



REVIEW ARTICLE

Application of targeted therapy strategies with nanomedicine delivery for atherosclerosis

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Atherosclerosis (AS) is the main pathological cause of coronary heart disease (CHD). Current clinical interventions including statin drugs can effectively reduce acute myocardial infarction and stroke to some extent, but residual risk remains high. The current clinical treatment regimens are relatively effective for early atherosclerotic plaques and can even reverse their progression. However, the effectiveness of these treatments for advanced AS is not ideal, and advanced atherosclerotic plaques—the pathological basis of residual risk—can still cause a recurrence of acute cardiovascular and cerebrovascular events. Recently, nanomedicine-based treatment strategies have been extensively used in antitumor therapy, and also shown great potential in anti-AS therapy. There are many microstructures in late-stage atherosclerotic plaques, such as neovascularization, micro-calcification, and cholesterol crystals, and these have become important foci for targeted nanomedicine delivery. The use of targeted nanoparticles has become an important strategy for the treatment of advanced AS to further reduce the residual risk of cardiovascular events. Furthermore, the feasibility and safety of nanotechnology in clinical treatment have been preliminarily confirmed. In this review, we summarize the application of nanomedicine delivery in the treatment of advanced AS and the clinical value of several promising nanodrugs.

Keywords: coronary heart disease; advanced atherosclerosis; vulnerable plaque; nanoparticles; targeted therapy; drug delivery

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INTRODUCTION

The incidence and mortality rates of arteriosclerotic cardiovascular disease (ASCVD) are increasing year by year. Currently, strategies for the prevention of cardiovascular events include lifestyle interventions, such as diet, exercise, and smoking cessation; control of the risk factors, including hypertension, serum cholesterol levels, and diabetes; and pharmacological interventions, including administering lipid-lowering drugs to prevent plaque growth and instability and the use of antiplatelet drugs in patients with chronic coronary syndromes, but the efficacy and safety of the antiplatelet therapy in patients require further assessment in view of a balance between the prevention of ischemic events and the increased risk of bleeding. We have used advanced imaging techniques, such as optical coherence tomography (OCT), intravascular ultrasound (IVUS), or near-infrared spectroscopy (NIRS), to demonstrate that current clinical coronary artery disease treatment programs are relatively effective for early atherosclerotic plaques and can even reverse their progression [1, 2]. However, the effectiveness of these treatments for advanced atherosclerosis (AS) is not ideal, and advanced atherosclerotic plaques—the pathological basis of residual risk—can still cause a recurrence of acute cardiovascular

and cerebrovascular events after the current treatment regimens [3–5].

AS is a form of chronic inflammatory disease that leads to the deposition of lipids on the vascular wall. AS plaques begin to form in areas of impaired endothelial function in large- to medium-sized arteries, forming areas of frequent lesions. Recently, we and other researchers found that 1-palmitoyl-2-(5-oxovaleroyl)-sn-glycero-3-phosphocholine, 25-hydroxycholesterol, and other types of lipid oxidation from oxidized low-density lipoprotein (oxLDL) can impair endothelial function significantly [6–8]. Impaired endothelial function may increase the penetration of macromolecules, such as lipoprotein, and increase the expression of chemotactic molecules, e.g., monocyte chemoattractant protein 1 (MCP-1), and adhesion molecules, e.g., intercellular adhesion molecule (ICAM-1), vascular cell adhesion molecule (VCAM-1), E-selectin, and P-selectin. Moreover, the increased recruitment and accumulation of monocytes are crucial pathophysiological factors in AS [9, 10]. Monocytes differentiate into macrophages, which convert to foam cells by modifying apolipoprotein B (APO-B) containing LDL (oxidation and acetylation). Lipoprotein and immune cells in the vascular wall are subcutaneously deposited in the arteries, and the deposited LDL is transformed into oxLDL

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through the action of reactive oxygen species (ROS) secreted by the vascular cells. Macrophages are further induced to take up lipoprotein and form foam cells. These cycles constitute the first stages of AS. Lipoprotein and immune cells may decrease as inflammation subsides or lead to plaque progression, apoptosis, and neovascularization over subsequent years or decades.

The pathological features of advanced atherosclerotic lesions are a lipid or necrotic core composed of a large amount of lipid or necrotic cell deposits, increased numbers of inflammatory cells, a thin fibrous cap, and unstable neovascularization in the plaques. Immune cells release inflammation-degrading enzymes, such as matrix metalloproteinases (MMPs), which increase plaque instability. The clinical manifestations are erosion and rupture of the atherosclerotic plaques followed by thrombosis, which eventually leads to coronary lumen obstruction and adverse cardiovascular events. We and others have found that the formation of the atherosclerotic plaque is accompanied by thickening of the arterial intima. When the intima thickens to a certain extent, local hypoxia occurs, and the microvessels that originate from the outer membrane and nourish the blood vessels extend to the bottom of the plaque [11–13]. Subsequently, the process of neovascularization begins in plaques as a compensatory defense mechanism to restore nutrient supply to the vascular wall [14]. Neovascularization can promote cell transport and the recruitment of immune cells, which in turn promote inflammation and plaque progression and can even lead to plaque rupture. The lack of vascular wall cells and the poor connectivity of surface endothelial cells results in the high permeability and osmotic brittleness of the plaques; the vascular wall becomes prone to leakage and rupture, leading to internal bleeding, which increases the instability of the late atherosclerotic plaque.

