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Nanomedicine Approaches for Advanced Diagnosis and Treatment of Atherosclerosis and Related Ischemic Diseases

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Cardiovascular diseases (CVDs) remain one of the major causes of mortality worldwide. In response to this and other worldwide health epidemics, nanomedicine has emerged as a rapidly evolving discipline that involves the development of innovative nanomaterials and nanotechnologies and their applications in therapy and diagnosis. Nanomedicine presents unique advantages over conventional medicines due to the superior properties intrinsic to nanoscopic therapies. Once used mainly for cancer therapies, recently, tremendous progress has been made in nanomedicine that has led to an overall improvement in the treatment and diagnosis of CVDs. This review elucidates the pathophysiology and potential targets of atherosclerosis and associated ischemic diseases. It may be fruitful to pursue future work in the nanomedicine-mediated treatment of CVDs based on these targets. A comprehensive overview is then provided featuring the latest preclinical and clinical outcomes in cardiovascular imaging, biomarker detection, tissue engineering, and nanoscale delivery, with specific emphasis on nanoparticles, nanostructured scaffolds, and nanosensors. Finally, the challenges and opportunities regarding the future development and clinical translation of nanomedicine in related fields are discussed. Overall, this review aims to provide a deep and thorough understanding of the design, application, and future development of nanomedicine for atherosclerosis and related ischemic diseases.

1. Introduction

1.1. Pathophysiology of Atherosclerosis and Related Ischemic Diseases

Atherosclerosis is the usual cause of myocardial infarction (MI).[1] An estimated 12 million deaths arising from coronary atherosclerosis are projected to occur by the end of 2030, mostly due to coronary disorders such as non-ST-segment elevation MI and ST-segment elevation MI.[2] Cardiac ischemia, if not treated early, results in a cascade of irremediable processes leading to cell apoptosis, tissue-damaging necrosis, and contractile dysfunction.[1] A thorough study on the cellular and molecular mechanisms that trigger atherosclerosis and related ischemic diseases has led to an agreed interpretation of these events (**Figure 1**).[3] The commencement and the development of the diseases are highly multifaceted, and this section seeks to provide mechanistic insights of atherosclerotic formation and progression.

The formation of an atherosclerotic plaque begins at lesion-prone regions in average and large-sized arteries where the endothelium is disordered. Plaque formation is influenced by cardiovascular risk factors such as chronic alcoholism, smoking, diabetes, hypertension, obesity, and hyperlipidemia.[4] Disordered endothelium is a vital pathophysiological element in atherosclerosis, causing heightened penetration of lipoproteins, proliferative expression of adhesion molecules (e.g., vascular cell adhesion molecule (VCAM)) and chemotactic molecules like monocyte chemotactic protein 1 (MCP-1) with enhanced monocyte recruitment and build-up.[4b] The monocytes successively differentiate into macrophages, which form foam cells by phagocytizing apolipoprotein B containing low-density lipoproteins (LDL).[5] The retention of immune cells together with lipoproteins in the vessel wall constitutes the principal stage of atherosclerosis development. The immune cells and lipoproteins within the subendothelium can either resolve the inflammation or result in the evolution of plaque, neovascularization, and cell death over a long period, in some cases over a period of decades.[6] The progressive atherosclerotic disease could

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ultimately contain a large proportion of necrotic cells and lipids in the plaque, known as the necrotic core.

The vasa vasorum (VV) are structural networks with effective end arteries which exist all over the human anatomy.[7] The development of the VV serves to remove excess lipoprotein in the disordered endothelium. A ruptured VV contributes to atherogenesis and it is the cause of the most obvious symptoms experienced in clinical atherosclerosis. The VV supply blood to the parent vessels, satisfy the nutritional request of the vessel wall, and eliminate unwanted substances formed by intramural cells or presented by the luminal endothelium. In the advancement of an atherosclerotic plaque, the intima thickens and leads to a reduced distance between the inner layers of the intima and the luminal surface. Once the oxygen diffusion threshold is exceeded, the local hypoxia arises. Previous studies suggest that neovascularization is a compensatory mechanism in reaction to malnourishment and hypoxia.[8] This process stabilizes plaque integrity. In advanced stages of atherosclerosis nevertheless, the neovessels turn out to be conduits for numerous inflammatory cells (particularly macrophages) and also allow cells to relocate in the intima, thus destabilizing the atherosclerotic plaque integrity.[8] Plaques with a high volume of new microvessels are at a major risk of rupture, hemorrhage, atherothrombosis, and expansion. Since they are conduits for transporting inflammatory cells, lipids, and erythrocytes, VV neovessels appear to be complex in atherosclerosis.

