEG-AT1 nanocarrier could accurately deliver AMO1 to ischemia myocardium, and reduce cardiomyocyte apoptosis.

5.2. Antioxidant nano-enzymes

Some nanomaterials, such as cerium oxide NPs and graphene derivatives, exhibit antioxidant enzyme-like activity. These nanomaterial-based artificial enzymes (nano-enzymes) can effectively maintain the redox imbalance by mimicking endogenous enzymes, and overcome the problems of natural enzymes including low stability and poor bioavailability [159,213–215]. Although nano-enzymes have a great

prospect, research studies in the field of myocardial ischemia reperfusion treatment remain at the initial stage. To date, these materials can be roughly divided into three categories, namely metal/metal-oxide nano-enzymes, carbon-based nano-enzymes and DNA nano-enzymes [28,213,216,217].

5.2.1. Metal and metal-oxide nano-enzymes

Currently, cerium oxide (ceria), iron oxide, gold, and copper have been used for myocardial ischemia reperfusion injury therapy [28,213, 218–220]. Ceria oxides have broad antioxidant activities due to the redox cycling between cerium (IV) and cerium (III). Loss of an oxygen atom leads to reduction of cerium ($Ce^{4+} \rightarrow Ce^{3+}$) and an increased oxygen vacancy in the lattice. The oxygen vacancy balances the reduction of the positive charge by Ce^{3+} , and stabilize the activity of the Ce^{3+} oxidation state [221–223]. Ceria exists as pure Ce^{4+} or Ce^{3+} at the bulk state. Interestingly, at the nanoscale, it has a mixture of both Ce^{3+} and Ce^{4+} on the surface of ceria. The Ce^{3+}/Ce^{4+} surface ratio is closely related to the antioxidant enzyme-mimetic activity towards ROS. Ceria nano-enzymes with high amounts of Ce^{3+} (40–60%) on the surface behave as SOD-mimics ($Ce^{3+} + 2H^+ + O_2^- \rightarrow H_2O_2 + Ce^{4+}$), whereas ceria nano-enzymes with high amounts of Ce^{4+} (70–80%) act as catalase-mimics ($Ce^{4+} + H_2O_2 + 2OH^- \rightarrow O_2 + 2Ce^{3+} + H_2O$). Meanwhile, ceria nano-enzymes can effectively scavenge $OH\cdot$ ($Ce^{3+} + 2OH\cdot \rightarrow 2Ce^{4+} + H_2O$) (Fig. 14A) [28,223]. Ceria nano-enzymes have been

