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REVIEW ARTICLE

Nanomedicine for Gene Delivery for the Treatment of Cardiovascular Diseases

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Abstract: ***Background*:** Myocardial infarction (MI) is the most severe ischemic heart disease and directly leads to heart failure till death. Target molecules have been identified in the event of MI including increasing angiogenesis, promoting cardiomyocyte survival, improving heart function and restraining inflammation and myocyte activation and subsequent fibrosis. All of which are substantial in cardiomyocyte protection and preservation of cardiac function.

***Methodology*:** To modulate target molecule expression, virus and non-virus-mediated gene transfer have been investigated. Despite successful in animal models of MI, virus-mediated gene transfer is hampered by poor targeting efficiency, low packaging capacity for large DNA sequences, immunogenicity induced by virus and random integration into the human genome.

***Discussion*:** Nanoparticles could be synthesized and equipped on purpose for large-scale production. They are relatively small in size and do not incorporate into the genome. They could carry DNA and drug within the same transfer. All of these properties make them an alternative strategy for gene transfer. In the review, we first introduce the pathological progression of MI. After concise discussion on the current status of virus-mediated gene therapy in treating MI, we overview the history and development of nanoparticle-based gene delivery system. We point out the limitations and future perspective in the field of nanoparticle vehicle.

***Conclusion*:** Ultimately, we hope that this review could help to better understand how far we are with nanoparticle-facilitated gene transfer strategy and what obstacles we need to solve for utilization of nanomedicine in the treatment of MI.

Keywords: Myocardial infarction, cardiomyocytes, angiogenesis, inflammation, gene transfer, nanoparticles.

1. INTRODUCTION

Ischemic heart disease (IHD) is the leading cause of morbidity and mortality for decades [1]. Among IHD, myocardial infarction is the most serious one and directly leads to heart failure. Atherosclerosis is the fundamental pathological basis of IHD, resulting from endothelial injury [2, 3], lipoprotein sub-endothelium retention [3, 4], inflammatory infiltration and accumulation [5-7] and collagen deposition [8]. As atherosclerotic plaque grows, it narrows coronary arteries and limits blood supply to cardiomyocytes. Myocardial infarction happens when severe coronary stenosis blocks blood flow and deprives oxygen and nutrient supply. Reperfusion exposes cardiomyocytes under oxidative stress which further accelerates cell death. In response to cell apoptosis, innate immune cells, and subsequently, T lymphocytes and B lymphocytes infiltrate into the infarcted area for further cardiomyocyte destruction. For instance, following myocardial infarction, chronic β-adrenergic activation is a potent stimulator for the production of TNF-α, IL-1β, IL-6 and IL-18, all of which contribute significantly to myocardial damage and accelerate fibrotic progression [9]. Adult mammalian cardiomyocytes carry low proliferative potential and could not replace the dead cardiomyocytes [10]. Therefore, insufficient healing results in scar formation and heart failure. By far, accumulated studies have demonstrated how cardiomyocytes are subjected to apoptosis and how inflammation stimulates fibrosis, the key questions/strategies focus on improving angiogenesis to protect ischemic cardiomyocytes and preserve heart function, regenerating cardiomyocytes and restraining inflammation and ventricular remodeling.

1.1. Angiogenesis for Cardiomyocytes Survival and Function

Early thrombolysis and stenting reopen stenosed coronary vessel and recapitulate the supply of oxygen and nutrients to promote cardiomyocyte survival in the acute phase. Nevertheless, some patients miss the best chance for thrombolysis and catheter intervention. When these patients are in a chronic stage, how to minimize cardiomyocyte damage, maintain cell function and inhibit the progression of fibrosis are under investigation.

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Manipulating key angiogenic factors not only protect coronary endothelial cells but also assist new vessel formation. In the mice received coronary ligation and lectin and hypoxyprobe injection, Kobayashi *et al.* demonstrated that new vessels developed from the endocardium on day 3 in the ischemic area and became mature on day 14. These primitive vessels are independent from coronary circulation but could perfuse ischemic area with oxygen supply. They further showed that VEGF-VEGFR2 signaling pathway was crucial in the formation of primitive vessels [11].