Nanomedicine is defined as the application of nanotechnology in the monitoring, control, diagnosis, and treatment of biological systems [15]. Nanomedicine was originally developed in the field of anticancer therapy research, and recent studies have indicated that nanomedicine shows great potential for the diagnosis and treatment of AS [16]. The mechanisms behind the formation of late-stage atherosclerotic plaques are complex, and multiple molecular targets are known to play important roles. The systemic treatment strategies used in clinical practice reflect the limitations and substantial systemic side effects of current therapies. Therefore, strategies that specifically target multiple molecular sites or the late-stage atherosclerotic plaque microstructure could help to combat these limitations. Furthermore, nanomedicines can overcome the problem of rapid kidney excretion, thus maintaining the drugs in the blood circulation for longer periods. This characteristic is conducive to the extravasation of the vascular system so that the nanomaterials can accumulate and be adequately distributed throughout the required tissues or organs, achieving the maximum therapeutic effect at the minimum drug dose. In this paper, the use of nanomedicines in the treatment of advanced AS is systematically summarized (Table 1), and the potential clinical application and value of several promising nanomedicines, as well as the challenges faced in the development of nanomedicines, are discussed in depth.

COMPOSITION OF NANOCARRIERS FOR THE TREATMENT OF ATHEROSCLEROSIS

Nanoparticles (NPs), which typically range from 1 to 100 nm, are similar in size to biological macromolecules, such as proteins and DNA. Unlike bulk materials, the intrinsic physical properties of nanomaterials vary. Nanocarriers may consist of organic materials (such as lipids, peptides, glycosylated compounds, hyaluronic acids, and even nucleic acids), metals, inorganic materials (such as iron oxide and gold), or different combinations of these materials. The NP surface can be modified with peptides, polymers, or antibodies that facilitate the selection of specific targets in cells or

tissues, increasing drug availability and avoiding systemic side effects. The most commonly used are FDA-approved polymer nanocarriers, which are combined with standard drugs to improve absorption and bioavailability. Among them, lipid NPs, represented by liposomes, are the most widely used targeting strategies for atherosclerotic plaques. In the early stages of AS, liposomes targeting the endothelial dysfunction and lipid accumulation of macrophages are used, and in the later stages, a combination of liposomes and drugs targeting the ruptured edges of vulnerable plaques has been proven to be effective at low doses [17]. Liposomes are a type of traditional nanomedicine delivery carrier composed of a lipid bilayer. Depending on whether the interior of the liposome is lipophilic or hydrophilic, they can be used to carry lipophilic or water-soluble medicine, and cationic liposomes can also carry DNA, RNA, and other nucleic acids [18, 19]. The problem with exogenous nucleic acids, especially siRNA, miRNA, and other RNA molecules, is that they are quickly degraded during transport and cannot easily pass through the target cell membranes; however, packaging nucleic acids in liposomes helps to combat these barriers, allowing them to carry out functions inside the target cells [20].

An important mechanism that limits the use of nanocarrier liposomes to deliver traditional medicines is the human mononuclear phagocyte system (MPS), which recognizes NPs as foreign substances and engulfs them, altering the duration of drug retention in the blood and affecting drug distribution to tissues [21, 22]. The standard technique used to solve this problem is to modify the surface of NPs with polymers, such as polyethylene glycol (PEG), a process known as pegylation [23]. Because of the highly flexible and hydrophilic properties of PEG, the hydration layer formed by PEG can effectively reduce the adsorption of undesirable proteins on the surface, avoiding immune recognition and clearance, prolonging drug circulation, and enhancing targeted medicine delivery [24]. In response to the problem that pegylated drug-carrying NPs trigger an immune response and increase the risk of being cleared by the immune system [25], many biomimetic membranes, such as those of red blood cells, white blood cells, platelets, and endothelial cells, have been used to encapsulate NPs. The use of these membranes can avoid macrophage phagocytosis and improve the utilization rate of drugs, and many studies have shown that the therapeutic effect is better than that with pure PEG nanocarriers [26, 27]. In addition to liposomes, various micelles, such as chitosan and mannitol micelles; polymers, such as polyglycolic acid, poly lactic-co-glycolic acid (PLGA), and β -cyclodextrin; lipoproteins (natural and artificially modified); and other materials have been increasingly used in recent years for nanomedicine delivery. Nanocarriers of the same drug that act on different molecular targets, cells, or tissues can exert a variety of different effects in the body. Composite nanomaterials, which are used in multiple structures or material combinations for different drug applications, also provide additional strategies for the treatment of AS [28].

