

# **Animal models used in the research of nanoparticles for cardiovascular diseases**

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**Abstract:** Cardiovascular disease (CVD) is the leading cause of mortality and morbidity worldwide. Tremendous progress has been made in the prevention and treatment of CVD, however there are still lots of limitations and new technology is needed. Nanoparticles has been studies recently for CVD due to their nano scale size and unique properties, and hold a potential to be a novel therapy for the treatment. To test the safety and effectiveness of drug-loaded nanoparticles for CVD prior to human studies, animal disease models are unavoidably needed. This review summarized the animal models used in the research of nanoparticles for CVD and provided a generic picture of current use of CVD animal models according to the different type of diseases which should be prioritized when considering the application of nanoparticles in treating CVD. This review would be useful resources not only for life science researchers and clinicians but also for those from chemistry and materials sciences background who may not have a systematic knowledge about CVD animal models.

**Key words:** animal model; cardiovascular disease; nanoparticles; drug delivery

## **1 Introduction**

Cardiovascular disease (CVD), including coronary artery disease, peripheral vascular disease, congestive heart failure and atrial fibrillation, is the leading cause of mortality and morbidity worldwide<sup>1</sup>. Substantial improvements have been achieved in life-expectancy and living quality due to better prevention and enhanced treatment for CVD, but there is still an urgent need for the drug innovation. Nanoparticles, defined as heterogeneous group of substances that vary in size (10–100 nm) have emerged as a potential candidate for drug delivery system. Nanoparticles possess several unique properties such as enhanced biocompatibility, reduced toxicity and prolonged retention time<sup>2</sup>. Besides, certain nanoparticles can serve as a drug due to their anti-inflammatory and antioxidant effects<sup>3</sup>. In this review, we summarized *in vivo* preclinical studies of nanoparticles for CVD using various animal models. These established animal models provided useful information to guide further experiments particularly when designing and choosing nanoparticles and would be useful resources not only for life science researchers and clinicians but also for those from chemistry and materials sciences background who may not have a systematic knowledge about CVD animal models<sup>4</sup>.

## **2 Opportunities and challenges of nanoparticles for CVD treatment**

The epidemiological transition in the 20th century was marked by a significant decrease in deaths and disability from communicable diseases and an increase in non-communicable diseases (NCDs). Among NCDs, CVD is the leading cause of mortality and morbidity worldwide and accounts for approximately one third of all deaths globally. According to a newly published data, prevalent cases of total CVD nearly doubled from 271 million in 1990 to 523 million in 2019, and the number of CVD deaths steadily increased from 12.1 million in 1990 to 18.6 million in 2019<sup>5</sup>. Both genetic and environmental factors are implicated in the development of CVD, which makes the treatment even more complicated.

Developing relevant CVD drugs is the common strategy for treatment. Aspirin, statins,  $\beta$ -blockers, angiotensin converting enzyme inhibitors /angiotensin receptor blockers and other drugs have been validated in many large clinical trials to improve clinical outcomes in individuals with stable CVD<sup>6</sup>. Further development on novel therapeutics such as using a delivery system to improve drug half-life and reduce side effects becomes a hot research topic. As the molecular processes underlying CVD become

better defined, there are growing studies to intervene CVD using gene therapy. However, nuclear acid is easy to be identified and diminished and requires a carrier to protect them interacting with internal environment. Finding a delivery machine also seems a logical tool to bring gene therapy to fruition<sup>7</sup>.