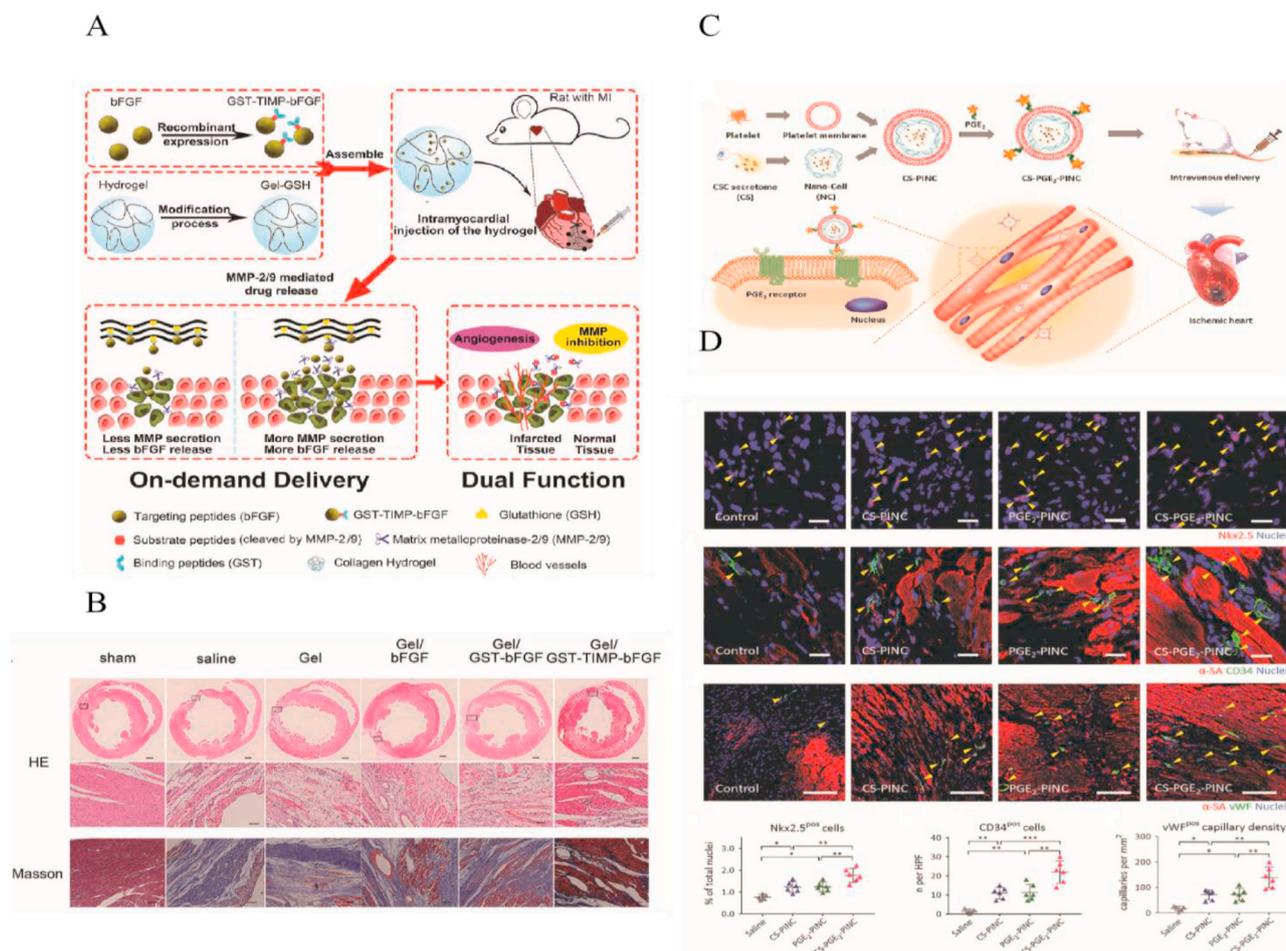


Fig. 12. (A) Schematic representations of MMP-responsive hydrogel preparation and the process of drug release in the myocardial ischemia model rats. (B) The Gel-GST-TIMP-bFGF group showed the least wall thinning compared to other myocardial ischemia rats, suggesting the most obvious inhibition of cardiac remodeling. Reprinted with permission from Ref. [198]. (C) Schematic illustration of the fabrication process of PINC and the therapeutic effects of PINC on mice with myocardial ischemia reperfusion injury. (D) Representative images and quantification of the number of Nkx2.5-positive cells, CD34-positive cells and vWF-positive capillaries in the infarct heart 4 weeks after PINC treatment. Reprinted with permission from Ref. [199].

applied to myocardial ischemia reperfusion injury therapy [224,225]. For example, Pagliar et al. found that ceria nano-enzymes could escape CPCs from oxidative stress, but did not alter the phenotype and growth of CPCs under treatment with H₂O₂ [223]. CPCs are the main cell source during heart regeneration and can differentiate into cardiomyocytes in a specific microenvironment. However, following the occurrence of severe cardiac injury, such as myocardial ischemia, this differentiation process is not sufficient to meet the need for heart tissue repairing. Therefore, delivery of CPCs to the damaged myocardium is a promising therapy for the treatment of myocardial ischemia reperfusion injury. Unfortunately, the growth and differentiation of CPCs are affected by the oxidative environment. Therefore, improving the resistance of CPCs to excessive ROS is necessary. The ceria nano-enzyme could significantly reduce ROS production and protect CPCs from oxidative injury (Fig. 14B–C). However, Wingard et al. have shown that exposure to the ceria Nano-enzyme can impair the structure and function of blood vessels and exacerbate myocardial ischemia reperfusion injury by inducing an inflammatory response [226]. Particularly, the inflammatory process largely depends on mast cell activation, which could produce numerous proinflammatory cytokines, including IL-6, IL-13, TNF- α , transforming growth factor- β (TGF- β) and osteopontin (Spp1). These results suggested that the ceria nano-enzyme has proinflammatory side effects during its clinical application (Fig. 14D–E).

Such nano-enzymes, including Fe₂O₃ and Fe₃O₄, can act as both oxidants and antioxidants in biological systems, given their pH-

dependent dual enzyme (peroxidase and catalase-like) activity [227–229]. After the internalization of an iron oxide nano-enzyme into cells, it is decomposed in acidic lysosomes and endosomes to release free iron ions (Fe²⁺ and Fe³⁺). Subsequently, these Fe²⁺/Fe³⁺ participate in Haber–Weiss–Fenton reactions to generate OH \cdot by catalyzing H₂O₂ (peroxidase), which lead to oxidative stress injury. However, there was no OH \cdot generation observed in the cytoplasmic neutral environment. Instead, the iron oxide nano-enzyme decomposes H₂O₂ into water and oxygen through its catalase-like activity [230]. For instance, Xiong et al. investigated the cardioprotective activity of an iron oxide nano-enzyme [229]. They found that the iron oxide nano-enzyme reduced the infarct size in rats after myocardial ischemia, while it increased the cellular viability in hypoxia and reoxygenation cardiomyocytes. Furthermore, the ROS levels were significantly decreased after iron oxide nano-enzymes treatment. However, myocardial delivery of the iron oxide nano-enzyme may also cause iron overload in the myocardium, which can paradoxically induce oxidative stress to exacerbate myocardial injury. The high concentration of iron oxide can release a large amount of iron ions to catalyze the Fenton reaction, causing the overproduction of OH \cdot [39,231,232]. For example, Zheng et al. also found that an iron oxide nano-enzyme induced ferroptosis in ischemic cardiomyocytes. Ferroptosis regulates cell death based on the iron-dependent accumulation of lipid peroxides [230,233]. Particularly, this contradictory phenomenon whether iron oxide nano-enzyme acts as antioxidant or induces ROS production is dose-dependent.