VEGF is a very potent factor to stimulate angiogenesis. Among these family members, VEGF-B is the most abundantly expressed in cardiomyocytes [12]. Huusko *et al.* injected adenoviral vector containing VEGF-A, or VEGF-B or VEGF-E into the anterior wall of the left ventricle in C57BL/6 mice. By ultrasound and perfusion analyses, they found that VEGF-B- and VEGF-E-induced angiogenesis was more physical than that of VEGF-A. Although neither injection altered left ventricular function, VEGF-A had more side effects than VEGF-B and VEGF-E [13]. In agreement with this report, when rats underwent I/R injury and then VEGF-B injection, it increased Akt phosphorylation and Bcl-2 expression, reduced p38MAPK phosphorylation, all of which contributed to the inhibition of autophagy for cell survival [14]. Topical expression of VEGF-B by adeno- or AAV-9-mediated gene transfer could increase the density of the capillary area and cardiomyocyte proliferation and enhance cardiac function in mice model with myocardial infarction [15, 16]. Unlike VEGF-B, the role of VEGF-C in cardiomyocytes is uncertain. On one hand, in a rat I/R model with pretreatment of VEGF-C in the left ventricle myocardium, VEGF-C/VEGFR2 activates Akt phosphorylation and inhibits Bax expression, leading to increased cardiomyocyte survival and function [17]. On the other hand, binding to its receptor VEGF-R3 on myofibroblasts, VEGF-C could activate TGF-β1 and ERK phosphorylation and participate fibrosis [18].

1.2. Improving Cardiac Function

Except angiogenesis that could promote cardiomyocyte survival with function, calcium stimulates cardiomyocyte contraction, and thus, is an important mediator for cardiac function. Cardiac action potential consists of two cycles, a rest phase and an active phase. Ca^{2+} influx into cytoplasmic compartment depolarizes cardiomyocyte contraction. Immediately after that, Ca^{2+} is removed from cytosol for Ca^{2+} homeostasis. The Ca^{2+} efflux is controlled by Sarco/Endoplasmic reticulum Ca2-ATPase (SERCA-2a), a calcium ATPase in the sarcoplasmic reticulum in cardiomyocytes. As the Ca^{2+} transporter, it facilitates Ca^{2+} transportation from cytosolic compartment to the Sarcoplasmic Reticulum. In cardiomyocyte-specific SERCA-2-/- mice, Ca^{2+} transient amplitude was reduced which was accompanied with O2 consumption dysfunction [19]. In the patients with heart failure, calcium cycling was impaired partially due to decreased SERCA-2 activity [20]. By contrast, direct [21] and indirect [22, 23] increase of SERCA-2 expression improved energy utilization and cardiac contractility. Apart from that, connexin 43 has been identified as the major mediator of intracellular Ca^{2+} propagation between cardiomyocytes [24]. Down-regulation of connexin 43 could enhance cardiomyocyte proliferation under myocardial infarction [24].

1.3. Restraining Inflammation and Myofibroblast Activation

Inflammation is the main drive for cardiomyocyte fibrosis and cardiac remodeling. In the presence of MI, endothelial cells become activated and express a series of adhesion molecules to attract neutrophils, macrophages, monocytes and lymphocytes for infiltrating into injured site [25, 26]. These inflammatory cells release inflammatory cytokines such as IL-1β, TNF-a and IL-17A that strengthen cardiomyocyte apoptosis [27-29], MMPs for matrix degradation [30, 31] and myofibroblast activation [32, 33].

Beside inflammatory cells, β1-adrenergic receptor (β1-AR) and mineralocorticoid receptor (MR) pathways are activated in cardiomyocytes, both of which stimulate inflammatory cytokine production to exaggerate inflammation cascade. From the mechanism view, stress activates β1-adrenergic receptor (β1-AR) on cardiomyocytes for reactive oxygen species production, which, in turn, increases inflammasome component NLRP3 production for caspase-1 activation. Activated caspase-1 cleaves pro-IL-18 into active IL-18 to further reinforce inflammation. In contrast, blockade of IL-18 by neutralizing antibody reverted cardiac inflammation and fibrosis [9].

The mineralocorticoid aldosterone is produced and secreted from adrenal gland to regulate water and electrolyte homeostasis. By cell-type-specific gene targeting, MR is detected in extra-renal cells including endothelial cells, vascular smooth cells, macrophages and cardiomyocytes in mice [34]. MR pathways are involved in inflammation and fibrosis in cardiomyocyte infarction by the following evidence: (1) activation of MR pathways in endothelial cells could stimulate adhesion molecule expressions such as vascular adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule-1 (ICAM-1) [35, 36]; (2) NGAL (neutrophil gelatinase-associated lipocalin) promotes cardiac damage and remodeling which is a downstream target of MR activation [37]; (3) deletion of MR in VSMCs improves left ventricular dysfunction in mice MI model [38]; and (4) binding of aldosterone to MR induces a panel of fibrotic molecule expression in cardiomyocytes including activation of extracellular signal-regulated protein kinase (ERK), c-Jun N-terminal kinase (JNK) and p38MAPK, transforming growth factor (TGF)-β1 pathways and increased production of collagen and α-smooth muscle actin (SMA). Inhibition of MR expression abolishes the above fibrotic marker protein expression [39].