TARGETING ADVANCED ATHEROSCLEROTIC PLAQUES WITH NANOPARTICLES

The ongoing research and development of nanomaterials means that the approaches and mechanisms for targeted nanomedicine delivery to atherosclerotic plaques are continuously evolving (Fig. 1). However, the strategies can be summarized as two main types [29].

The first is nonspecific targeting, also known as passive targeting. The tight connectivity of normal endothelial cells (<2 nm) limits the distribution of NPs, whereas the presence of large gaps in dysfunctional endothelial cells allows large molecules and NPs to seep out of local areas of plaque neovascularization. Arterial wall endothelial permeability increases in atherosclerotic lesions, allowing more lipoproteins and small particles, such as NPs, to migrate to the intimal layer [30]. Sites of neovascularization are

Table 1. Use of nano-drugs in the treatment of advanced AS.

Therapeutic strategy	Drug	NPs	Target	Drug delivery	Animal model	In vivo fundings
Regulation of lipid metabolism	miR-33, miR-206, miR-223 [52]	chNPs: PEG and chitosan	Macrophage	Intrinsic targeting	C57BL/6 mice	In vivo, mice treated with miR-33-chNPs showed decreased reverse cholesterol transport (RCT) to the plasma, liver, and feces.
	HDL [48]	rHDL: phospholipids, cholesterol and Apo-AI	ABCA1 transporter	Intrinsic targeting	Sprague-Dawley rats; ApoE ^{-/-} mice	In rats, release of inflammatory factors↓. In ApoE ^{-/-} mice, the aortic plaque area↓, lipid-rich plaques↓
	PCSK9 siRNA [44]	Lipidoids: cholesterol, the phospholipid DSPC PEG2000-DMG and siRNA	Hepatocytes and immune cell	Intrinsic targeting	C57BL/6 mice; nonhuman primates	PCSK9 expression and plasma LDL-C levels↓
	Na ₂ SeO ₃ [53]	Selenium nanoparticles (SeNPs): GSH and BSA	Liver and blood vessel	Intrinsic targeting	ApoE ^{-/-} mice	Serum total cholesterol, triglyceride, LDL level↓, HDL level↑; the NO level and the activities of glutathione peroxidase (GPx), superoxide dismutase (SOD) and catalase in the serum and liver↑; foam cells and lipids in aortic plaques↓
Reduction of dead cell accumulation and plaque necrotic cores	IL-10 [55]	Col-IV IL-10 NP22 PEG and PLGA	Type IV collagen	Specific targeting	LDLR ^{-/-} mice	Efferocytosis in plaques in the aortic root↑; thickness of the fibrous-cap↑; size of necrotic cores↓
	Rapamycin [60]	RBC/RAP@PLGA: PLGA nanoparticles cloaked with the cell membrane of RBCs	Pathological lesion	Non-specific targeting	ApoE ^{-/-} mice	Atherosclerotic plaques area of aortic↓; lipid-rich plaques↓; size of necrotic cores↓; macrophage infiltration and MMP-9 expression in plaques↓
	Rapamycin [61]	PNP: platelet membrane-coated nanoparticles	Pathological lesion	Non-specific targeting	ApoE ^{-/-} mice	Atherosclerotic plaques area of aortic↓; lipid-rich plaques↓; size of necrotic cores↓; macrophages in plaque↓; collagen fibers and smooth muscle cells in the plaque↑
	Rapamycin [34]	ROS-sensitive material (Ox-bCD) and acid-labile material (Ac-bCD), both by functional modification of cyclodextrins	Pathological lesion	Non-specific targeting	ApoE ^{-/-} mice	Atherosclerotic plaques area of aortic↓; lipid-rich plaques↓; size of necrotic cores↓; macrophage infiltration and MMP-9 expression in plaques↓; collagen fibers in the plaque and thickness of the fibrous-cap↑
	TPCD NP [33]	TPCD NP: conjugating a superoxide dismutase mimetic agent Tempol and a hydrogen peroxide-eliminating compound of phenylboronic acid pinacol ester onto a cyclic polysaccharide β-cyclodextrin	Macrophages and vascular smooth muscle cells	Non-specific targeting	ApoE ^{-/-} mice	Cholesterol crystals in plaques↓; size of necrotic cores↓; macrophage infiltration and MMP-9 expression in plaques↓; thickness of the fibrous-cap↑
Selective anti-inflammatory therapy	Rapamycin [71]	Leukosomes: integrates leukocyte-derived membrane proteins into the phospholipid bilayer.	Endothelium at the pathological lesion	Non-specific targeting	ApoE ^{-/-} mice	Proliferation of macrophages in aortic↓; the release of inflammatory factors and proinflammatory factor↓; atherosclerotic plaques area of aortic↓; MMP-9 activity↓
	Hyaluronan [70]	Hyaluronan Nanoparticles (HA-NPs)	CD44 cell surface receptor	Specific targeting	ApoE ^{-/-} mice	Atherosclerotic plaques area of aortic↓; macrophages in plaque↓; thickness of the fibrous-cap↑
	Paclitaxel and methotrexate [67]	LDE: lipid core nanoparticles resemble the low-density lipoprotein structure	LDL receptor	Specific targeting	New Zealand white rabbits	Under LDE-PTX + LDE-MTX treatment, atherosclerotic plaques area of aortic and intima area↓; macrophage infiltration and MMP-9 expression in plaques↓
	MnO ₂ [73]	EC-BSA-MnO ₂ : dissociable MnO ₂ nanoparticle core shielded in the plasma membrane of endothelial cells (ECs).	Integrin β3 on EC surfaces	Specific targeting	ApoE ^{-/-} mice	Macrophage accumulation in plaques↓; atherosclerotic plaques area of aortic↓
Targeting neovascularization in plaques	Anti-miR-712 [76]	Cationic lipoparticles (CCLs) coated, decorated with peptide (VHPK)	Vascular cell adhesion molecule 1 (VCAM-1)	Specific targeting	ApoE ^{-/-} mice	Silencing of inflammatory endothelial cells↓; Plaque area and degree of left common carotid artery stenosis↓
	Fumagillin [79]	αvβ3-targeted paramagnetic nanoparticles	αvβ3-integrin	Specific targeting	Hyperlipidemic rabbits	Angiogenesis in plaque↓; combined with atorvastatin, the antiangiogenic effect was more significant and sustained