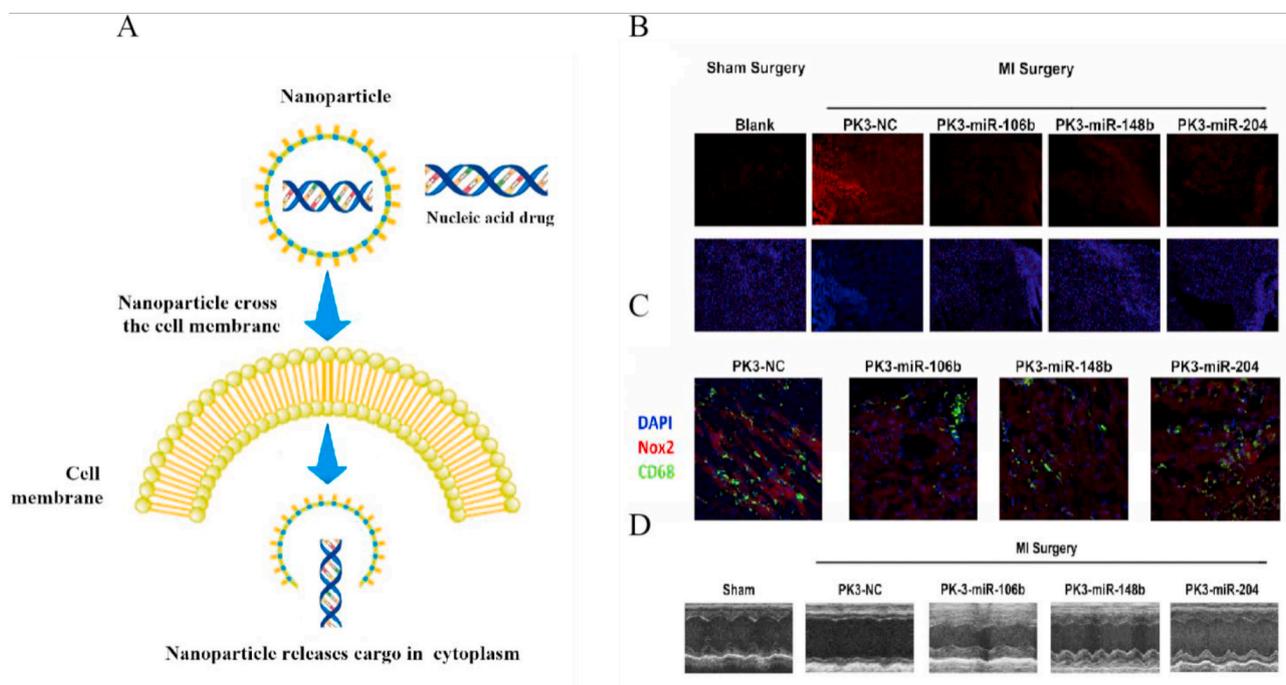


Fig. 13. (A) Schematic illustrations of nucleic acid drug-nanoparticle formation and delivery. Nanocarrier contributes to nucleic acid drug cross cell membrane, and then release nucleic acid drug in cytoplasm to exert therapeutic effects. (B) Dihydroethidium staining demonstrated that PK3-miRNA treatment significantly reduced ROS production of mice with myocardial ischemia. (C) PK3-miRNA treatment inhibited NOX2 expression of myocardial ischemia mice. (D) PK3-miRNA particle improved cardiac function of mice with myocardial ischemia including a stronger the amplitude of the left ventricular wall motion. Reprinted with permission from Ref. [207].

Cardiomyocytes stimulated with iron oxide nano-enzyme (0.01, 0.1, and 0.5 mg/ml) did not show marked differences in cell viability, indicating that these doses of iron oxide enzyme were non-toxic to normal cardiomyocytes. In contrast, within a 30-day treatment period, intraperitoneal injections of the iron oxide nano-enzyme at a dosage of 25 mg/kg and 50 mg/kg caused cardiovascular system dysfunction by inducing the death of cardiomyocytes in mice [234]. Therefore, the dose of the iron oxide enzyme represents a key factor in determining its application in myocardial ischemia reperfusion injury therapy. In addition, surface modification (e.g., N-acetylcysteine) can be employed to significantly suppress the toxic effects of an iron oxide nano-enzyme. N-acetylcysteine could attenuate iron-induced oxidative damage in myocardial cells via increasing SOD and catalase expression and decreasing oxidative damaged products [234]. Other surface modifications, such as graphene, can increase the antioxidant capacity of an iron oxide enzyme, which may be related to the formation of a radical adduct at the sp^2 carbon site of graphene leading to radical destruction [235]. Overall, iron oxide nano-enzymes have shown potential for the clinical treatment of myocardial ischemia reperfusion injury. However, Iron oxide NPs can cause necrosis of cancer cells by inducing ROS production and autophagy activation [236]. But this reaction cannot be observed in normal cells, which may be attributed to the different intracellular environment.