Nanoparticles including inorganic nanoparticles, liposomes and extracellular vesicles seem to be a good choice for this purpose. The nano scale of nanoparticles enable themselves to cross natural barriers and enter into target cells more easily. Hence, drugs wrapped in nanoparticles could protect themselves from interactions with the surrounding environment and degradation, and further enhance the concentration in the targeted tissues. Additionally, nanoparticles can achieve controlled drug release by constructing a release system in response of internal stimuli such as pH, redox state and the presence of biomolecules as well as external stimuli such as light and magnetic field<sup>2</sup>. For example, lipid nanoparticles are the carriers most used in clinical trials for therapeutics, due to their pronounced advantages over other delivery systems such as easy preparation, high biocompatibility and biodegradability. Unfortunately, being synthetic and quite “unnatural”, lipid nanoparticles can be toxic and induce immune response at high doses, particularly in the condition where the required dose is high or frequent application is required. However, these effects could be overcome by selecting proper lipid components<sup>8,9</sup>. Up to now, several nano-formulations of lipid nanoparticles have been approved for human use. For example, BNT162b2, a kind of vaccine against COVID-19 and adopted lipid nanoparticles as vehicles, have been put into clinical use<sup>10</sup>. All these predict a promise application of using nanoparticles for the treatment of CVD.

### **3 Animal models for studying nanoparticles as a treatment for CVD**

Animal models remain the best tool to investigate the novel pharmacological treatment of nanoparticles for CVD. The complicated nature of CVD present challenges to test the application of nanoparticles in treating CVD. Better understanding of all animal models used is therefore the key in the study. In this paper, the most commonly used CVD animal models and their usages in testing nanoparticles applications for the treatment of CVD are reviewed. We focused on the animal models according to the type of CVDs and explained the complexity of choosing a proper animal models to best mimic the pathological conditions of CVDs. We did not exam and compare which nanoparticle should be chosen and their potentially complicated properties when

interacting with animals due to very random data in the limited applications. However, the properties of nanoparticles, especially when interacting with physiological system is the important aspects to be investigated in each individual study. Interdisciplinary collaboration with experts in chemistry and materials would greatly improve our understanding of how to best use nanoparticles to treat CVDs in this aspect.

The common animal models are mainly created based on cardiovascular pathological conditions, such as hypertension, atherosclerosis, myocardial infarction and myocardial ischemia-reperfusion etc (Table 1), and the availability and feasibility of choice of animals for these cardiovascular pathological conditions.

Table1 Common animal models for CVDs

Cardiovascular disease or pathological conditions	Intervention	Typical features
Hypertension	spontaneous; fructose; dexamethasone; DOCA	Elevated blood pressure
Atherosclerosis	high cholesterol; continuous intimal injury through catheter, balloon angioplasty or nitrogen exposure	lipid abnormality with elevated triglyceride and other liver enzymes; atherosclerotic plaques on the wall of arteries featured by foam cells
Myocardial infarction	Permanent LAD ligation; ISO	elevated myocardial enzymes and inflammatory cytokines; reduced ejection fraction; macroscopic appearance of scar; obvious histological alterations such as fibrosis and left ventricular remodeling.
Myocardial Ischemia-Reperfusion	partial LAD ligation and blood reperfusion	similar to MI model
Pulmonary arterial hypertension	monocrotaline; hypoxia;	thick wall of the pulmonary peripheral arteries; right ventricular remodeling
Arrhythmia	calcium chloride; barium chloride;	disorders of heart rhythm
Abdominal aortic aneurysm	chronic infusion of angiotensin II into low density-lipoprotein receptor-deficient (LDLr <sup>-/-</sup> ) mice in combination with a high-fat diet; elastase infusion of the aorta;	macroscopic appearance of the abdominal aorta; macrophage activation within the aortic media

Abbreviations: DOCA, deoxycorticosterone acetate-salt; LAD, anterior descending coronary artery.

Both small animals such as mice, rats and large animals such as rabbits, canines, swine,

sheep, non-human primates are used in the study and they have their own advantages and disadvantages. For example, small animals are easier to handle and house, have a shorter gestation time, and have lower maintenance cost than larger animal models<sup>4</sup>. These features make small animals the most used model for cardiac physiology and pathology, genetics, pharmacology, and long-term survival studies. In addition, the availability of transgenic and knockout strains and little difficulty in creating new genetic modifications make the mouse one of the most attractive models for research. Nevertheless, large animals have similar cardiovascular system to human at both the organ and cellular levels, thus transitions from the researches in those large animals to human researches are more likely to repeat and the results obtained are also ready to use. In the following sections, we summarise all those animal models according to the type of CVD and how they have been used to test the applications of nanoparticles in treating CVD.

