Cardiovascular disease (CVD) accounts for almost half of all deaths worldwide and has now surpassed infectious disease as the leading cause of death and disability in developing countries. At present, therapies such as low-density lipoprotein—lowering statins and antihypertensive drugs have begun to bend the morality curve for coronary artery disease (CAD); yet, as we come to appreciate the more complex pathophysiological processes in the vessel wall, there is an opportunity to fine-tune therapies to more directly target mechanisms that drive CAD. MicroRNAs (miRNAs) have been identified that control vascular cell homeostasis, lipoprotein metabolism, and inflammatory cell function. Despite the importance of these miRNAs in driving atherosclerosis and vascular dysfunction, therapeutic modulation of miRNAs in a cell- and context-specific manner has been a challenge. In this review, we summarize the emergence of miRNA-based therapies as an approach to treat CAD by specifically targeting the pathways leading to the disease. We focus on the latest development of nanoparticles (NPs) as a means to specifically target the vessel wall and what the future of these nanomedicines may hold for the treatment of CAD.

miRNAs and CAD

miRNAs (miRs) belong to a class of noncoding RNA that is highly conserved across species. miRNAs are short and single-stranded, between 20 and 22 nucleotides (nt) in their mature form, and form duplexes with complementary sequences in the 3' untranslated region of mRNAs. The specificity of the miRNA:mRNA interaction is derived from the seed region of the miRNA, a 5–7nt region that is either partially or totally complementary to its target mRNA sequence. Target gene repression is achieved through either miRNA degradation or translational inhibition, the former occurring only when the miRNA:mRNA duplex is perfectly complementary. Despite being only 5–7nt in length, miRNA seed sequences result in remarkable specificity, and even a single nucleotide base change can completely abolish an miRNA:mRNA interaction.

Conversely, multiple genes within a given pathway can contain targeting sequences for the same miRNA, allowing a single miRNA to repress an entire gene set simultaneously. This coordinate genetic regulation makes miRNAs uniquely powerful as functional modulators that can be targeted therapeutically.

During atherogenesis, excess circulating lipids activate endothelial cells to produce proinflammatory factors that promote the recruitment of monocytes into the subendothelial space. Because monocyte-derived macrophages engulf accumulated lipids, transforming into inflammatory foam cells, they secrete factors that activate smooth muscle cells (SMCs), induce further monocyte recruitment, and promote macrophage proliferation. At each of these stages, miRNAs have been shown to both promote and protect from atherogenesis (Figure). The details outlining the mechanisms of miRNA function in CAD have been reviewed extensively elsewhere; therefore, we instead highlight miRNAs whose therapeutic targeting in the vessel wall shows promise but may benefit from NP-based targeted delivery to avoid the accompanying risks of systemic or nonvascular manipulation.

miR-33

Discovered simultaneously by several groups, miR-33 was identified in a feedback loop with its host gene SREBP-2 to attenuate cholesterol efflux from cells during times of sterol depletion.5,7,11 Since its initial characterization as an ABCA1 (ABC-binding cassette transporter 1)-targeting miRNA, multiple other lipid and energy metabolism genes have been identified as miR-33 targets.12–14 Blocking miR-33 using either genetic or antisense approaches reduces lesion burden in mouse models of progressing and regressing atherosclerosis.13,15–18 Some of these benefits may be derived from the high-density lipoprotein–raising effects of miR-33 antagonism, but in many cases, miR-33 inhibition protected from atherosclerosis in the absence of increases in high-density lipoprotein. However, longer-term studies in mice of miR-33 blockade raised concerns over the development of fatty liver and elevated circulating triglycerides,19–21 though this did not occur in all mouse studies nor was it observed in nonhuman primates.22,23 Although this may be related to variations in biodistribution of different chemically modified antisense oligonucleotides, as well as differences in total versus partial knockdown, there is, nonetheless, concern that what may be good for the atherosclerotic plaque may not be good for the liver.

miR-21

miR-21 was identified as being elevated in several vascular diseases, suggesting that its modulation may offer some protection against disease.24 In SMCs, miR-21 serves to block the expression of its target genes PTEN, SPRY1, PDCD4, and...
ABCA1 and enhanced cholesterol efflux. These studies highlight the cell-specific nature of miR-143/145 function and its potential opposing roles. miR-143/145 are also potent tumor suppressors and play a significant role in the development of carcinomas potentially via activation of the RAS pathway. As such, therapies aimed at delivering and inhibiting miR143/145 in the vessel wall must take into account its diverse and potentially beneficial roles in other tissues.