2. VIRUS-MEDIATED GENE TRANSFER IN THE TREATMENT OF MI

2.1. Virus as Vectors for Modulating Candidate Protein Expression

As we described above, MI is a complicated process, among which, key factors are critically involved in cardiomyocyte death, calcium handling, inflammation and scar formation. Selective overexpression or deletion of the key factor could promote cardiomyocyte survival and attenuate inflammation and fibrosis.

By far, virus-mediated gene transfer remains the most potent means to modulate certain protein expression in animal models. Both systemic and topical gene transfers by different types of viral vectors have shown beneficial effects in various animal MI models. Adenoviruses and adenovirus-associated viruses are more frequently used than retroviruses and lentiviruses, because these vectors could induce sustained gene expression for months and they do not integrate into the host genome [40-43]. Table **1** lists the effects of virus-mediated gene transfer in animal models of MI. As shown in Table **1**, they can be generalized as promotion of angiogenesis, [44-62] regulation of SERCA2a expression, [22, 63-67] and enhancement of cardiac functions partially *via* modifying β2-adrenergic signaling pathways [68-71] by virus-mediated gene transfer.

2.2. Virus-mediated Gene Transfer in Reprogramming Stem Cells for Cardiac Repair

Following the appearance of induced pluripotent stem cells (iPSCs), reprogramming of cardiac fibroblasts into induced cardiomyocytes holds great promise for regenerating functional cardiomyocytes after MI.

When fibroblasts are induced with Gata4, Mef2c and Tbx5 expression, these factors are sufficient to drive fibroblasts transdifferentiating into cardiomyocytes [72-74]. As adenovirus and lentivirus vectors are often used in reprogramming, Mathison *et al.* compared their potency and efficacy in a rat model of MI. Three weeks after coronary ligation, rats were injected with adenovirus or Lentivirus, encoding the cocktail of Gata4, Mef2c and Tbx5. By immunohistochemistry, cardiomyocyte marker troponin T expression was comparable and injection fraction was increased in a similar extent in both Ad and lentivirus gene transfer groups shown by echocardiography. These data imply that Adeno and lentiviral vectors are equally effective in inducing fibroblast transdifferentiating into induced cardiomyocyte-like cells with function [72]. Despite promising, the main obstacles in cell reprogramming are low transfection efficiency, time-consumption and genome integration.

Recently, Miyamoto *et al.* demonstrated that Sendai vectors encoding Gata4, Mef2c and Tbf5 could rapidly reprogram fibroblasts into induced cardiomyocyte-like cells without any sign of integration. In mouse fibroblasts, Sendai virus system generated 100-fold more beating induced cardiomyocyte-like cells than retroviral-GMT and the duration to induce beating cells was shortened from 30 to 10 days. Accordingly, by *in vivo* lineage tracing, injection of Sentai virus encoding the factors above was more potent to induce cardiomyocyte-like cell expression with improved cardiac function and reduced fibrosis when compared with retroviral-Gata4/Mef2c/Tbf5 group [74].

2.3. Genetic Modified MSC for MI Treatment

Except reprogramming, mesenchymal stem cells (MSCs) are multipotent stem cells derived from the mesoderm of early-phase embryos. They are self-renewable and capable of differentiating into a variety of cell types, such as osteoblasts, cartilage, skeletal muscle, tendon, fat, endothelial cells and nerves [75]. In addition, MSCs could produce a variety of proteins and RNAs, so-called secretome and exome, to attenuate inflammation and enhance angiogenesis [76, 77]. Delivery of MSCs or subpopulation of MSCs to animal models of acute myocardial infarction improves left ventricular function ejection fraction and increases blood vessel density in the ischemic area and reduces collagen deposition [78, 79].

To gain better homing and angiogenesis efficiency, MSCs are genetically equipped with certain character prior to injection in MI animals. For instance, when MSCs were transfected with VEGF cDNA or hepatic growth factor cDNA and then injected into the border of infarcted cardiomyocytes individually, MSC-HGF and MSC-VEGF injection showed the most advantageous effect than other groups [46]. In line with this, using the facially amphipathic bile acid-modified polyethyleneimine (BA-PEI) conjugates, transfection efficiency was further improved, resulting in higher VEGF expression in MSCs and increased angiogenesis in infarcted cardiomyocytes [80].