### **3.1 Hypertension animal models**

Hypertension is known for persistently elevated blood pressure in the arteries. High blood pressure is a major risk factor for many diseases such as coronary artery diseases and stroke which usually doesn't cause obvious symptoms. Hypertension is classified into essential hypertension and secondary hypertension. Human essential hypertension is a complicated disease involved both genetic and environmental factors. It has been demonstrated that genetic predisposition, excessive salt intake, over-stress and hyperactivation of renin-angiotensin-aldosterone system are involved in the development of hypertension. Therefore, hypertension animal models are established mainly based on the above etiology.

There are four major hypertension models produced so far. Spontaneously Hypertensive Rat (SHR) is the internationally recognized animal model which is closest to human essential hypertension and has been widely used in basic research on essential hypertension and antihypertensive drug screening<sup>11</sup>. Fructose<sup>-12</sup>, dexamethasone<sup>-13</sup> and DOCA<sup>-14</sup> induced hypertension model are all secondary hypertension models, which can mimic hypertension combined with insulin resistance, hypertension caused by activation of the mineralocorticoid receptor and elevated aldosterone, respectively. The hypertension animal model is characterized with elevated blood pressure.

As mentioned above, human essential hypertension is a complex disease associated with both genetic and environmental factors. Therefore, one single animal model may not be able to fully mimic the hypertensive responses and is therefore not sufficient to explain the antihypertensive effects of drugs when using nanoparticle as its delivery system. Therefore, various hypertension animal models are needed and results from these different models need to be compared before any conclusion can be drawn.

The current hypertension animal models used in the study of nanoparticles for the treatment of hypertension was summarized in Table 2. The nanoparticles combined with antihypertensive drugs such as nifedipine and lercanidipine hydrochloride enhanced the drug efficacy by prolonging the retention time *in vivo*. But those effects have not been tested for a long period of time and this is the same to their toxicity and side effect. Generally, antihypertensive drugs need to be continuously used and even for the rest of life. The animal models used at the moment normally last for couple of months as its maximum. Therefore, conclusion obtained should be further tested and verified. Nevertheless, these applications suggested that it was feasible to using drug-loaded nanoparticles for the treatment of hypertension.

**Table2. Hypertension animal models used in the study of nanoparticles for the treatment of hypertension**

Type	Animal	Nano-delivery system	Carried drugs	Administrati on route	Measure ment	Results	Refs
Spontaneously Hypertension Model	Male, adult rats	Polymeric nanoparticle (PCL, PLAGA, Eudragit RL/RS)	Nifedipine	Oral	systolic arterial blood pressure by tail-cuff method	BP within normal range after 10h of dosing with all three NPs while PEG solution failed to achieve such sustained effect	15
	Male rats	PLA nanoparticles	Aliskire	by gavage		BP was lower in both powdered and nanoparticle-loaded aliskiren groups with a more pronounced effect in the latter case. Only nanoparticle-loaded aliskiren increased the expression of nNOS along with increased NOS activity in the heart (by 30%).	16
Fructose-Induced Hypertension Model	Wistar rats	SLN	Isradipine	oral		Isradipine nanoparticles showed a decrease in systolic blood pressure for 36h, while suspension showed a decrease in systolic blood pressure for only 2h.	17
	Male SD rats	NLC	Lercanidipine hydrochloride	Oral		NLCs released lercanidipine hydrochloride for a longer period of time in a controlled manner than plain drug.	18
Dexamethasone -Induced Hypertension Model	Unisex Wistar rats	SNEDDS (SNEOF and CSNEOF)	Olmесartan Medoxomil	Intravenous injection		After 48 h, rats were found normotensive (BP < 130 mm Hg) with SNEOF and CSNEOF, while rats with marketed formulation didn't show such lasting effect. .	13
DOCA-Induced Hypertension Model	Rats	lecithin/chitosan nanoparticles	HCT and HCT-β-CD			HCT and HCT-β-CD loaded nanoparticles demonstrate 1.5-fold percentage decrease in systolic blood pressure and a prolonged duration of action.	14
<p>SLN: DOCA, deoxycorticosterone acetate-salt; Solid lipid nanoparticles; NLC: Nano structured lipid carriers; HCT: Hydrochlorothiazide; HCT-β-CD: hydro-chlorothi-azide complexed with β-cyclodextrin</p>							