Inflammatory miRNAs

Several miRNAs have been identified that regulate the immune response in the vessel wall during inflammation and atherosclerosis. Thus far, the most well characterized are miR-155, miR-146, and miR-33. miR-155 has been described as a multifaceted immune regulatory miRNA that functions in monocytes, T cells, and B cells. In macrophages, miR-155 promotes inflammation and inhibition leads to improved cardiac responses to hypertrophy. However, the role of miR-155 in atherosclerosis is stage-dependent, promoting atherosclerosis at early stages but protecting from it at later stages. Indeed, a recent study demonstrated the opposing roles for miR-155 in endothelial cells versus monocytes and suggest that the balance of expression of this miRNA has the potential to dramatically alter the inflammatory response in the artery wall. Similarly, miR-146a is a potent anti-inflammatory miRNA that serves to limit NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) activation in a feedback loop, and in the atherosclerotic plaque, delivery of miR-146a mimics limits the development of lesions in mice. However, as miR-146a acts to limit inflammation in response to bacterial infection and other stimuli, the utility of miR-146a replacement therapy for a chronic disease like atherosclerosis would be limited.

These miRNAs provide examples of attractive therapeutic targets to treat CAD via overexpression or inhibition strategies, yet are hampered by their cell-, tissue- and context-specific roles. Below we describe some of the challenges associated with miRNA-targeting therapies and provide some insight into how NPs are emerging as a critical tool to increase the therapeutic promise of these potent modulators of CAD.

Challenges of miRNA-Targeting Therapies

Although both miRNA mimics and anti-miRs have great therapeutic potential, each of these miRNA modulators have unique challenges that must be addressed for successful development of miRNA therapies. To be recognized correctly in the cytosol by the RNA-induced silencing complex, the cellular machinery responsible for the miRNA- and siRNA-mediated gene repression, synthetic miRNA mimics must be double-stranded. This results in the potential of immune reactivity in response to dsRNA, which triggers an antiviral response. Moreover, miRNA mimics have short half-life in the circulation, and any chemical modification to improve their stability might interfere with their processing into RNA-induced silencing complex and decreased effector function. On the contrary, miRNA inhibitors (anti-miRs) need only bind to a single-stranded mature miRNA to block their function and, thus, generally do not activate the immune system. As a result, anti-miRs can be chemically modified to improve their stability and affinity for the targeted miRNA. These chemical modifications are focused on the sugar, the base or the linkage between nucleotides of miRNA. Because the nuclease action involves 2′-OH in the ribose ring, substitution of

Figure. Top, MicroRNAs in the atherosclerotic plaque that would benefit from nanoparticle (NP) delivery of RNA therapeutics. Bottom, Schematic representation of different NP platforms. PEG indicates polyethylene glycol.
This 2′-OH group with 2′-O-methyl, 2′-O-methoxyethyl, or 2′-fluoro groups has been explored to increase the stability. Other modifications include changing phosphodiester linkage to phosphorothioate bonds by replacing nonbridging oxygen atom with a sulfur atom and locking the ribose rings via methylene bridges connecting the 2′-O and the 4′-C atoms, constraining the ribose to C3′-endo conformation (locked nucleic acid). Currently, locked nucleic acid–based miRNA modulators show promising results in different tumor mouse models. Although these modifications improve resistance to nuclease degradation, they also increased possible cytotoxicity and side effects because of the sustained retention of anti-miRs in the body. Despite the advantages of chemical modifications of miRNA modulators, cellular uptake, biodistribution, off-target effects, and toxicity still remain the major limitations for miRNA therapies. Because of the phosphate groups on the back bone, both miRNA mimics and anti-miRs are negatively charged, making it difficult for them to leave the circulation and enter the cells. To circumvent these challenges, both viral and nonviral nucleotide delivery platforms have been explored as a delivery vehicles for miRNA therapeutics. However, these approaches have their limitations, and hence, nonviral delivery strategies have been highly sought after. Nevertheless, choosing the appropriate delivery platform to improve the stability in the circulation, augment the spatiotemporal delivery of the miRNAs, and successful endosomal escape seem to be most important factors influencing miRNA therapeutics.