Similarly, gold also has antioxidant effects [28,34,237,238]. Gold nano-enzymes can improve cardiac injury by increasing the antioxidant capacity. The levels of antioxidant enzyme SOD and glutathione peroxidase were significantly increased after the administration of a gold Nano-enzyme [238–240]. Meanwhile, gold Nano-enzymes are thought to be relatively non-cytotoxic compared with other metal Nano-enzymes [238,241]. In addition, copper Nano-enzymes also possess antioxidant and anti-inflammatory properties [242]. Copper Nano-enzymes can diminish oxidative stress and inflammatory cytokines and increase nitric oxide by activating the glycogen synthase kinase-3 β (GSK3 β) signaling pathway.

5.2.2. Carbon-based nano-enzymes

The second group is carbon-based nanomaterials, which can be divided into three categories: graphene, fullerene, and carbon nanotubes [28,243–245]. Particularly, graphene and fullerene nano-enzymes have received considerable attention for myocardial ischemia reperfusion injury therapy because of their ability to mimic the SOD and catalase enzymes [213].

Mesenchymal stem cells (MSCs) are a type of adult stem cells derived from the mesoderm, which can be easily cultured *in vitro* for a period without alterations in their characteristics (i.e., self-renewal and multiple differentiation potential) [246–249]. MSC implantation may be a promising therapeutic approach, as these cells can differentiate into cardiomyocytes and vascular structure [161,249]. Meanwhile, MSCs can facilitate the secretion of cardiac-regenerating and immunomodulatory factors, which attenuate myocardial ischemia reperfusion injury [250]. Unfortunately, the stem cell therapy is usually unsatisfactory because of the low survival rates of the transplanted cells in the infarcted area after transplantation [22]. After reperfusion, the high levels of ROS in the infarcted area can inhibit the interactions between MSCs and the myocardial ECM, which lead to low survival of MSCs. Recently, Choe et al. developed a new MSC-Graphene oxide (GO)/microgel delivery system with strong antioxidant activity [161]. GO have lots of sp^2 -hybridized carbon network, which can induce electron transfer to neutralize ROS [251–254]. Therefore, encapsulation of MSCs in GO NPs could protect them from oxidative stress injury. In addition, GO could absorb ECM proteins from serum due to its unique surface chemistry. The MSCs-GO-microgel composite significantly decreased the infarct size and improved cardiac function in mice with myocardial ischemia by improving the viability MSCs (Fig. 15A–B).

Recent studies have found that GO could also induce M2 macrophage polarization. For example, Han et al. prepared a macrophage-targeting/polarizing GO nanocomposite (MGC), which encapsulated IL-4 plasmid DNA (pDNA) [255]. GO exerts dual functions: direct ROS scavenging and delivery of IL-4 pDNA to cardiac tissue. Notably, scavenging of ROS can induce macrophage polarization from M1 to M2. MGC reduced the