3. NANOPARTICLES AS VEHICLES FOR GENE DELIVERY

Although virus-mediated gene transfer is successful in promoting cardiomyocyte survival, improving cardiac function and mitigating fibrosis in mice, the safety issues are the most concerned that hinder its application moving from animal studies to clinical practice. The safety issues mainly comprise poor targeting efficiency, immunogenicity and unpredicted insertion site of the human genome. As an alternative approach, nanoparticles provide another option for gene delivery.

3.1. General Introduction

As how it is named, nanoparticles are small particles between 1 and 100 nm in size. The interfacial layer typically consists of ions, inorganic and organic molecules, which affects the properties of nanoparticles. They do not belong to modern science. In fact, the history is traced back to the fourth century as a component for dichroic glass by artisans in Roma. But till 1857, its scientific terms were first described as the optical properties of nanometer-scale metals. Nanoparticles elicited biologists' interest because they could be linked to biological molecules such as tags that direct nanoparticles to specific sites within living cells for tracing [81-83]. Nowadays, the applications of nanoparticles have been extended for imaging, drug and gene delivery system.

3.2. Types of Nanoparticles

Nanoparticles used in medical research consist of micelles or liposomes, polymers, dendrimers, carbon nanotubes and metallic nanoparticles. Micelles are hydrophilic. When micelles are incorporated with hydrophobic therapeutic agents, the solubility problems are solved [84]. Liposomes are compatible with the cell membrane and thus the most popular carriers for cell endocytosis [85, 86]. Polymers could incorporate both hydrophilic and hydrophobic agents to increase solubility [87, 88]. Among polymers, hydrogels have been used in patients for wound healing, antibacterial infection and hemostasis [88-91]. Dendrimers are organic nanoparticles, synthesized step by step to finely tune their properties and formed in the three-dimensional structure

Table 1. Summary of the effects of virus-mediated gene transfer in animal models of myocardial infarction.

Molecular Target	Vector Type	Animal MI Models	Results
Angiogenesis			
CD151 [44]	rAAV, local	Rat	Increased VEGF expression and improved left ventricular function
VEGF [45-49]	Adenovirus, local	Rat, sheep	Improved ejection fraction; reduced myocardial fibrosis
β-adrenoceptor [50]	Ad	rats	Activation of VEGF pathway for increased angiogenesis and global contractility
Fibroblast growth factor 9 [51, 52]	Ad	mice	Conditional expression of FGF9 promotes myocardial vascularization and hypertrophy with enhanced systolic function and reduced heart failure mortality after MI.
Endothelial nitric oxide synthase [53-56]	Ad	rat	eNOS provided cardiac protection after myocardial infarction injury through inhibition of cardiac apoptosis and collagen deposition, and suppression of TGF-β1
Stromal cell-derived factor or CXCR4 [57, 58]	Ad	Rat	SDF-1 alpha could improve cardiac structure and function after Myocardial infarction through angiogenic and anti-fibrotic actions.
Hepatic growth factor [59-61]	Ad	Rabbit, rat, canine	improved left ventricular ejection fraction and fractional shortening, reduced the fibrotic area, and increased the capillary density in the risk area.
apoA-I [62]	Ad	mice	Increased endothelial progenitor number and function and the peak rate of isovolumetric relaxation by AdapoA-I
SERCA2a expression			
SERCA2a [63, 64]	AAV1; Lentivirus	Sheep, rat	Improved contractility, reduced myocyte apoptosis and myocyte hypertrophy
antisense phospholamban (asPLB) [22]	AAV	rat	enhanced myocardium SERCA activity; prevented the progression of heart failure
Urocortin-2 [65]	AAV8	mice	increased LV systolic and diastolic function
Small ubiquitin-like modifier 1 (SUMO-1) [66]	AAV1	swine	improved cardiac function and stabilized LV volumes
S100A1 [67]	AAV9,	porcine	Prevented heart failure and reduced scar side
Cardiomyocyte preservation and regeneration			
Stem cell factor [68]	Adenovirus	Swine	Recruitment of cKit+ cells, improved cardiac function
Connexin43 [69]	Ad	pig	Targeted manipulation of Cx43 levels improved conduction velocity and reduced ventricular tachycardia susceptibility.
G protein-coupled receptor kinase 2 (GRK2) [70, 71]	scAAV	Sheep, pig	preservation of regional and global systolic function

[92]. Carbon nanotubes are graphite sheets rolled up into a tubular form in which drugs are filled [93]. Different from others, metallic nanoparticles are functional themselves. The conjugation of metal nanoparticles with biomolecules could be used in biosensing, bioimaging and tissue engineering [94]. When nanoparticles circulate in the peripheral blood, it could be taken by white blood cells, leading to reduced homing. To reduce phagocytosis and obtain better targeting efficiency, nanoparticles are pegylated on the surface to escape the recognition by circulating and resident phagocytes [95].