The artery wall is altered during the progression of an atherosclerotic lesion due to the accumulation of plaque caused by lipoproteins, immune cells, and VV rupture within the arterial wall. The alteration occurs either outwardly or inwardly. With outward remodeling blood continuously flows to the extremities while inward remodeling results in a deficiency in blood supply to cardiac tissue leading to chest pain. Due to the peculiar nature of the visceral pain which initiates from the pericardium, angina can radiate to the left arm or jaw. The release of mediators (especially adenosine) from ischemic myocardium is the cause of this pain. Thrombus formation inside the lumen of the blood vessel is a result of plaque rupture.[9] Unexpected disastrous events, such as sudden cardiac arrest or stroke may be caused by the thrombus constricting the lumen of the artery. Vulnerable plaques are predisposed to thrombosis and rupture.[10] Plaque rupture may occur in a previously narrowed lumen, with a reduction of about 70% or more of the cross-sectional area.[11] Alarmingly, such plaque rupture may also occur where there is less than 65% of chronic occlusion in previously severe asymptomatic ischemia.[12] The resultant thrombus may completely or partially block the artery. Regardless, specks of the thrombus can cause embolism in target organs and occlude smaller vessels eventually leading to tissue ischemia and cell death.

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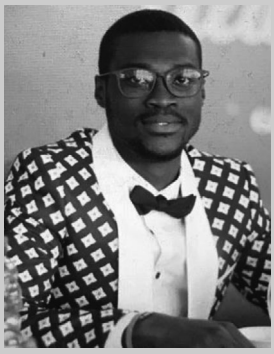

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1.2. Currently Available Therapeutic and Diagnostic Regimens

Clinical management of atherosclerosis mainly consists of surgery and pharmacological interventions. Surgical treatments are conducted under acute and aggravated conditions when atherosclerotic plaque ruptures or arterial clogging threatens the survival of local tissues. Based on the severity of the patient's arterial and tissular states, surgical therapies including angioplasty, stent placement, endarterectomy, coronary artery bypass surgery, atherectomy, etc., are conducted to ensure proper blood flow.[13] The pharmacological intervention serves as an essential way to control blood lipids, blood pressure, thrombus development, and other risk factors related to the pathological process. Small molecule drugs are most widely used in clinical applications and are classified into lipid-lowering drugs (e.g., statins), antiplatelet drugs (e.g., aspirin and clopidogrel), anticoagulants