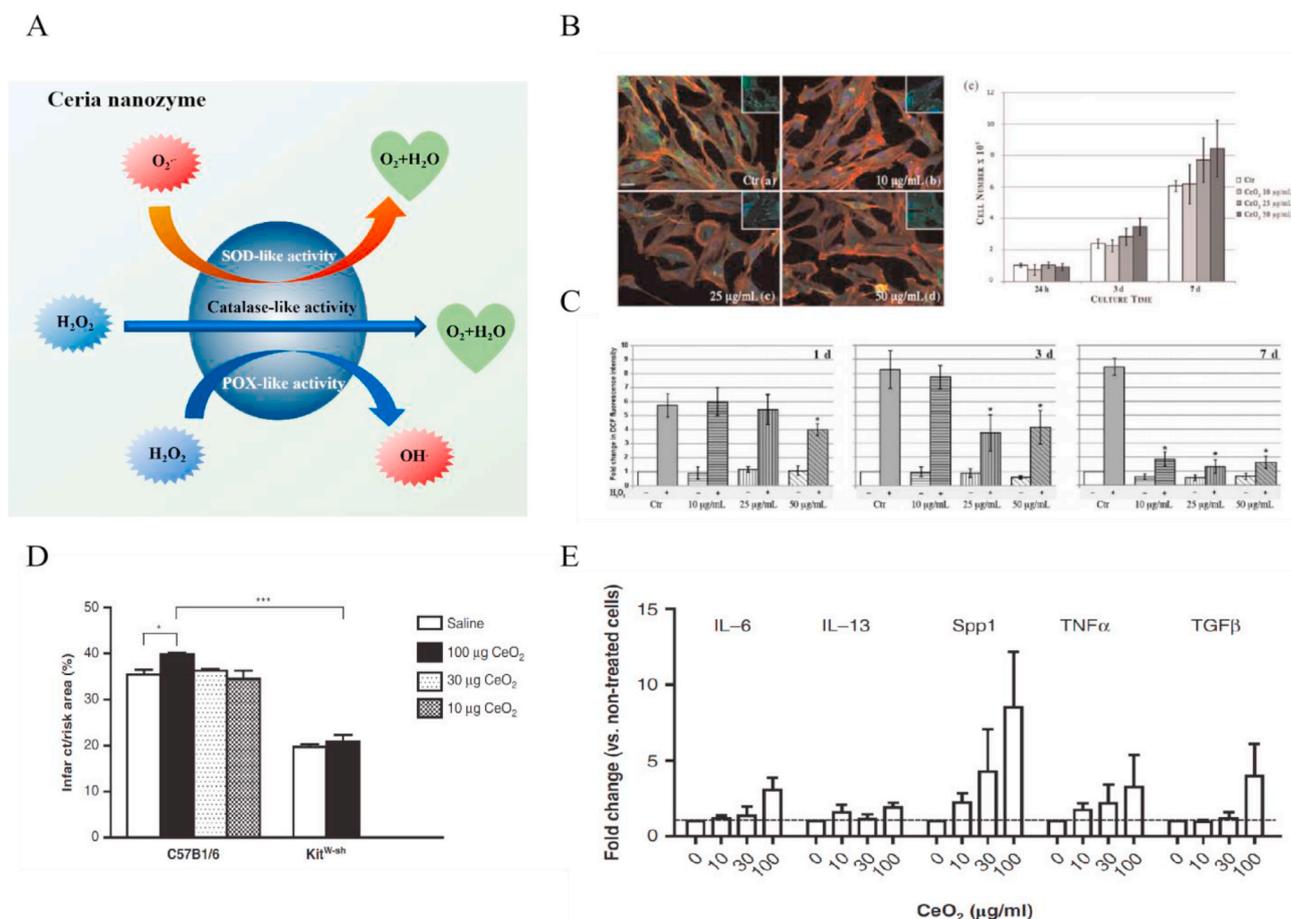


Fig. 14. (A) Ceria nano-enzyme acts as efficient antioxidant SOD-mimetics ($\text{Ce}^{3+} + 2\text{H}^+ + \text{O}_2^- \rightarrow \text{H}_2\text{O}_2 + \text{Ce}^{4+}$) or catalase-mimetics ($\text{Ce}^{4+} + \text{H}_2\text{O}_2 + 2\text{OH}^- \rightarrow \text{O}_2 + 2\text{Ce}^{3+} + \text{H}_2\text{O}$). (B) Ceria nano-enzyme does not alter CPC phenotype and growth, as shown by phalloidin staining of actin filaments and CPCs counts. (C) The effect of ceria nano-enzyme on ROS production under H_2O_2 exposure. Reprinted with permission from Ref. [223]. (D) Comparison of the size of myocardial infarction in C57BL/6 exposed to 10, 30 and 100 μg of ceria and Cg-Kit^{W-sh} mice exposed to 100 μg of ceria. (E) Ceria increased mRNA expression of several inflammatory cytokines including IL-6, IL-13, TNF- α , TGF- β and Spp1. Reprinted with permission from Ref. [226].

ROS levels in mouse bone marrow-derived macrophages, and promoted M2 macrophage differentiation. MGC also promoted cardiac repair as indicated by the upregulated expression of connexin 43 (Cx43) and downregulated expression of phosphorylated c-Jun N-terminal kinase (pJNK). Cx43 is responsible for transmitting information between cardiomyocytes, while the activation of pJNK induced by intracellular ROS is closely associated with the decreased expression of Cx43. Moreover, IL-4 is an anti-inflammatory cytokine, and could selectively induce M2 macrophage polarization. Therefore, GO and IL-4 pDNA exert synergistic effects on inducing the polarization of M1 macrophages into M2 macrophages. Collectively, the MGC can effectively control inflammatory and oxidative injury after myocardial ischemia. Particularly, MGC restored the structure and function of the cardiovascular system, including ventricular remodeling and vessel density (Fig. 15C–E).

The antioxidant activity of fullerenes largely depends on their electron donation/acceptance capacity through the three-dimensional π -conjugated structure [250,256,257]. Recently, Hao et al. prepared an injectable fullerene/alginate hydrogel to load brown adipose-derived stem cells (BADSCs) [258]. Fullerene is a fullerene derivative with excellent water solubility, and can be effectively scavenge ROS. The prepared fullerene hydrogels exhibited superior compressive strength and injectable performance. BADSCs have high cardiomyocyte differentiation potential for myocardial regeneration. The fullerene/alginate hydrogel could alleviate oxidative stress damage, promote the viability of BADSCs, and induce myocardial differentiation of BADSCs. The combination of nanotechnology and cell therapy

represents a promising therapeutic strategy [229,259].

5.2.3. DNA nano-enzymes

DNA nanomaterials have also shown great potential in the treatment of myocardial ischemia reperfusion injury, with superior biocompatibility, stability, and easy absorption by cells compared with traditional nanomaterials [63]. Recently, Zhang et al. developed tetrahedral DNA nanostructures (TDNs) with antioxidant and antiapoptotic effects to attenuate the myocardial injury [63]. The antioxidant and antiapoptotic therapeutic effects of TDNs are primarily originated from the activation of the Akt/Nrf2 signaling pathway. Nrf2 is an important nuclear transcription factor, which can upregulate the expression of antioxidant genes to enhance cell survival under oxidative stress [54]. Furthermore, the activation of Akt can promote Nrf2 gene expression to induce expression of antioxidant enzyme heme oxygenase-1 (HO-1) [260]. In addition, HO-1 could decrease the expression of ROS-induced proapoptotic proteins. Collectively, the antioxidant and antiapoptotic activity of TDNs render it a promising novel therapeutic agent against myocardial ischemia reperfusion injury.