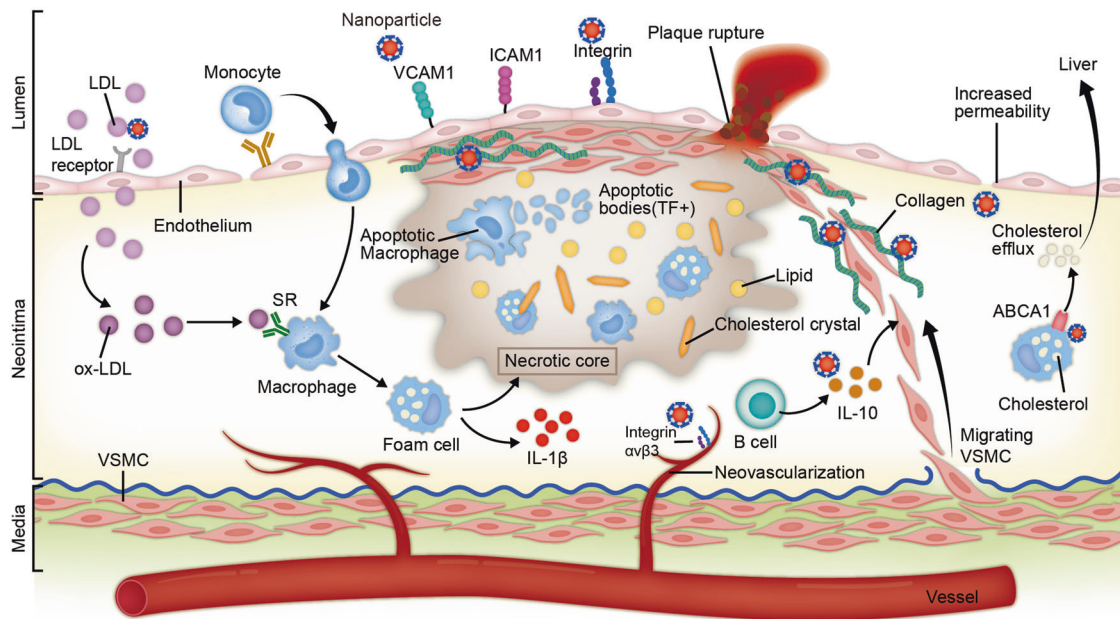


Fig. 1 Nanomedicine-based strategies for targeting advanced atherosclerotic plaques. The nanoparticles (NPs) circulating in the blood enter plaques either through increased permeability (nonspecific targeting) or through specific receptors (e.g., vascular adhesion molecule 1 (VCAM-1), low-density lipoprotein (LDL) receptor and integrin) on the surface of vascular endothelial cells (specific targeting). After entering the plaque, some nanoparticles were targeted to the lesion through nonspecific targeting due to their own characteristics (such as the use of responsive nanomaterials to target the acidic or inflammatory microenvironments). Others specifically target certain cells (e.g., macrophages, endothelium and vascular smooth muscle cells) or the extracellular matrix, such as collagen, with targeting peptides on their surfaces.