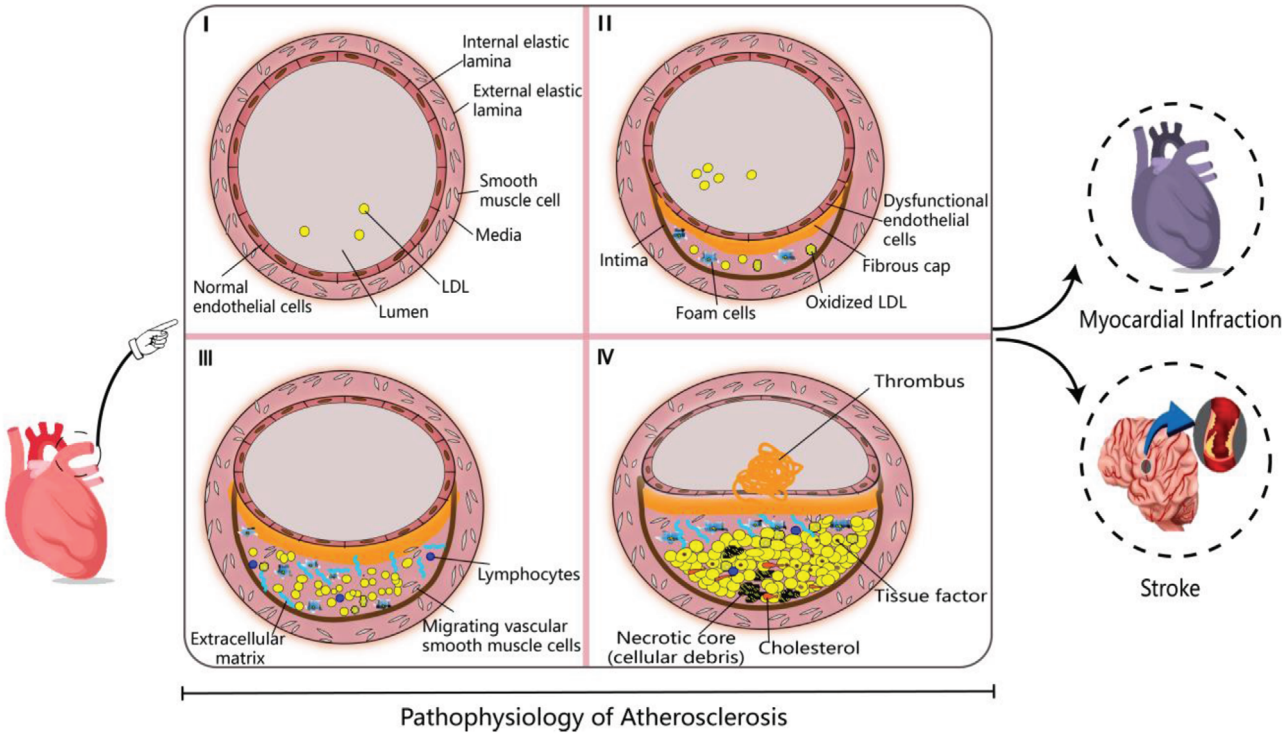

Figure 1. A schematic presentation of the pathophysiology of atherosclerosis and related ischemic diseases.

(e.g., warfarin and heparin), beta-blockers, angiotensin converting enzyme inhibitor, etc. However, most of these drugs are confronted with the challenges of low aqueous solubility and extensive first-pass effect, resulting in undesirable systemic bioavailability and severe adverse drug effects. For example, with limited water solubility and considerable molecular weight, some statins exhibit an oral bioavailability as low as 30%.[14]

The most commonplace diagnostic techniques applied in the clinical settings are referred to as arteriography which includes computed tomography (CT), magnetic resonance imaging (MRI), positron emission tomography (PET), and single-photon emission computed tomography (SPECT). Contrast agents play a significant role during imaging. Nevertheless, clinically frequently-used contrast agents, such as gadolinium-diethylenetriamine pentaacetic acid, present disadvantages of short retention time, weak signal, and potential toxic side effects and are not able to target specific organs or tissues.[15] Additionally, currently available imaging techniques perform well in the visualization of atherosclerotic plaque morphology but fail to precisely foresee the threats of plaque rupture.[16]

Despite all the advances in clinical practices, the suboptimal outcome of currently available therapeutic and diagnostic regimens for patients with atherosclerosis and related ischemic diseases requires continuous efforts to develop novel and effective resolutions.

1.3. Nanomedicine

Nanotechnology has generated revolutionary effects on many medical fields. With increasing potential in the development of novel therapeutic options, the area which involves nanotechnology and its impacts on medicine has been extensively characterized as nanomedicine. Through ingenious design and uses of nanostructured materials and nano-electronic devices, nanomedicine provides safe, efficient, precise, and personalized approaches for treatments and holds great promise to overcome the obstacles existing in currently available therapies.[17] Nanostructured materials are defined as materials/devices of nanometer size or containing nanometer-sized structures.[18] Different forms of nanostructured materials, including nanoparticles, nanocapsules, nanotubes, nanogels, and nanofibrils, have been studied for various medical applications following their biochemical and dimensional properties.[19] Nanostructured materials are also fabricated to immobilize biomolecules, serving as nanobiosensors, to analyze typical biomarkers and developmental stages of specific diseases, such as glucose nanosensors and triglyceride nanosensors.[20] More sophisticated nanodevices are manipulated to accomplish delicate tasks. For example, implantable or wearable medical devices containing nanoelectronic components can be exploited for real-time monitoring of particular physiological status.[21]