Can NPs Offer a Solution for Therapeutic miRNA Modulation?

NP-Based Therapeutic Platforms: Composition, Synthesis, and Biophysical and Chemical Properties
To date, numerous engineered NP platforms have been developed for wide variety of disease applications, and the following have made a significant advances toward the clinic: liposomes, polymeric NPs, dendrimers, and metal-based and carbon-based materials (Figure). Liposomes are vesicle-like structures consisting of a single or multiple bilayered membranes composed of natural or synthetic lipids. They were one of the first nanostructured platforms discovered to have potential as drug delivery vehicles. Typically, liposomes are spherical in shape comprising an aqueous core and a lipid bilayer shell, and their size can vary from 20 nm to several micrometers. Liposomes are extensively used in a variety of biomedical and pharmaceutical applications mainly because of their biocompatibility, biodegradability, and ability to encapsulate both hydrophobic and hydrophilic payloads. To improve the half-life in the circulation and decrease phagocytic clearance, polyethylene glycol was introduced on the surface of liposomes by using polyethylene glycol–modified lipids as one of the starting components. Additionally, the surface of liposomes can be decorated with targeted ligands for site-specific drug delivery. Doxil was the first Federal Drug Agency–approved liposomal formulation entered into clinic for the treatment of AIDS-related Kaposi’s syndrome over 20 years ago. Polymeric NPs along with liposomes are widely used in nanomedicinal research and consist of biodegradable, biocompatible polymeric materials that are either hydrophobic, hydrophilic, or amphiphilic in nature. Most of the polymeric NPs reported in the literature are based on polylactide, polyglycolide, polycaprolactone, and poly lactide-co-glycolide. Polymeric NPs are versatile in nature with tunable sizes, shapes, and drug release properties and, as such, are used as drug delivery vehicles for wide range of therapeutics, such as hydrophobic and hydrophilic small molecules, proteins, nucleotides, and peptides, and comprise the majority of nanomedicines currently in clinical trials. Polymeric NP–based nanomedicines are being tested for use in cancer, CVD, diabetes mellitus, bone-healing therapies, and vaccinations. They can also be used as so-called theranostics by conjugating contrast agents to the surface of polymeric NPs for visualization by magnetic resonance imaging or other noninvasive imaging modality.

Dendrimers named after the Greek word dendron for tree are chemically synthesized macromolecules with well-defined tree-like architectures. Typically, dendrimers consist of a central core with 2 or more reactive functional groups that are covalently attached to repeating chemical units. They can either encapsulate or covalently conjugate a variety of therapeutic and imaging agents in their dendric core or on the surface for various biomedical applications. Lipoprotein-modified NPs are high-density lipoprotein–based natural NPs. Although the high-density lipoprotein is known for its role in cholesterol transport, it can also act as carriers for systemic delivery of drugs and imaging agents. Inorganic metal-oxide NPs, such as mesoporous silica NPs, iron oxide, and gold NPs, are physicochemically and biochemically stable NP platforms with high surface area and less drug leakage. These NP platforms are extensively used in theranostics and multimodality imaging applications. Carbon-based materials, such as fullerenes, carbon nanotubes, and graphene sheets are widely used for various clinical applications, including the delivery of nucleic acids for the purpose of gene therapy.