### 3.2 Atherosclerosis animal models

Atherosclerosis, characterized by sub-endothelial accumulation of fatty substances called plaques, is the main underlying cause of myocardial infarction and stroke<sup>19</sup>. It usually takes decades to form plaques with various inflammatory cytokines and immune cells contributing to the process. Rats and mice were inherently resistant to atherosclerosis. Therefore, transgenic model such as LDLR<sup>-/-</sup> mouse<sup>20</sup> and apoE<sup>-/-</sup> mouse were established to develop atherosclerosis through gene knockout technology. However, gene knockout animals are rather expensive and may impose a substantial financial pressure to any research as well as limit the number of samples obtained. The rabbit is used as the ideal alternate animal for experimental atherosclerosis by high-cholesterol diet or repeated or continuous intimal injury through catheter, balloon angioplasty or nitrogen exposure<sup>4</sup>. The rabbit atherosclerosis model is a no plaque rupture model while porcine atherosclerosis models are valid to investigate the plaque rupture and restenosis. The high-cholesterol diet induced atherosclerosis models present lipid abnormality with elevated triglyceride and other liver enzymes, and atherosclerotic plaques on the wall of arteries featured by foam cells. The external injury induced atherosclerosis models exhibit atherosclerotic plaques without abnormal lipid profiles.

Table 3 summarized the studies using nanoparticles for the treatment of atherosclerosis. Some modified nanoparticles like oligonucleotide functionalized nanoparticles could be specifically recognized by foam cells, which may serve as a potential delivery vehicle and therefore is worth for future study<sup>21</sup>. Superparamagnetic iron oxide nanoparticles for example could reach the target region under external magnetic gradient. This opens up the opportunity for further investigations aiming both to improve targeting efficacy and to develop the potential “magnetic drug targeting”-based approaches to therapy of arterial injuries and/or atherosclerotic plaques<sup>22</sup>. In most of the study, drug-loaded with nanoparticles showed reduced lesion size and inflammatory responses than free drug in atherosclerosis animal model, suggesting that nanoparticles may be a promising strategy to prevent atherosclerosis. However, there are also some studies which didn't show any anti-atherogenic effects with administration of nano-formulated drugs while enhanced accumulation of drugs in the desired regions were observed<sup>22</sup>. Therefore, selecting optimal drugs turns to be crucial and important.



**Table 3. Atherosclerosis animal models used in the study of nanoparticles for the treatment of atherosclerosis.**

Type of Atherosclerosis Model	Animal	Nano-delivery system	Carried drugs	Administration route	Measurement	Results	Refs
Gene Knockout mice	ApoE null C57Bl/6 mice	Oligonucleotide functionalized nanoparticles	None	intravenous injection	1) lipid analysis; 2) immunohistochemistry;	Oligonucleotide functionalized nanoparticles were specifically recognized by the scavenger receptors on lipid-laden foam cells, providing a strategy for targeting atherosclerotic lesions	21
	Ldlr <sup>-/-</sup> mice	(PLGA-b-PEG) copolymer	LXR agonist GW3965	retro-orbital injection		NP-LXR was more effective than free GW3965 at inducing LXR target gene expression and suppressing inflammatory factors in macrophages. Treatment with NP-LXR over two weeks markedly reduced the CD68-positive cell (macrophage) content of plaques (by 50%) without increasing total cholesterol or triglycerides in the liver and plasma.	23
	ApoE <sup>-/-</sup> mice	lipid nanoparticles	siCCR2	intravenous injection		Lipid nanoparticles encapsulated siCCR2 showed a marked reduction of inflammatory Ly-6C <sup>high</sup> monocytes, 46% reduced presence of myeloid cells, and a 38% reduction of lesion size in the aortic root.	24
Atherosclerosis model induced by balloon injury, high cholesterol diet or their combination.	Rabbit	Lipid nanoparticles	DTX	intravenous injection		LDE-DTX treated group showed reduced atheroma area and lowered expression of pro-inflammatory markers compared to controls, without hematological, hepatic or renal toxicity consequent to LDE-DTX treatment.	25
	Rabbit	superparamagnetic iron oxide nanoparticles	Dexamethasone	intra-arterial infusion		Enhanced inflammatory burden in the plaques, increased macrophage content and larger intima-media thickness were observed in animals treated with SPION-DEXA compared with controls.	22
PLGA-b-PEG: poly(lactide-co-glycolide)-b-poly (ethylene glycol); LXR:Liver X receptor; DTX, docetaxel							