5.3. Biomimetic NPs for myocardial ischemia reperfusion injury

Theoretically, the intelligence of these smart NPs depends largely on their chemical and physical changes to stimuli, which are generally “mechanical” and fundamentally different from biological intelligence. Although these traditional smart NPs have improved the efficacy of

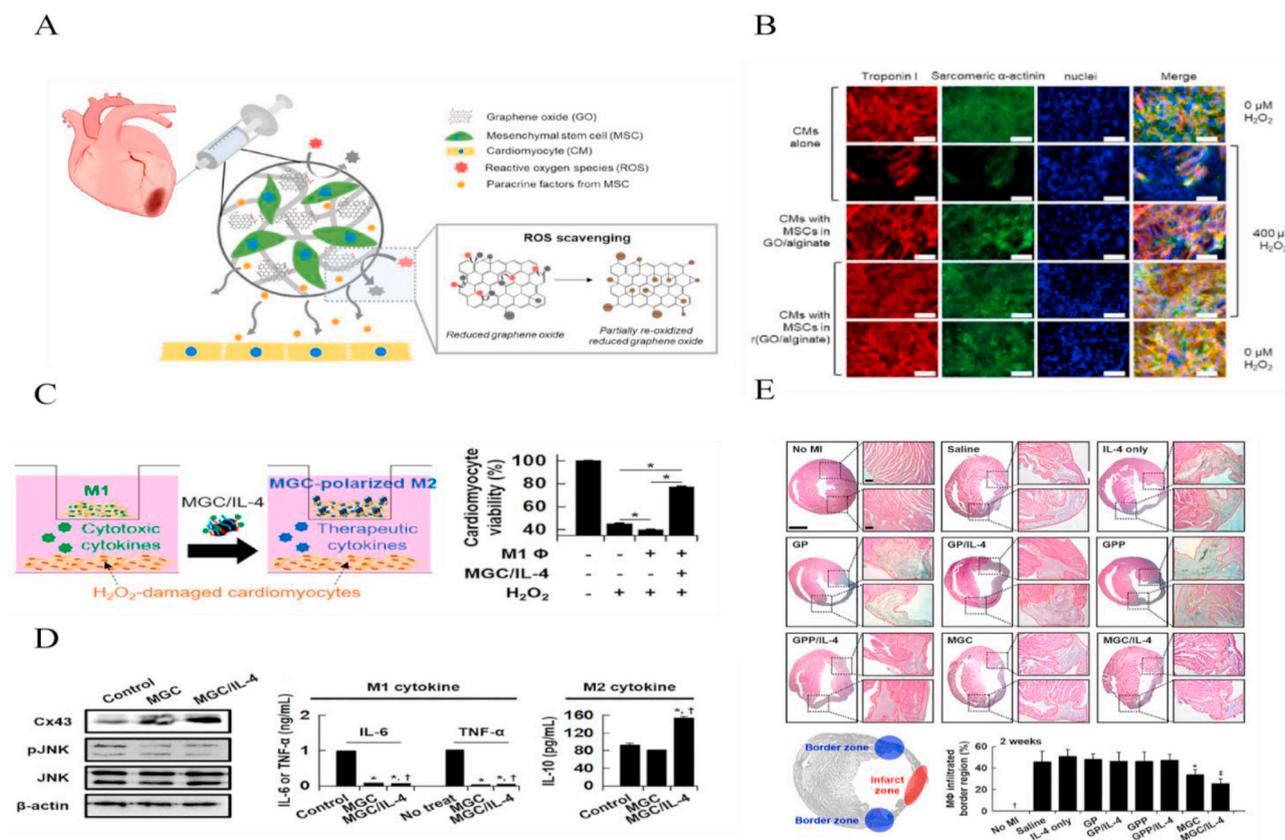


Fig. 15. (A) Schematic illustration of the functions of GO/alginate microgel embedding MSCs for cardiac tissue repair for ischemia reperfusion injury treatment. (B) Immunostaining of cardiomyocytes for troponin I (red) and sarcomeric α -actin (green) (cardiac marker), suggesting that GO/alginate or r(GO/alginate) helped differentiation potential of cardiomyocytes co-cultured. Reprinted with permission from Ref. [161]. (C) The cardioprotective effect of MGC/IL-4 pDNA-polarized M2 macrophage as indicated by the viability of H_2O_2 -treated cardiomyocytes after coculture with M1 macrophages or MGC/IL-4 pDNA-polarized M2 macrophages for 48h. (D) The expression of Cx43 and pJNK/JNK and the secretion of anti-inflammatory and inflammatory cytokines in LPS-stimulated M1 macrophage after MGC or MGC/IL-4 treatment. (E) Hematoxylin-eosin staining was used to assess the area of inflammatory cell infiltration near the border zone. Further, the quantitative densitometry analysis indicated that MGC or MGC/IL-4 group showed a significant reduction in the inflammatory cell population. Reprinted with permission from Ref. [255].

nanotherapy to a certain extent, they cannot meet the needs of the complex microenvironment under ischemic injury [261–265]. Biomimetic nanomaterials simulate the design and function of specific cells, and can intelligently respond to the pathological microenvironment to effective treatment of myocardial ischemia reperfusion injury.