The applications of nanomedicine in the diagnosis, treatment, and prevention of atherosclerosis and related ischemic diseases have been under investigation with impressive outcomes. Nanomedicine presents advantages over conventional therapies for its superior properties. With inherently small dimensions (1–1000 nm), most nanostructured particles succeed in minimizing internal clearance mechanisms and prolonging blood circulation,[22] which improves the solubility and bioavailability of therapeutic agents. For example, nanoformulations of fenofibrate available in the market (TriCor) are used to manage

cholesterol and triglyceride of hypertriglyceridemia patients with ameliorative drug solubility and absorption.[23] The ideal size distribution of nanostructured materials also endows desired permeability through physiological barriers if designed rationally. Rapidly proliferated endothelial cells at the atherosclerotic plaque site generate deficient and leaky blood vessels, and such pathological characteristics allow the entrance of nanoparticles into the interstitial tissue and local accumulation of therapeutics with the assistance of immature lymphatic drainage system.[24] Another remarkable feature of nanoparticles is their considerable surface area.[25] This often endows the particles with more sensitive and diversified reactivity compared to bulk materials.[26] As a result, it provides more possibilities to functionalize nanoparticles, including targeting, imaging, improvement of pharmacokinetic characteristics, and integration of diagnosis and treatment compared to the currently available categories of drugs for the management of atherosclerosis. For example, Ye and co-workers developed nanoparticles combined with the phase transitional material perfluorohexane and dextran sulfate targeting class A scavenger receptors (SR-A) as a theranostic approach for diagnosis and treatment of atherosclerotic plaques.[27] Active accumulation of nanoparticles was achieved at the sites of SR-A expressed macrophages for ultrasound imaging in an atherosclerotic plaque model. From an overall perspective, nanomedicine effectively manages atherosclerosis and related ischemic diseases from different aspects of the physiological disposition of payload, including enabling stable, prolonged, and controlled delivery of payload, allowing specific interactions towards CVDs components and ensuring effective elimination within a particular time window.[28]

There have been some previously published review articles outlining the development of nanomedicine and its applications for early diagnosis or treatment of CVDs.[29] In this review, we expand on previous work and present the latest advances in nanomedicine-based regimens for atherosclerosis and associated ischemic diseases, such as restenosis and MI. In pursuit of this goal, the pathophysiology of these diseases is introduced first, followed by the elaboration of the potential targets for treatment and diagnostic purposes. The applications of nanomedicine are then discussed in detail with the most recent research outcomes in noninvasive cardiovascular imaging, biomarker detection, tissue engineering, and nanoscale delivery (**Figure 2**). We also provide an overview of the most relevant nanomedicines which have progressed through the different stages of clinical development up to the present day (**Table 1**). Finally, the challenges and opportunities of nanomedicine in related fields are discussed and our opinion on the future development of the field is proposed. This review is focused on the specialized application of nanomedicine for atherosclerosis and its related ischemic diseases in both preclinical and clinical practice, and providing milestones to accelerate the clinical translation of nanomedicine.