### **3.3 Myocardial Infarction animal models**

Myocardial infarction (MI) occurs when blood flow decreases or stops at certain part of the heart, causing damage to cardiomyocytes. MI is a life-threatening disease and can cause sudden death. Victims survived from MI often suffer from adverse ventricular remodeling, which is also known as heart failure. Therefore, it's essential to explore new therapy strategies to rescue the ischemic cardiomyocytes and attenuate ventricular remodeling. It has been revealed that myocardial hypertrophy, fibrosis, inflammation, mitochondrial dysfunction and autophagy/apoptosis are all involved in the progression of post-infarct ventricular remodeling. Using drug delivery system of nanoparticles carrying with cardioprotective agents to target any of the mechanisms above will benefit the dying cardiomyocytes. To examine their effectiveness, appropriate animal models need to be chosen to imitate human myocardial infarction as much as possible. Surgical ligation MI model and isoproterenol induced MI model are current well received models.

The surgical ligation MI model is made by ligating the left anterior descending coronary artery (LAD). Rat is dominated used in the respect of heart damage because, while rats share many of the advantages with mice, their larger size greatly facilitates surgical operations. The mortality in surgical ligation MI models are higher than other non-traumatic MI models because the animals receiving thoracotomy often die from complications such as pneumothorax. However, after training and practice, the ligation method has higher stability than other methods. The myocardial infarction is more thoroughly, and the size of the infarct size is positively correlated with the height of the ligation site.

Isoproterenol (ISO) induced MI model mimics sympathetic activation in the pathogenesis of myocardial infarction. ISO induced MI model is widely adopted in promoting chronic myocardial infarction due to the lower mortality rate and less complexed procedure compared to the surgical ligation MI model. However, the infarct area caused by this method occasionally shows non-necrotic myocardium. Besides, the infarct area mainly distributes on the left ventricular wall and septum, especially near the apex. This method is also used for establishing the heart failure model and hyperthyroid heart disease model<sup>3</sup>.

The preferred large animal model of heart damage is the pig, because the collateral coronary circulation and arterial anatomy of pigs and humans are very similar and infarct size can be accurately predicted. This MI animal models present elevated myocardial enzymes, reduced ejection fraction and obvious histological alterations such as fibrosis and left ventricular remodeling.

Table 4 briefly summarized the MI animal models used in the study of nanoparticles for the treatment of acute myocardial infarction or post-infarct heart failure. Improved cardiac function and decreased infarct size were noticed in the MI animal model treated with empty nanoparticles or drug -loaded nanoparticles. Nucleic acid nanotherapies were investigated in MI animal model as well. Nox2-siRNA nanoparticles were observed to prevent upregulation of Nox2 and significantly recover cardiac function in mice receiving LAD ligation. In the past few years, several cardiac gene therapy clinical trials were conducted with aim at inducing therapeutic angiogenesis in the ischemic heart. However, the efficiency of cardiac gene delivery remains a major hurdle preventing success<sup>26</sup>. Nanoparticles could address a breakthrough in delivery efficiency and hold a great promise for cardiac gene therapy.