vulnerable to leakage and to the enhanced permeability and retention effect (EPR effect), which leads to NP aggregation in the lesion [31]. The use of responsive nanomaterials to target the common microenvironments of different diseases is a nonspecific targeting method that has emerged in recent years and is achieving significant therapeutic effects in the fields of tumor [32] and cardiovascular treatment [33]. Dou et al. used β -cyclodextrin (β -CD)-based pH-reactive NP (Ac-bCD) and ROS-reactive NP (Ox-bCD) to encapsulate rapamycin (RAPA) to treat AS model mice; the therapeutic effect was found to be better than RAPA monotherapy and nonresponsive NP (PLGA)-wrapped RAPA [34].

The second is specific targeting, which is also known as active targeting. NPs bind to specific cells or molecules in the lesion through their surface ligands, and thus, the process of reaching the lesion site is considered active targeting. Scavenger receptors on the surface of macrophages (MSR-A, SR-BI, and CD-36), mannose receptors on the surface of macrophages (CD206), (VCAM-1) secreted by endothelial cells, a specific marker of neovascular endothelial cells in plaques known as integrin $\alpha v \beta 3$, and type IV collagen exposed on the surface of advanced atherosclerotic plaques, as a result of endothelial connection disruption, can all be used as targets to direct nanomedicine into plaques, allowing it to play a local therapeutic role [35]. In addition, specific accumulation of therapeutic agents can also be achieved through the intrinsic targeting of natural NPs. Artificially modified lipoproteins retard the progression of AS by increasing serum APOA1 levels, reversing cholesterol transport, and regulating inflammation [36]. An siRNA for the PCSK9 gene encapsulated in a cationic liposome significantly reduced PCSK9 expression and plasma LDL-C levels [37].

DIFFERENT STRATEGIES FOR STABILIZING ADVANCED ATHEROSCLEROTIC PLAQUES

Regulation of lipid metabolism and reduction of lipid-rich plaques
As an independent risk factor for AS, we found that LDL deposition not only induces oxidative stress but also damages

the endothelium [38], induces platelet activation and promotes the formation of vulnerable plaques, which is associated with the occurrence of adverse cardiovascular events, such as myocardial infarction (MI). In addition, NIRS was used to detect lipid-rich plaques associated with acute coronary syndrome and MI [3]. Our and many other studies have shown that cholesterol crystallization in plaques is also an important factor leading to plaque instability and local inflammation [39, 40]. Currently, commonly used lipid-lowering drugs, such as statins, can stabilize atherosclerotic plaques and reduce the incidence of MI. However, the residual risk associated with advanced AS plaques is still large, and the recurrence rate of cardiovascular events remains high [41]. Furthermore, side effects, such as liver damage, should not be ignored. The PCSK9-inhibitor evolocumab, a pro-protein converting enzyme, can significantly reduce circulating LDL cholesterol levels by reducing LDLR degradation and increasing LDL-C uptake; the enzyme has been approved for use in patients with familial hypercholesterolemia and cardiovascular disease who still require further LDL-C reduction at the maximum tolerated dose of statins [42]. In comparison, inclisiran, a novel PCSK9 inhibitor that silences the PCSK9 gene with siRNA, has a faster and more long-lasting therapeutic effect than evolocumab [43]. PCSK9 expression and plasma LDL-C levels were significantly reduced in high-risk cardiovascular patients in phase II clinical trials. The key to overcoming the difficulties with using siRNA, such as the impenetrability of cell membranes and the instability of exogenous RNA in the blood, is to coat the siRNA with lipidoids [44], as this facilitates their efficient delivery to the liver and inhibits the synthesis of PCSK9 in the liver.

Serum high-density lipoprotein (HDL) levels negatively correlate with the risk of coronary heart disease (CHD), mainly via reverse cholesterol transport (RCT), by preventing lipid accumulation by transporting excess cholesterol from surrounding cells and tissues to the liver for excretion and modulating inflammation to prevent cardiovascular disease. However, we found that normal anti-inflammatory HDL converts to proinflammatory HDL in AS, which not only loses the function of protecting blood vessels but also

damages the vascular endothelium [16, 45, 46]. Therefore, cardiovascular events cannot be prevented by raising HDL cholesterol levels. The direct injection of recombinant HDL (rHDL) particles and boosting of APOA1 levels can have antiatherosclerosis effects. A recent work suggested the value of HDL-mimicking NPs for AS [47]. rHDL particles are self-assembled from phospholipids, cholesterol, and apo-AI, and those particles composed of different phospholipids have different inhibitory effects on the release of inflammatory factors, narrowing the aortic atherosclerotic plaque area, reducing the content of lipid-rich plaques, and delaying the progression of AS in ApoE^{-/-} mice [48]. Several clinical studies also confirmed the efficacy of different types of rHDL for plaque regression and modification of the lipoprotein composition in patients [49, 50].