The physicochemical properties (size, shape, surface charge, and rigidity) of the NP platforms can influence their uptake by different organs, tissues, and cells. Therefore, substantial efforts have been made in the optimization of these properties through the effective design of NPs. Based on the application as well as physicochemical properties of the payload, several preparation methods were developed for different NP platforms, which vary in the method of emulsification, solvent evaporation, and film hydration. A detailed discussion of these primary properties of NPs, the optimization of which is essential for the development of efficient therapeutic NPs, have been reported elsewhere. Although delivery to the atherosclerotic plaque has yet to be perfected, the flexibility in NP design will likely allow for fine-tuning NP properties to achieve the more desirable and on-target effects.

Lessons Learned From siRNA Therapies for CVD Applications
Given that miRNA and siRNA share several structural similarities, the development of siRNA delivery methods has the potential to be extrapolated to miRNAs. In mammalian...
systems, miRNAs are encoded in the genome and are processed in the nucleus by the cell of origin, whereas siRNAs primarily occur after exogenous delivery. To functionally bind its target mRNA(s), both classes of small RNA need to be single-stranded, yet require efficient and physiological loading into the RNA-induced silencing complex. Although siRNAs lead to degradation of target mRNA, miRNAs lead to either degradation or translational inhibition of target mRNA. In both cases, an ideal delivery platform would be one that achieves a high load of RNA per NP, but efficiently releases these RNAs from the platform where they can successfully escape from the endosomes and distribute evenly in the cytosol. In recent years, different siRNA nanodelivery approaches have been used to target CVDs. Systemic delivery of siRNA nanovehicles has been explored for CVDs applications. But in most cases, even though respective gene knockdown was achieved, these NPs accumulated to a greater extent in organs such as liver and spleen than the target tissue (heart or plaques). Some of the initial strategies were focused on local injection of nanoplatforms into target sites and were moderately successful. Local delivery of the siRNA-containing polyethyleneimine polymer-based NPs or pH-sensitive polyketal-based NPs into the myocardium resulted in appropriate target gene knockdown and validated the delivery platforms for siRNA; however, more studies are needed with systemic administration of these NPs to evaluate their potential for broad applicability.

Taking advantage of the pattern of NP organ accumulation observed with systemic delivery, different approaches were developed to indirectly target the cardiovascular system. In one example, intravenous administration of cationic liposomal NPs containing siRNA against C–C chemokine receptor 2 accumulated more in the spleen and bone marrow and were up-taken by monocytes. These NPs effectively silenced the C–C chemokine receptor 2 mRNA in inflammatory monocyte subsets and inhibited their migration, leading to decreased inflammatory monocytes in atherosclerotic plaques, reduced infarct size after coronary artery occlusion, and prolonged normoglycemia in diabetic mice after pancreatic islet transplantation. Also recently, polyethyleneimine-based polymeric endothelial-avid NPs containing multiple different siRNAs that can effectively silence multiple targets was reported. Here, NPs were loaded with 5 siRNAs targeting 5 endothelial cellular adhesion molecules (CAMs; ie, ICAM1, ICAM2, VCAM1) and E-selectin and P-selectin and simultaneously reduced their expression in apolipoprotein E−/− mice with accelerated atherosclerosis after myocardial infarction. These latest studies show tremendous potential for the development of vascular cell– and inflammatory cell–specific NPs that can target more than one gene at once and provide excitement for the possibility that multiple miRNAs may be targeted simultaneously using similar technology.

**Other NP Applications in CVD**

There are a growing number of examples of drugs that could have enormous benefit in the atherosclerotic plaque but have untoward effects on other organs, namely the liver. Therefore, methods of targeting only the cardiovascular system without unwanted systemic effects have been highly sought after. Over the last decade, the progress in cancer nanotechnology improved our understanding of nano-bio interactions and made inroads in developing nanomedicine-based CVD therapies and diagnostics. A variety of NP platforms containing drugs, proteins, and biologics have been tested preclinically and provide some encouraging results that NPs may soon be used for cardiovascular-specific applications.