**Table4. MI models used in the study of nanoparticles for the treatment of acute myocardial infarction or post-infarct heart failure**

Type of MI model	Animal	Nano-delivery system	Carried drugs	Administration route/Dose	Measurement	Results	Refs
Surgical Ligation MI Model	Adult male C57BL/6 mice	polyketal nanoparticles	Nox2-NADPH oxidase siRNA	intramyocardial injection	1) electrocardiogram 2) echocardiogramphy 3) cardiac MRI 3) serum parameters measurement 4) histological analysis	Nox2-siRNA particles prevent upregulation of Nox2 and significantly recovered cardiac function.	27
	Male FVB mice	PLGA nanoparticles	Insulin-like growth factor	intramyocardial injection		PLGA-IGF-1 NPs was sufficient to prevent cardiomyocyte apoptosis, reduce infarct size, and improve left ventricle ejection fraction 21 days after experimental MI in mice.	28
	Male C57BL/6 mice	PLGA nanoparticles	Pitavastatin	intravenous injection		Treatment with Pitavastatin-NPs attenuated post-infarct left ventricular remodeling accompanied by a reduction of monocytes/macrophages in the heart, whereas pitavastatin solution treatment did not.	29
	Wistar rats	LDE	MTX	intravenous injection		LDE-MTX treatment achieved a 40% improvement in left ventricular systolic function and reduced the infarction size, myocyte hypertrophy and necrosis, number of inflammatory cells, and myocardial fibrosis.	30
	Sprague Dawley rats	PEG-modified SLNs	Sch B	intravenous injection		Treatment with Sch B loaded SLNs exhibited higher heart drug concentration and longer blood circulation time than the drug solution. MMP-Sch B SLNs also showed the best therapeutic efficacy by reducing the infarction size to the greatest extent	31
	Female	PLGA	FGF1	intramyocardial		PLGA nanoparticles formulated with CHIR99021 and	32

	York-shire swine	nanoparticles	and CHIR99021	injection		FGF1 provided an effective slow-release system for up to 4 weeks. Intramyocardial injection of CHIR + FGF1-NPs reduced infarct size by 20%–30% and preserved cardiac contractile function in I/R pig models.	
Isoproterenol Induced MI Model	Wistar albino rats	copper nanoparticles	None	oral		Low-dose copper nanoparticles and exercise training significantly prevented ISO-induced MI through preconditioning and GSK-3b inhibition.	33
	Adult male Wistar albino rats	gold nanoparticles	None	intravenous injection		Gold nanoparticles of 50nm diameter improved myocardial injury after ISO-induced myocardial infarction in rats.	34
	Male Wistar rats	cerium oxide nanoparticles	None	Intraperitoneal administration		Nano-ceria showed a promising ameliorative and prophylactic effect against cardiac toxicity compared to Captopril reference drug.	35
	Male BALB/c mice	nanoliposome	SMV	intragastric (i.g.) / and intraperitoneal administration		By i.p. administration, the SMV-Lipo at an equal SMV dose exhibited more noticeable inhibitory effects than the crude SMV on cardiac remodeling. In addition, SMV-Lipo administrated by either i.p. or i.g. more significantly improved the plasma SMV concentration than the crude SMV.	36
	adult male guinea pigs	poly lactic acid nanoparticle	curcumin and nisin	subcutaneous injection		Curcumin-nisin based nanoparticle showed significant cardio-protection in the guinea pig and was nontoxic.	37
<p>PLGA: poly D, L lactide-co-glycolide; PEG: polyethylene glycol; LDE: lipid core nanoparticles; SLN:solid lipid nanoparticles; MTX: Methotrexate; Sch B: Schisandrin B; SMV:Simvastatin</p>							