The cholesterol efflux of macrophages is the first and most important step of RCT and is related to the protective role of a unique form of selective autophagy called lipophagy against atherosclerosis in macrophages. Excess lipids in atherosclerotic plaques are carried to lysosomes and cleared by lipophagy [51]. Recently, researchers have used chitosan nanoparticles (chNPs) composed of PEG and chitosan polymers to transfer exogenous miR-33 into macrophages [52], reducing the expression of the target gene ABCA1 and the outflow of cholesterol from macrophages to apolipoprotein A1 (ApoA1). In contrast, chNPs coated with pro-exudative miRNAs, such as miR-206 and miR-223, had anti-AS effects in mice by upregulating ABCA1 expression and RCT. This confirms that miRNAs in NPs can be efficiently delivered to macrophages, where they can regulate ABCA1 expression and RCT. Recently, Guo et al. confirmed for the first time that selenium nanoparticles (SeNPs: synthesized from Na₂SeO₃ and other materials) regulate cholesterol metabolism through antioxidant selenium protein, reduce oxidative stress and therefore reduce hyperlipidemia and vascular damage in ApoE^{-/-} mice on a high-cholesterol and high-fat diet [53]. SeNPs that focus on oxidative stress and treat AS through multiple pathways, such as metabolism and immunity, have promising potential applications for future AS treatment strategies.

Reduction in dead cell accumulation and plaque necrotic cores
The large necrotic core and thin-cap fibroatheromas (TCFAs) are key components of atherosclerotic vulnerable plaques. TCFAs with large necrotic cores are associated with poor prognosis in AS [9]. Reducing the size of the necrotic core or increasing the thickness of the fibrous cap undoubtedly increases the stability of advanced atherosclerotic plaques. The increased senescence and apoptosis of smooth muscle cells and the accumulation of oxLDL and other kinds of lipoproteins promoted the transformation of macrophages into foam cells, all of which increased the instability of late atherosclerotic plaques [54]. Macrophages with impaired function in advanced AS cannot clear a large number of dead cells that have engulfed lipids through efferocytosis, which results in the outflow of a large number of lipid necrotic cells and the infiltration of a large number of inflammatory cells. The anti-inflammatory cytokine interleukin-10 (IL-10) can block the accumulation of inflammatory cells and promote their excretion; remove pathogens, cell debris, and inflammatory cytokines; stimulate efferocytosis; and repair damaged tissues. Kamaly et al. developed a kind of sustained-release polymer NP (Col-IV IL-10 NP22) consisting of PEG and PLGA and containing IL-10. The NPs can enter atherosclerotic plaques through disturbed endothelial connections to bind with exposed type IV collagen (Col-IV) and release IL-10; they can be used to treat LDL^{-/-} mice with advanced AS by increasing efferocytosis in plaques in the aortic root, increasing the thickness of the fibrous cap, and shrinking necrotic cores to reduce the formation of vulnerable plaques [55].

Autophagy plays a unique role in the delicate control of cell fate in the development of AS and neointimal hyperplasia in postpercutaneous coronary intervention restenosis [56]. In

advanced AS, dysfunctional macroautophagy can make cells susceptible to apoptosis stimulation and impaired clearance of apoptotic cells, thus accelerating the progression of plaque [57]. RAPA, a targeted inhibitor of the mammalian target of RAPA (mTOR) pathway, induces autophagy to promote macrophage clearance, maintains plaque stability through autophagy-mediated cell death, reduces lipid accumulation, and reduces inflammation in plaques. Because of the side effects of the systemic administration of RAPA, such as hypertriglyceridemia, hypercholesterolemia, and interstitial lung disease, RAPA drug-eluting stents are currently used to reduce vascular inflammation and prevent restenosis after angioplasty by retarding vascular smooth muscle cell phenotype switching and hyperproliferation [58]. However, RAPA may cause systemic hyperlipidemia possibly because it suppresses LDL receptor expression in the liver. Furthermore, in advanced atherosclerosis plaques, excessive autophagy stimulation may present obstacles for the treatment of AS. Therefore, the optimal blood concentration of RAPA needs to be determined, and better delivery methods are needed to reduce systemic side effects. Studies have suggested that RAPA-containing liposomes have a high encapsulation rate of RAPA and a sustained-release effect of the release of RAPA [59]. Furthermore, numerous studies have demonstrated the antiatherogenic effectiveness of nanocarriers combined with RAPA in vivo; at the same time, there was no significant change in plasma lipoprotein and cholesterol and no significant adverse effects with long-term treatment [34, 60, 61].