Exosomes are nano-sized membrane vesicles derived from a variety of cell types, and are thought to act as important intermediate carriers for intracellular communication and material exchange [266–268]. Specially, Exosomes secreted by MSCs contain a complex cargo including various proteins and miRNAs with the potential to be a source of cardiac regeneration and resistance to oxidative stress. Moreover, exosomes therapy avoids the defects of MSCs transplantation, such as low percentage of local engraftment and survival rate. MSCs-derived exosomes have been demonstrated their beneficial effects for myocardial ischemia reperfusion injury therapy [266–270]. For example, Liu et al. found that MSCs-derived exosomes significantly reduced cardiomyocyte apoptosis and the myocardial infarct size by inducing cardiomyocytes autophagy via targeting AMPK and Akt pathway [267]. MSCs-derived exosomes also have good biocompatibility and are adopted as nanocarrier for various molecules such as nucleic acid drugs. For example, Wang et al. prepared miRNA-19a/19b-loaded exosomes for the treatment of myocardial infarction [271]. miRNA-19a/19b can induce the proliferation of cardiomyocytes and promote cardiac regeneration [266]. Therefore, encapsulation of miRNA-19a/19b into MSC-derived exosomes could effectively accumulate in the ischemic myocardium, and significantly improve cardiac functional recovery.

Very recently, melatonin-biosmart NPs with microenvironment targeting and self-adaptive capacity (MTSNP) have been developed by imitating the structure and function of mitochondria for the treatment of myocardial ischemia [261]. Melatonin has antioxidant function and can maintain the stability of mitochondrial membrane. MTSNP is composed of the double PLGA shells, which correspond to the two-layered membranes of mitochondria (Fig. 16). The melatonin-loaded cores are like the mitochondrial matrix to simulated the cell-protective mechanism of mitochondria. The circular DNA located between the two PLGA shells is like the function of mitochondrial DNA. After intramyocardial injection of MTSNP, melatonin was rapidly released from MTSNP to inhibit Cyt c release by binding to melatonin receptor I on mitochondrial membrane, which effectively prevent cardiomyocytes apoptosis at the acute stage of ischemia. And then, the circular DNA could sense hypoxia and produce VEGF for revascularization. MTSNP treatment could decrease infarct size, and induce revascularization in the mouse model of myocardial ischemia.

6. Summary and outlook

In the past decades, numerous attempts have been made to improve the efficacy of therapeutic agents for myocardial ischemia reperfusion injury [8,65]. Unfortunately, numerous cardioprotective drugs and cell therapies have failed to show benefits in the treatment of ischemic regions due to the intrinsic limitations [9]. The presence of high levels of ROS is the core factor in myocardial ischemia reperfusion injury. ROS