### 3.4 Myocardial Ischemia-Reperfusion animal models

Myocardial ischemia-reperfusion (I/R) injury occurs when blood supply returns to heart after a period of ischemia<sup>38</sup>. The possible mechanisms of myocardial I/R injury are: 1) the outbreak of oxygen free radicals; 2) Ca<sup>2+</sup> overload; 3) mitochondrial dysfunction; 4) inflammation; 5) energy metabolism disorders. Myocardial I/R injury is a vital negative factor influencing the therapeutic efficacy for the MI victims who accept revascularization such as percutaneous transluminal coronary intervention (PCI) and coronary artery bypass grafting (CABG). The I/R animal model used is normally based on surgical ligation MI model. The difference is that left anterior descending coronary artery is partially ligated to achieve reperfusion in I/R model, while in MI model, it is completely ligated. The myocardial I/R animal model established by this method can better simulate human myocardial ischemia-reperfusion. Besides, isolated Langendorff I/R heart is often used for the *ex-vivo* study of myocardial I/R injury. Since the isolated heart is detached from nerve innervation and systemic humoral factors, and the heart rate, pre- and post-load, perfusion pressure and other factors can be controlled. It is therefore especially suitable to study the effects of some single factors on cardiac function and metabolism without interference effects of related factors. However, the conclusions from isolated I/R heart are worthy of consideration when extending to humans because this model is isolated from inner environment. Small animals such as mice and rats are admired by researchers to build myocardial I/R animal model and the reasons are similar to MI animal model. At present, there is no agreeable conclusion on the exposure time of ischemia and reperfusion because they are affected by the size of the animal and the tolerance of the organ or tissue to ischemia and hypoxia.

Table 5 briefly reviewed the I/R animal models used in the study of nanoparticles for the treatment of myocardial I/R injury. PLGA nanoparticles and other inorganic nanoparticles were tested either as empty or loaded with most used clinical drugs on these animal models. For majority of the study, reduced inflammatory responses, apoptosis and lesion size were observed in myocardial I/R animal models treated with nano-formulated drugs, indicating the potential of nano-drug delivery system for the treatment of I/R injury. For example, silver nanoparticles increased the level of cytokines and aggravated I/R injury. For the concern of safety, silver nanoparticles are not proper nanoparticles for the treatment of I/R injury.

**Table5. I/R animal models used in the study of nanoparticles for the treatment of myocardial I/R injury**

Type of IR model	Animal	Nano-delivery system	Carried drugs	Administration route/Dose	Measurement	Results	Refs
I/R Animal	Adult male Sprague-Dawley (SD) rats	PLGA nanoparticles	Pitavastatin	intravenous injection	1) electrocardiogram; 2) echocardiography; 3) serum parameters measurement; 4) histological analysis	Pitavastatin-NP induced phosphorylation of Akt and GSK3 $\beta$ , and inhibited inflammation and cardiomyocyte apoptosis in the IR myocardium.	39
	Male Sprague Dawley rats	silver nanoparticles (AgNP)	None	intratracheal instillation		AgNP increased circulating levels of several cytokines, which may contribute to persistent expansion of I/R injury.	40
	Male FVB mice	stock and PEG-modified polystyrene nanoparticles	None	intravenous injection		Nanoparticles with a core diameter in the 20–200 nm range were optimal for rapid passive targeting of the I/R-injured left ventricle.	41
	Male C57/B mice	PVAX and HPOX Nanoparticles	None	intraperitoneal injection		PVAX effectively suppressed the generation of ROS caused by I/R, and PVAX significantly reduced the level of NADPH oxidase (NOX) 2 and 4 expression, which favors the reduction in ROS generation after I/R.	42
	Adult male C57BL/6J mice	PLGA nanoparticles	Irbesartan	intravenous injection		Treatment with irbesartan-NP, but not with control nanoparticles or irbesartan solution inhibited the recruitment of inflammatory monocytes to the I/R heart, and reduced the infarct size via PPAR $\gamma$ -dependent anti-	43