3.3. Nanomedicine in Imaging

Magnetic resonance imaging (MRI) allows us to visualize the structure and characteristic of atherosclerotic plaques, coronary stenosis and the extent of infarcted cardiomyocyte area. Three MRI techniques are T1, T2 and off-resonance. In off-resonance, pulse sequences excite and refocus off-resonance water to give the positive contrast. Paramagnetic contrast agents including gadolinium chelates and nanoparticles enhance T1 contrast to give bright contrast in MR im-

age. In addition, iron oxide nanoparticles enhance predominantly T2 contrast and give dark contrast. Some nanoparticles contain several contrast agents such as 18F-cross-linked iron oxide which is formed by superparamagnetic iron oxide core and functionalized with the radionuclide 18F. When iron oxide nanoparticles are injected to animals with MI, macrophages take up these nanoparticles and thus become labeled by MRI. From the detected signals on MRI, we could know how severe the inflammation is, present in the infarcted area and plaque and whether the plaques are stable or not [96]. Another substantial application of nanomedicine in imaging is cell tracing. When stem cells are labeled with certain nanoparticles before injection, they could be followed up by MRI for weeks to assess homing efficiency and fate of the injected stem cells [97].

3.4. Drug Delivery System Using Nanoparticles

The golden criteria of drug delivery system include specific targeting, controlled drug release, pharmaceutical efficacy and degradable delivery material for safety. Receptors and intracellular molecules are equally important mediators for cell function. Using antibodies immune therapies, antibodies could recognize receptors on the cell surface and a limited number of extracellular targets [98, 99]. Nevertheless, how to control intracellular targets is more demanding. These issues could not be reached by conventional drug assembling system. The development of nanotechnology has brought a solution to solve these issues.

Accumulated studies have consistently delineated the therapeutic effects in the treatment of cardiovascular diseases using nanoparticle-based drug delivery system (nano-DDS). Using microchip technology and 3D dynamic contrast-enhanced MRI, Kim *et al*., found that nanoparticles could translocate over endothelium with controllable permeability in rabbits [100]. These data imply that nanoparticles delivery systems could target molecules inside the cells.

Recently, two polymers, polylactide (PLA) and poly(lactide-co-glycolide) (PLGA) were approved by the FDA for nanoparticle synthesis. PLGA polymers could incorporate hydrophilic and hydrophilic agents and become biodegradable. In a mouse model of atherosclerotic plaque rupture, a single injection of PLGA nanoparticles containing pioglitazone significantly reduced the number of Ly6chigh monocytes. After weekly intravenous injection for 4 weeks, the fibrous cap in atherosclerotic plaque became thickened and stabilized [101]. Similarly, when mice were subjected with coronary ligation and injected with pitavastatin alone or with pitavastatin-incorporating nanoparticles for consecutive 3 to 5 days, administration of pitavastatin-incorporating nanoparticles decreased the number of monocytes/macrophages in the infarcted heart and inhibited left ventricular remodeling whereas pitavastatin alone did not [102]. These data indicate that nanoparticles are more efficient than traditional drug delivery system.

3.5. Gene Delivery System Using Nanoparticles

Besides for imaging and drug delivery, nanoparticles hold some advantages for gene transfer. First, they are small molecules that easily and efficiently penetrate to target cells; second, nanoparticles could be covalently linked to specific tags in a controlled number per nanoparticle and thus they could be taken by target cells; third, multivalent nanoparticles could cluster receptor to activate signaling pathways; and fourth, the synthesis of nanoparticles would be faster than the virus packaging system [103, 104].