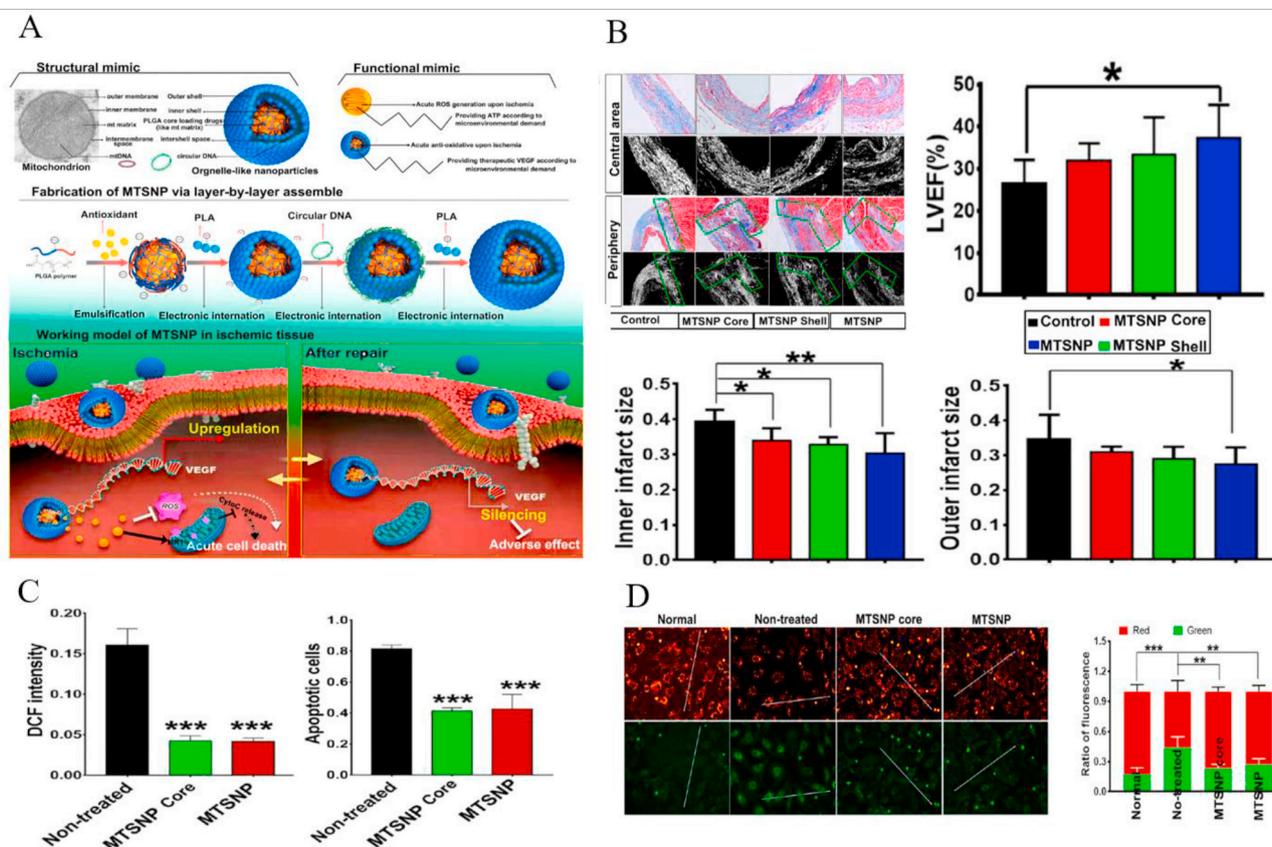


Fig. 16. (A) Schematic illustration of the preparation of MTSNPs and their working mechanism in ischemic myocardium. (B) MTSNPs improved the structural and functional injury in ischemic myocardium, including myocardial fibrosis, left ventricular ejection fraction (LVEF), and infarct size. (C) The protection of MTSNPs on cardiac cells by MTSNPs *in vitro*, including decreasing the levels of ROS and cell apoptosis. (D) The protective roles of MTSNPs on mitochondria measured by JC-1 staining. Reprinted with permission from Ref. [261].

from different sources can induce damage to cardiac tissue by directly affecting material and energy metabolism and inducing myocardial cell death. Therefore, the scavenging of ROS can be regarded as a promising strategy for suppressing myocardial ischemia reperfusion injury [8,28,65]. Nanomedicines have shown great potential in overcoming these limitations and provided a new direction for ischemic therapy. The application of nanomaterials in myocardial ischemia reperfusion injury could be divided into four aspects: (i) nanocarriers, (ii) antioxidant Nano-enzymes, (iii) biological nanomaterials, (iv) smart NPs [28,116,261,266]. Nanomedicines have some unique effects (like size effects, surface effects, and interface effects, etc.), and exhibit many excellent properties and new functions. Specially designed nanomaterials have the properties of artificial antioxidant enzymes and can be used to eliminate a variety of ROS in myocardial infarction reperfusion injury. In addition, Nanomedicines can significantly overcome the short blood circulation time and poor efficacy of small molecule drugs, protein drugs, and nucleic acid drugs. The selective accumulation of nanomedicines in the myocardial infarction is realized through myocardial damage site targeting and inflammation targeting to reduce oxidative damage, eliminate inflammation, and repair damaged myocardial tissue more effectively. Encouragingly, initial success results have obtained from nanotechnology-based cardiac protection.

However, the challenges regarding the translation from the basic research on nanotherapies to clinical trials remain to be solved. First, all current research on ROS-based nanotherapies is done at the small animal mode. There are huge differences between small animal models and patients. For example, most animal models of myocardial infarction reperfusion are made with young animals. These young animals usually have very good physical functions, such as sound immunity and strong repair ability. Most patients with myocardial infarction are elderly

people, and their repair ability is generally weak. Some of patients have basic diseases such as high blood pressure and diabetes. These ROS-based nanotherapies need to be further optimized for clinical application based on fully understanding the pathological and physiological differences between patients and small animals. Secondly, the nanotherapies are limited by the potential toxicity for myocardial ischemia reperfusion injury therapy [224,272,273]. For example, the modification of the size, shape, and surface of metal Nano-enzymes needs to be further optimized. For example, the 60 nm gold Nano-enzyme has demonstrated relatively low cytotoxicity compared with the 10 nm and 30 nm gold Nano-enzyme [199,274]. Furthermore, the delivery of therapeutic agents by nanocarriers remains at an early stage, and there are some shortcomings in practical applications. The controlled and reproducible manufacture of nanomedicines in terms of the subtle variations in size, stability, solubility, drug release, and sterility in the manufacturing process, which result in uncontrolled efficacy or unwanted toxicity, remains a challenge. Meanwhile, the introduction of novel stimuli-responsive nanocarriers for the delivery of therapeutic agents generally complicates the development process. The dosage, time, and administration route of drugs loaded in nanocarriers also need to be further optimized for their practical applications [275]. Improving the delivery efficacy of nanocarriers is also important for their clinical applications. In conclusion, further investigations are warranted to optimize the application of nanomaterials in the treatment of myocardial ischemia reperfusion injury [276].

Declaration of competing interest

The authors declare no conflict of interest, financial or otherwise.

Acknowledgements

This work was supported by the National Natural Science Foundation of China, China (No.21974134, 81974508, 81673492, 81873581) and Innovation-Driven Project of Central South University (No. 202045005), Special Science and Technology Plan of Changsha City. (No. kq2001048), Key Research Project of Ningxia Hui Autonomous Region (Major Project) (2021BEG01001).

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