Liver X receptors (LXRs) decrease inflammation by modulating the expression of key inflammatory genes, making LXRs and their ligands attractive candidates for therapeutic intervention in CVD. However, LXR activation in the liver promotes SREBP1-driven lipogenesis, which results in hepatosteatosis, making systemic delivery of LXR agonists an impossibility for the treatment of atherosclerosis. In this context, potential LXR-based nanotherapies for the management of inflammation and atherosclerosis were explored by delivery of LXR agonists specifically to sites of inflammation. The synthetic LXR agonist GW3965 containing NPs were significantly more effective than the naked drug alone in inducing LXR target genes while downregulating proinflammatory mediators. Interestingly, in mice with existing atherosclerotic plaques, systemic administration of LXR agonist GW3965 containing NPs resulted in significant reduction in atherosclerotic lesion area without increasing total cholesterol or triglycerides in the liver and plasma. In other strategies, NP platforms were used in modulating the polarity of monocytes and macrophages toward a less inflammatory phenotype to prevent plaque destabilization and markers of rupture. Here, polymeric NPs were loaded with peroxisome proliferator–activated receptor-γ agonist pioglitazone (pio-NPs) and delivered to circulating monocytes. In atherosclerotic mice studies, pio-NPs reduced markers of plaque rupture in mouse brachiocephalic arteries by decreasing circulating inflammatory monocytes and suppressing the protease activity of plaque macrophages.

More recently, the importance of the family of proresolving mediators as potent suppressors of inflammation has been revealed, and as such, therapies aimed to deliver these specialized proresolution mediators could have significant potential to suppress inflammation and promote anti-inflammatory mechanisms. Spatiotemporal delivery of specialized proresolution mediators to atherosclerotic plaques using NP platforms improved the bioavailability of the mediators and enhanced the therapeutic efficacy. In a recent proof-of-concept study, proresolving mediator Ac2-26-containing polymeric NP platforms showed enhanced resolution to a much greater extent than free Ac2-26 peptide in acute inflammation settings. These NPs were decorated with collagen IV-binding peptide on the surface of the NPs for the targeted delivery to sites of injury. When evaluated for their therapeutic effect on chronic, nonresolving inflammatory conditions, collagen IV–Ac2-26 NPs showed promise in mice with advanced atherosclerotic plaques. Collagen IV–Ac2-26 NPs homed to atherosclerotic lesions and stabilized vulnerable lesions by reducing oxidative stress and collagenase activity in a myeloid-FPR2/ALX–dependent manner. These findings suggest a new modality to combat inflammation in atherosclerosis.
without compromising the host defense system by targeted delivery of proresolving mediators. In a separate study, collagen IV–targeted NPs containing anti-inflammatory cytokine interleukin 10 (IL-10) showed similar potential in the treatment of atherosclerosis and further augmented the strategy of tempo-spatial delivery of proresolving mediators. These examples of NP-delivered therapeutics provide support for the potential of plaque-specific delivery of therapeutics with less than desirable whole-body effects.

**Future Perspectives**

miRNAs have established themselves as key regulators of vascular disease and have generated tremendous excitement for their potential to be therapeutically manipulated. And although the identification of unique miRNAs continues to grow, and gene knockout and antisense models have provided some insight into the role of specific miRNAs in disease, our understanding of the cell- and tissue-specific role of miRNAs is lagging. Similarly, the presence or change in expression of a particular miRNA does not always lead to changes in its target gene expression because not all miRNAs bound to Ago2 are functionally active. On the contrary, the translation of NP-delivered miRNA therapies will require several improvements in NP platforms to maximize tissue-specific delivery, improve miRNA stability, and customize the pharmacokinetics/pharmacodynamics and biodistribution profiles. Major advances in the development of NPs for cardiovascular applications have been made in the area of diagnostic imaging, and theranostics, and developing miRNA and NPs tagged with imaging agents and studying them in animal models will improve our understanding of factors that drive plaque NP accumulation and the fate of miRNAs and vehicles once inside the plaque. In contrast to small molecules and peptides, which may readily pass through cellular compartments, miRNAs are relatively unstable and may avoid degradation during transit through the endosomal pathway to load into the RNA-induced silencing complex. These unique features of RNA-based therapies make the design of NP platforms a challenge. However the understanding of miRNAs and advances in nanomedicine technology are both moving at a rapid pace, and merging of the 2 concepts will help in the translation of both of these powerful therapeutic tools.

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