There are two types of nanoparticle systems carrying DNA or RNA: an entrapping system which is a reservoir type nanosphere system and surface binding system which supports an ionic interaction between the cationic polymer and the anionic nucleic acid. The entrapping system could protect DNA or RNA from degradation. How to synthesize and modify nanoparticles for higher transfection efficiency with the least toxicity is under investigation. A substantial progress has been made on how to modify nanoparticles for better gene delivery, which is summarized below:

3.5.1. PEGylation

The complex of PEG-polycation block copolymers and DNA are water soluble, colloidally stable, non-toxic and effective in transfection. Based on this, conjugation of polylysine to PEG further condenses plasma DNA into DNA nanoparticles. When transfected, the transgene expression was 10- fold higher than controls [105]. In parallel, Dasari *et al.* linked PEG on the N-terminal cysteine of a peptide for ocular delivery. This construct enables gene transfer in the retina. Using the reducible PEG-POD/DNA nanoparticles, FLT1 cDNA was transfected intro retina cells *in vitro* and FLT1 expression was induced without any change of LDH activity. When tested *in vivo*, the reducible PEG-POD/DNA induced 21- fold increase in transgene expression which resulted in 50% reduction in choroidal neovascularization in a murine model of age-related macular degeneration [106]. Because it is quickly degraded by the extracellular environment, this reducible PEG-SS-POD/DNA nanoparticle is a powerful and safe gene delivery system.

3.5.2. Chitosan

Chitosan is a cationic polysaccharide derived from partial deacetylation of chitin. It is an ideal carrier for drug, DNA and siRNA delivery because of good incorporation and long-term release [107-109]. The strategies of making better chitosan/DNA nanoparticles have been revolutionized in the aspects of size, concentration and the stoichiometry of polymer for better efficiency and safety. Chitosan nanoparticle systems have been applied in vaccines and intranasal delivery of chitosan-DNA complex against Coxsackievirus B and hepatitis B infections [110-112]. Intriguingly, Mannosylated chitosan nanoparticles are preferentially taken by macrophages [113]. Thus, whether Mannosylated chitosan nanoparticles could be used for suppressing inflammation and attenuation of myocardial infarction awaits for future exploration.

3.5.3. Polyethyleneimine (PEI)

Polyethyleneimine (PEI) is one of the most widely studied cationic polymeric vectors. An advanced strategy was reported in which low molecular weight PEI was linked with succinic acid which improved the hydrophilic and hydrophobic balance within the polymer and in the meantime, minimized the toxicity. The modified PEI could condense plas-

mid DNA and the formed complex was approximately 130 nm in size. When tested using the CD200 gene as the reporter, the transgene expression was increased 1.5-fold than controls *in vitro* and the expression pattern was distributed in a variety of organs and could even penetrate the blood-brain barrier [114].

3.5.4. Solid Lipid Nanoparticles

Due to shared compatibility to the cell membrane, liposomes have been used extensively for gene transfer for research. As alternatives, Solid Lipid Nanoparticles (SLNs) and Nanostructure Lipid Carriers (NLCs) have been developed since the 1990s. Because they could be equipped on purpose, they could protect DNA/RNA from degradation during delivery, reach specific target cells, and pass through all barriers, resulting in better transgene expression as desired. SLNs have a solid lipid core with a surfactant layer in an aqueous dispersion whereas NLCs are mixtures of solid and liquid lipids.

SLNs and NLCs carry several properties superior to liposome in gene transfer: (1) Depending on the charges of nucleic acids to be transferred, SLNs and NLCs could either be cationic or anionic to obtain stable binding in an electrostatic manner which helps DNA/RNA condensation and protects them from being degraded by enzymes in the environment [115]; (2) After injection, they bind to serum proteins which serve as carriers and deliver them to cells. When they reach the cells, the positively charged SLCs and NLCs interact with the negatively charged cell membrane to mediate endocytosis. Once they are equipped with the target ligands that recognize the receptors on cell and/or nuclear membrane, the transfection efficiency would increase by receptor and ligand interaction on top of endocytosis [116, 117]; and (3) in addition to gene transfer, SLNs and possibly NLCs are also carriers for drug delivery. Currently, SLNs and NLCs have been widely tested in the field of cancer, infectious disease and ocular disease [118-120]. Nevertheless, limited studies have been performed using SLCs and NCLs for treating ischemic heart. Notably, modification of SLCs and NLCs makes them feasible for penetration of cell and nuclear membranes. Different pathways are involved in different types of cells. The endocytosis pathways have to be clarified before SLCs and NCLs are designed for drug and gene dual transfer into cardiomyocytes.

3.5.5. Magnetic Nanoparticles

As introduced earlier, magnetic nanoparticles such as iron oxide and Fe_3O_4 could accumulate to favored cells for imaging-based cell tracing. When they are coated by different polymers, they become stabilized and activated by pH, temperature and microwave [121]. Coating strategies are the main issues in preparing these nanoparticles. Recently, a type of mesoporous silica-coated magnetic nanoparticles was reported. Their magnetic targeting abilities, magnetic hyperthermia performance and MRI properties have made them a superior candidate for suicide gene therapy in cancer treatment [122]. Considering the different nature and mechanisms between cancer and infarcted cardiomyocytes, whether magnetic nanoparticles could be used for gene therapy is not yet known in myocardial infarction.