2. Therapeutic Targets for Atherosclerosis and Related Ischemic Diseases

Various factors, including inflammation, endothelium disorders, and abnormal blood lipid levels, are involved significantly in the pathogenesis of atherosclerosis and related ischemic diseases. Inflammation is caused by innate immune cells such as inflammatory cells secreting specific mediators.[30] Furthermore, the dysfunction of cellular components, including mitochondria and endoplasmic reticulum (ER), disrupts the homeostasis of the cellular environment and contributes to the generation of inflammation and ischemia. Injury to the arterial wall of the blood vessels causes the alteration of the extracellular matrix (ECM) which is comprised of non-cellular components and impairs blood flow to major organs of the body, especially the heart.[31] In addition, certain micro-ribonucleic acids (miRNAs) and proteins are dysregulated during the atherosclerotic disease progression, affecting the blood lipid profile.[32] Therefore, inflammatory cells and their mediators, cellular and non-cellular components, miRNAs, and proteins could potentially serve as the therapeutic targets for these diseases and are discussed below.

2.1. Inflammation: Cells and Mediators

2.1.1. Cells

Atherosclerosis is a disease of chronic inflammation that involves several cell types, including monocytes, endothelial cells, macrophages, and mast cells. Monocytes are short-lived cells and do not proliferate in the blood.[33] These cells are involved in scavenging other cells (especially dead cells) and toxic substances like oxidized LDL to produce inflammatory cytokines which later cause them to differentiate into inflammatory macrophages and dendritic cells.[34] In the development of atherosclerosis, monocytes infiltrate into the intima and subintima of the arterial wall.[35] Their scavenger receptors enable the monocytes to phagocytize oxidized lipoproteins, thus it is postulated that their accumulation and activation in the vessel wall are precipitated by fat deposits.[36]

The atherosclerotic lesion site offers an enabling microenvironment for oxidized lipoproteins, apoptotic cells, and proinflammatory mediators. Similarly, the macrophages present in atherosclerotic plaques are diverse.[37] The existence of relatively large amounts of pro-inflammatory macrophages (M1 subtype) in the atherosclerotic plaques is well documented.[38] In one example Bianca et al. prepared lipid core nanoparticles for the delivery of docetaxel (LDE-DTX) for the treatment of atherosclerosis.[39] In comparison with the control treatment options, LDE-DTX significantly reduced M1 pro-inflammatory markers leading to atherosclerotic regression. The presence of anti-inflammatory macrophages (M2 subtype) is also comparatively high during atherosclerotic disease regression.[40] The two subtypes have opposing roles in atherosclerotic lesions. The M1 subtypes are responsible for plaque rupture,[41] whereas the M2 subtypes reside in stable regions of the plaque and prevent foam cells from forming. In macrophages, liver X receptors (LXR) activation exerts a variety of atheroprotective effects by promoting cholesterol efflux, suppressing inflammation, and enhancing efferocytosis.[5] There are multiple examples of nanoparticles encapsulating a synthetic LXR agonist such as GW3965 to enhance drug delivery to atherosclerotic plaque macrophages, offering the benefits of LXR activation on atherosclerosis without adverse hepatic effects.[42]