Excess ROS can cause oxidative stress by acting as a signal to maintain physiological vascular homeostasis. Oxidative stress can promote the accumulation of dead cells in plaques by mediating the apoptosis of macrophage foam cells, thus causing secondary necrosis and increasing plaque instability [62]. Zhang et al. [33] modified β -cyclodextrin (β -CD) using a pinol hydroperoxide scavenging compound (a ROS response unit) of benzene borate and developed a broad-spectrum ROS scavenging nanomedicine system with antioxidant stress activity and a targeted effect, which was named TPCD NP. TPCD NP alleviated ROS-induced macrophage inflammation and apoptosis and effectively inhibited foam cell formation. Furthermore, TPCD NPs increased the stability of plaques in the aortic root of ApoE^{-/-} mice fed a high-fat diet for 12 weeks, reduced cholesterol crystals in plaques, shrunk necrotic cores, reduced macrophage infiltration and MMP-9 expression in AS plaques, and increased the thickness of the fibrous cap.

Selective anti-inflammatory therapy

The inflammatory reaction is involved throughout the formation and development of AS. Macrophages and inflammatory factors play key roles in endothelial dysfunction, the lipid deposition of foam cells, and necrotic core formation. We found that reducing chronic inflammation and modulating immune system responses are likely to be the most promising therapeutic strategies, and they are currently the most popular targets for the treatment of AS [63, 64]. The Canakinumab Anti-inflammatory Thrombosis Outcome Study (CANTOS) demonstrated that systemic anti-inflammatory therapy, i.e., interleukin-1 β antibody canakinumab, reduced the incidence of cardiovascular disease in high-risk populations [65]. However, systemic targeted inflammation has the potential to inhibit innate immunity and disrupt host defenses against infection, and infections treated with anti-inflammatory therapy in the CANTOS trial had a higher mortality rate. Nanomedicine delivery can reduce systemic side effects by targeting anti-inflammatory agents locally. Methotrexate (MTX), a commonly used anti-inflammatory drug for autoimmune diseases, has been shown in recent years to reduce the risk of cardiovascular disease due to chronic inflammation. A previous clinical trial using a lipid nuclear nanoparticle (LDE) with a similar structure to LDL to carry paclitaxel (PTX) showed that these compounds reduce the toxicity of anticancer chemotherapeutic

agents and have the potential to reduce the atherosclerotic lesion size in patients with cardiovascular disease [66]. Furthermore, Gomes et al. combined LDE-PTX treatment with methotrexate (MTX) associated with LDE (LDE-MTX) to treat atherosclerotic lesions in rabbits fed a high-fat diet and showed that the therapeutic effect of LDE-PTX was enhanced when combined with LDE-MTX [67]. However, the effectiveness of the treatment has yet to be confirmed in clinical trials. In addition to the delivery of anti-inflammatory drugs, NPs can be used to target tissues via their own anti-inflammatory properties. For AS, these properties can lead NPs to accumulate in the lesion to provide a therapeutic effect. Hyaluronic acid regulates cell adhesion, migration, and proliferation by binding to CD44 expressed on the surface of endothelial cells at the site of atherosclerotic inflammation. At sites of inflammation, the hyaluronic acid-rich microenvironment promotes tissue infiltration through the division of immune cells. Moreover, the biological characteristics of hyaluronic acid are largely determined by its polymerization, and highly polymerized hyaluronic acid has been proven to inhibit inflammation and have antiangiogenesis effects [68, 69]. Hyaluronic acid nanoparticles (HA-NPs) prepared with hyaluronic acid as the framework have been proven to have good plaque stabilization and anti-inflammatory effects in ApoE^{-/-} mice [70]. Chronic inflammation leads to impaired endothelial function, and when overexpressed, activated adhesion molecules, such as VCAM-1 and ICAM-1, can bind to white blood cells and transfer directly to the vascular wall. Leukosomes, biomimetic NPs designed using this approach, can accumulate at the site of vascular injury by simulating the distribution of white blood cells in AS, deliver drugs to the lesion site and exert an anti-inflammatory effect on the vessel wall. For example, delivery of RAPA can treat AS by inhibiting the release of inflammatory factors, inhibiting the proliferation of macrophages, and narrowing the necrotic core of plaques [71]. This is one example of successful synergistic complementary treatment of a disease by combining the properties of NPs with the properties of drugs.

In recent years, our knowledge of plaque rupture mechanisms, including hemodynamic and physiological factors, such as shear stress and fluid dynamics, has increased. Endothelial shear stress caused by endothelial surface friction is closely related to the pathogenesis of AS, the formation of plaques, and the development of plaque vulnerability [40]. Laminar shear stress (LSS) induced by stable blood flow has anti-inflammatory and protective effects on AS. LSS rapidly stimulates the conformational activation of the shear force sensor integrin β 3, inhibits downstream inflammatory signals, reduces mononuclear cell infiltration, and delays the occurrence of AS [72]. Gao et al. designed a type of MnO₂ NP that accurately activates integrin β 3. The biomimetic NPs form on the surface of endothelial cell membranes, preventing the removal of circulating NPs by the MPS; the biomimetic NPs, with reductive dissociation properties, have been shown to be effective high-efficiency integrin activators to inhibit downstream inflammatory signals and plaque formation [73].