All types of nanoparticles that could be applied for the treatment of cardiovascular diseases are summarized in Table **2**.

3.5.6. Limitations and Future Perspective

In virus-mediated gene transfer, there were potential limitations in nanoparticle-facilitated gene therapy as well. First, although nanoparticles-based gene delivery could induce faster and higher transgene expression than controls, the duration of transgene expression is relatively short and lasts for days. Second, the nucleic acids that nanoparticles carry do not integrate into human genome which makes it safer than virus vectors. However, the cellular toxicity is still present and could not be ignored. The toxic effects of engineered nanoparticles on germ cells, embryos and reproductive systems have been noted [123]. And third, detailed understanding of how nanoparticles are distributed, metabolized and eliminated is demanding to enhance targeting efficiency and prolonged efficacy with least of toxicity. Huge amount of work is anticipated for improving nanoparticle-mediated gene therapy for better targeting and least side effects.

Nonetheless, nanoparticles are potent in gene therapy. The dual properties in drug and gene transfer by nanoparticles could not be replaced by any other delivery systems. Here is an example, demonstrating elaborately how to utilize nanomedicine for better treatment. Tang *et al*., set up a combinatorial library of 15 high-density lipoprotein-inspired nanoparticles, a PEGylated micellar and a long-circulating liposomal nanoparticle. All of which had distinct physiochemical properties such as size and chemical composition. They screened the injected nanoparticles by half-life and accumulation in the organs by near-infrared fluorescence imaging and assessed their cholesterol efflux capacity *in vitro*. They further evaluated the effects of nanoparticles on inflammatory cells in aorta, spleen and blood by flow cytometry. Thus, the best candidate was screened which had high cholesterol efflux capacity, relatively long half-life, predominantly accumulated in aorta and liver and a high relative aortic-to-splenic macrophage association ratio. They formulated the candidate with a Liver X receptor agonist GW3965 (Rx-HDL) and confirmed its effects on atherosclerosis in apoE-/- mice. As expected, this nanoparticle processed all the characters above and inhibited atherosclerosis with the least liver toxicity [124]. This study gives a direction to utilize nanoparticles to deliver a gene of interest and drug of interest to the targeted cells for specificity, accuracy, efficacy and safety.

Based on their unique characters, hybrid nanoparticles could inherit both advantages to achieve better gene delivery efficiency. For instance, polymer-lipid hybrid nanoparticles hold the property of polymeric materials and also a lipid formulation. When they were incorporated with PEG-distearoyphosphatidylethanolamine, gene transfer efficiency was 3-fold higher than conventional transfection method [125]. Another elaborate example is the incorporation of gold nanoparticles (Au NPs) into the liposome. Once administered under near-infrared irradiation, liposomes are fragmented and gold nanoparticles are released and penetrated into tumor tissues to proceed photothermal treatment, leading to superior inhibition of tumor cell growth *in vitro* and

Table 2. Summary of the nanoparticles that could be applied for the treatment of cardiovascular disease.

Type	Structure and Compositions	Size	Modification for Improved Efficiency
Micelle [128]	Lipids and synthetic amphiphilic polymeric molecules dispersed in a liquid colloid	10-100 nanometers	Micelle-like nanoparticles: half micelle and half polymeric.
Liposome [129]	Phospholipid bilayers and an aqueous core	Up to thousands of nanometers	polyethylene glycol (PEG)
Polymeric nanosphere [129, 130]	Polymeric materials	An average of 700 nanometers	Poly(lactide-co-glycolide) (PLGA) or polylactide (PLA)-based biodegradable nanoparticles. Could carry both hydrophobic and hydraulic drugs
Dendrimer [131, 132]	A monodisperse assembly with complex structure	Starting from several nanometers	GPIb to PLGA; probucol loaded PLGA
Metallic nanoparticle [133, 134]	Could be gold, iron oxide to superparamagnetic iron oxide	Varied	Dextran or poly(ethyleneglycol); suitable for magnetic resonance imaging
Nanogel [135, 136]	A nanosized spherical hydrogel	Varied	Thermally responsive manipulation for better delivery