					inflammatory mechanisms, and ameliorated left ventricular remodeling 21 days after I/R.	
Male C57BL/6J (wild-type; WT) and cyclophilin D knockout (CypD-KO) mice	PLGA Nanoparticles	Mdivi1	intravenous injection		Mdivi1-NPs had cardioprotective effect against I/R injury through inhibition of Drp1-mediated Bax translocation to the mitochondria, namely, through MOMP, even in mice lacking a CypD/MPTP opening.	44
Male C57BL/6J and cyclophilin D <sup>-/-</sup> mice	PLGA Nanoparticles	CsA	intravenous injection		Treatment with CsA-NP at the onset of reperfusion enhanced cardioprotection against I/R injury through the inhibition of mitochondrial permeability transition pore opening.	45
Male Wistar rats	silica nanoparticles	Adenosine	intravenous infusion		Immobilization of adenosine on the surface of silica nanoparticles decreased the infarct size in the rat model.	46
Male Sprague-Dawley rats	NIPAAm-MMA nanoparticles	Visnagin	intravenous injection		Visnagin-NP treatment induced cardioprotection, reducing the size of the MI and ameliorating cardiac dysfunction through the induction of autophagy and the inhibition of apoptosis.	47
Male Sprague Dawley rats	Plasm exosomes	None	intravenous injection		The exosome-rich fraction was powerfully cardioprotective in I/R rats.	48
Bama mini-	PLGA	Pitavastatin	intravenous		Pitavastatin-NP significantly reduced infarct	49



	pigs	nanoparticles		injection		size without obvious adverse side effects.	
Langen- dorff I/R Heart	Male Wistar rats	PGMA nanoparticles	AID Peptide	Via a recirculation System		Curcumin and the AID peptide in combination effectively reduced muscle damage, decreased oxidative stress and superoxide production in cardiac myocytes.	50
	Male C57BL/6J (wild-type) and cyclophilin D knockout (CypD-KO) mice	PLGA Nanoparticles	Mdivi1	intravenous injection		Mdivi1-NPs have cardioprotective effect against IR injury through inhibition of Drp1-mediated Bax translocation to the mitochondria, namely, through MOMP, even in mice lacking a CypD/MPTP opening.	44
	Male Sprague Dawley rats	Plasm exosomes	None	Via a recirculation System		The exosome-rich fraction was powerfully cardioprotective in isolated I/R hearts.	48
<p>PLGA: poly-lactic/glycolic acid; PGMA: poly (glycidyl methacrylate); Mdivi1: Mitochondrial division inhibitor 1; CsA: Cyclosporine A; NIPAAm-MMA: N-isopropylacrylamide and methacrylic acid;</p>							

### **3.5 Others animal models**

There are some other animal models adopted to study the effect of nanoparticles for CVD, such as pulmonary arterial hypertension model, arrhythmia model and abdominal aortic aneurysm model. These animal models are more specific targeted for individual CVD conditions. Overall, drug-loaded nanoparticles showed cardioprotective effects in these animal models. Prostaglandin I<sub>2</sub> and its analogues (such as beraprost sodium, BPS) are beneficial for the treatment of PAH. Intravenous administration of BPS-nanoparticles (once per week, 20 µg/kg) protected against monocrotaline-induced and hypoxia-induced pulmonary arterial remodeling and right ventricular hypertrophy. The extent of this protection was similar to that observed with oral administration (once per day, 100 µg/kg) of BPS alone<sup>51</sup>. The beneficial effects of BPS-NP on PAH animal models seem to be mediated by its sustained release and tissue targeting profiles. BPS-nanoparticles may be useful for the treatment of PAH patients due to reduced dosages and frequency of BPS administration.

### **4 Conclusion**

CVD animal models remain the best tool to understand the mechanism of human CVD and validate the novel pharmacological therapeutics. No single animal model can perfectly duplicate the human disease and there are other concerns to be considered when choosing animal models such as cost, infrastructure and the requirement for specialized personnel. Choosing a model that best reflect on the aspect of disease being investigated can certainly help to explore how nanoparticles can be best used for CVD. The effects of nanoparticles in various preclinical animal models of CVD approved that nanoparticles as a delivery vehicle can significantly improve the therapeutic efficacy of the drug and reduce doses and frequency of drug administration through sustained release and tissue-specific targeting. Nanoparticles are also ideal delivery carrier for the gene therapy. Therefore, nanoparticles possess a strong potential for clinical translation in CVD. However, establishing more reliable large animal models and choosing more effective therapeutic genes or drugs should be prioritized when testing the nanoparticles application.

### **Conflict of Interest**

The authors declare no conflict of interest.

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