Targeting neovascularization in plaques

Using OCT and IVUS to explore the characteristics of vulnerable plaques, we and other researchers have shown that patients with CHD with neovascularization in nonculprit plaques show more vulnerable OCT features [74, 75]. Moreover, plaques containing neovascularization are still at high risk even under statin therapy [74], and the multiple molecular targets of neovascularization in plaques highlight the need for nanomedicine therapy. VCAM-1, an adhesion molecule secreted by activated vascular endothelial cells at the site of atherosclerotic lesions, recruits inflammatory cells to the activated endothelial surface. Therefore, nanocarriers can target endothelial cells in AS plaques by carrying peptides specifically identified by VCAM-1 [76]. Kheiriloom et al. modified the VHPK peptide on the surface of cationic liposomes

containing anti-miR-712 molecules to construct a VHPK-CCL-anti-miR-712 NP that specifically targeted the aortic endothelial cells of mice. Furthermore, *in vivo* studies showed that selective and efficient silencing of inflammatory endothelial cells played an anti-AS role, and miRNA delivery was highly selective to the target organ [77].

Angiogenesis in atherosclerotic plaques is regulated by a variety of cytokines and platelet growth factors, one of which is vascular endothelial growth factor (VEGF). Anti-VEGF drugs and many other antiangiogenic drugs have been successfully used in a number of studies to combat tumor angiogenesis and extend the survival of patients with cancer [78]. In addition, integrin α v β 3 has long interested researchers as a target in atherosclerotic plaques, and the delivery of fumrithromycin-NPs combined with atorvastatin to combat angiogenesis in plaques has the potential to substantially improve the stability of plaques [79]. However, whether the targeted inhibition of neovascularization in plaques in the early stages of AS can reduce the occurrence of adverse clinical events to improve the prognosis of atherosclerotic heart disease remains to be further studied and tested.

FUTURE DIRECTIONS AND CHALLENGES

The use of targeted NPs in the treatment of AS has become a hot topic and has provided many improved strategies and new research avenues for the treatment of patients with cardiovascular diseases. The precise therapeutic targets and the successful delivery of small molecules *in vivo*, even nucleic acids, provided by nanomedicine have created a potential therapeutic approach for AS that uses genetic information for accurate medicine and customized personalized therapy to improve the efficacy and reduce adverse reactions. For example, numerous genetic studies have demonstrated the role of target genes such as LDLR, APO-B, and PCSK9 and established the association between high LDL cholesterol levels and ASCVD [80]. In addition, a variety of miRNAs serve a vital cellular and molecular regulatory function in the formation and development of atherosclerotic plaques [81]. Using regulatory genes encapsulated in NPs to regulate the expression of target genes has been implemented in studies [37, 52]; perhaps we can modify NPs to carry out individualized treatment for different genetic backgrounds in the future. Therefore, nanomedicine-aided gene regulation is a potential therapeutic strategy in AS.

With the development of nanotechnology and the demand for effective treatments for cardiovascular diseases, a variety of new nanomaterials combined with different therapeutic drugs and new therapy targets are continuously being discovered and applied in animal models, many of which have shown potent and specific therapy effects, while some have been used in clinical trials for the treatment of AS [49, 50, 66]. Nanointervention with the delivery of silica-gold NPs has been proven to reduce total atheroma volume more in patients who have CAD with target lesions than in those who accepted stent implantation [82]. This study showed that the delivery of NPs with a bioengineered patch has a more pronounced effect and a more significant level of safety for AS, but the optimal technique of delivering NPs into the target tissue is still a key limitation. In addition, nanointervention is an invasive treatment. The feasibility and safety of NPs in clinical treatment have been preliminarily confirmed, but certain limitations remain in further clinical development with regard to their structural design, stability, targeting specificity, and toxicity. Therefore, to develop nanomedicine-based therapies as treatment methods that can be used in clinical practice, they need to be continuously optimized and subjected to medicine-based testing. Currently, nanotechnology has shown great potential for the diagnosis and treatment of cardiovascular diseases, and further research should focus on improving its stability and clinical translation effectiveness by increasing studies on the interaction

between bionanomaterials and various components of plaques. As our knowledge increases and the therapeutic mechanisms involved are further explored and improved, nanomaterials have the potential to unlock an array of innovative research fields in the diagnosis and treatment of cardiovascular diseases.

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ADDITIONAL INFORMATION

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