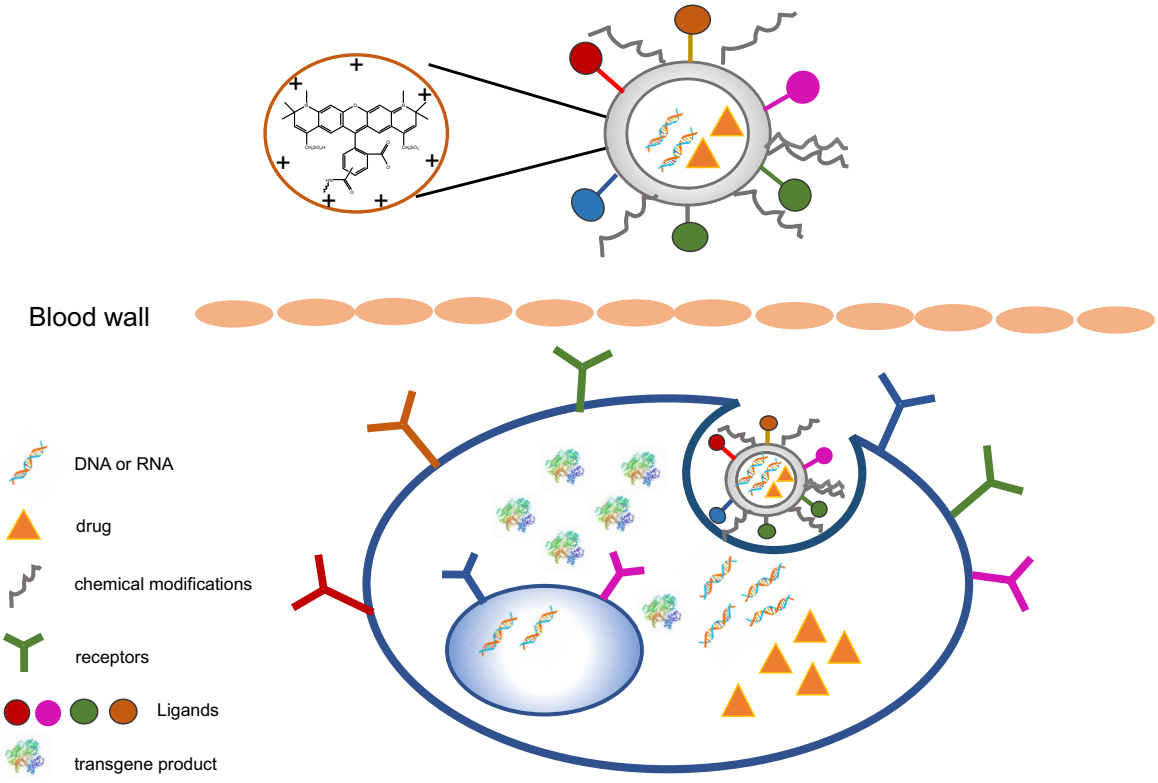

Fig. (1). Proposed nanoparticle-based gene transfer system. Nanoparticle-based delivery system could carry transferred genes and drug at the same time whereas other systems could not. The basic structure of nanoparticles could be manipulated by charges that regulate interaction between nanoparticles and DNA to be transferred and by chemical modifications on the surface, leading to DNA stability, resistance to enzyme destruction in the environment and improved cellular uptake. To further enhance uptake, ligands could be wrapped on the surface that could specific recognize the receptors on the target cells.

in vivo [126]. Therefore, how to make the best use of hybrid nanoparticles for gene delivery is under investigation. When polyethyleneimine particles are loaded with modified cholesterol, they could bind DNA with more affinity and protect DNA from degradation [127]. Therefore, these "lipopolyplexes" would be essential tools for more efficient gene delivery to target cells.

CONCLUSION AND FUTURE OUTLOOK

In conclusion, nanoparticles possess several advantages while other vectors do not. They could be synthesized in a

controlled manner and produced on a large scale. They are biocompatible and could be designed to carry certain properties to protect DNA/RNA content from enzyme-induced degradation, recognize target of interest more specifically, and efficiently penetrate cell and nuclear membrane (Fig. **1**). They could facilitate dual transfer-gene and drug at once. The biggest challenge that nanoparticles need to overcome is coating. With the development of technologies and knowledge on cell biology and pathology, nanoparticles would be desirable for clinical purpose. Different from cancer treatment, application of nanoparticle-mediated gene transfer in cardiomyocyte infarction is relatively rare which definitely opens the space for future exploration.

CONSENT FOR PUBLICATION

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CONFLICT OF INTEREST

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

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