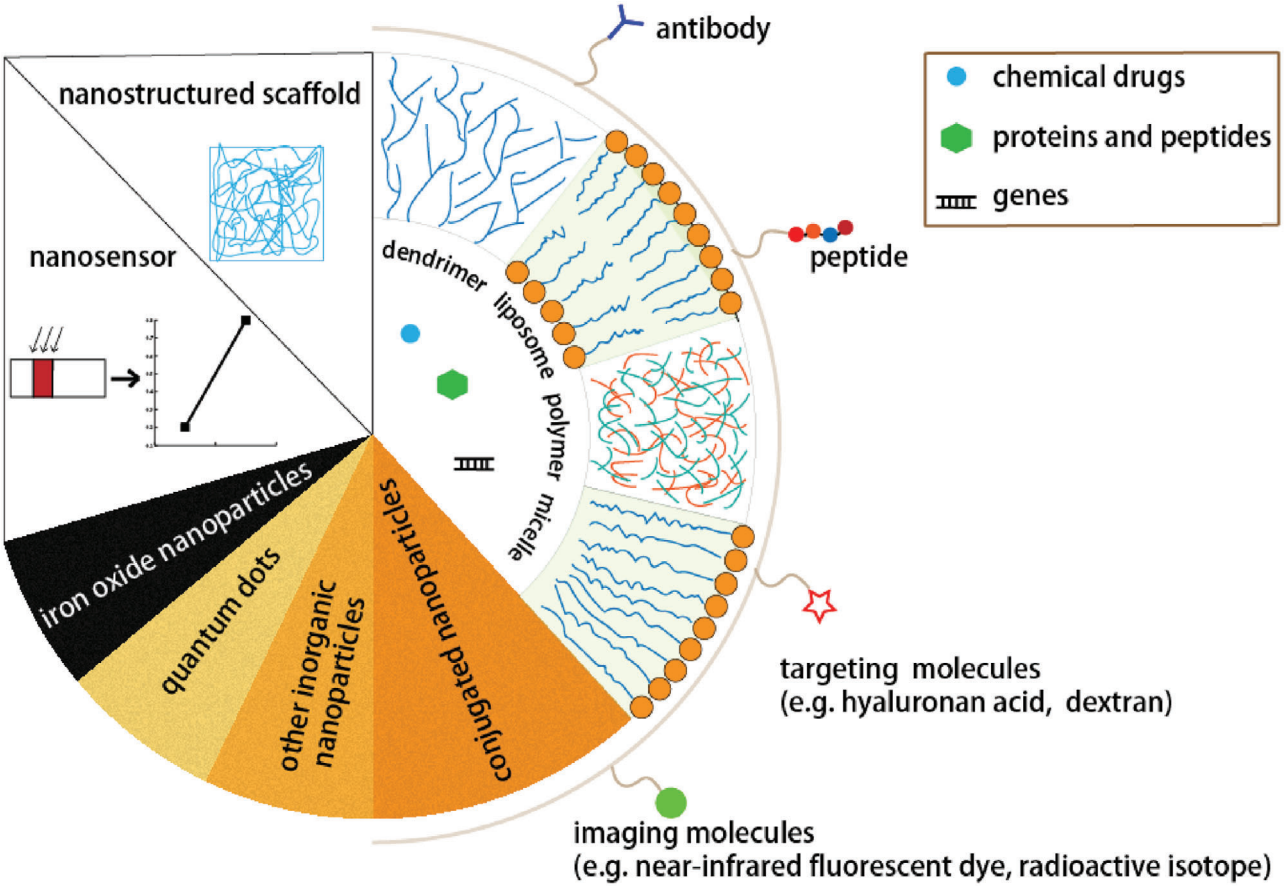

Figure 2. Diagram representing a wide range of nanostructured materials for the diagnosis and treatment of atherosclerosis and related ischemic diseases. Different types of nanoparticles, such as iron oxide nanoparticles, quantum dots (QDs), nanoparticles conjugated with imaging molecules, etc., have been investigated as noninvasive diagnostic tools for detecting pathological changes in diseases. Nanosensors facilitate the detection of CVD biomarkers at an early stage. Nanostructured scaffolds enhance the regeneration of injured cardiovascular tissues. A wide range of nanocarriers (dendrimers, liposomes, polymers, etc.) customized with diverse payloads and varying surface modifications opens up many opportunities for new and synergistic properties to emerge. Successful development may improve the overall therapeutic or diagnostic effectiveness of a nanomedicine.

Endothelial cells generate chemical vasodilators and vasoconstrictors to regulate vascular tone and structure. The vasodilators released from the healthy endothelium include nitric oxide, prostacyclin, and bradykinin, which prevent platelet aggregation and also cause fibrinolysis.[43] Production of vasoconstrictors such as endothelin and angiotensin II is also derived from the endothelium. These two mediators contribute to the proliferation of smooth muscle cells which consequently leads to plaque formation by releasing fat-soluble substances. Injury to the endothelium destabilizes the equilibrium between vasodilation and vasoconstriction and hence gives rise to events that aggravate atherosclerosis, such as platelet aggregation, endothelial permeability, leukocyte adhesion, and cytokine formation.[44] For example, Hashi et al. developed nano-fibrous scaffolds to expedite the process of curing endothelial cells in the presence of a stent by discharging nitric oxide to inhibit endothelial cell loss and platelet adhesion.[45]

Mast cells are mediators of both innate and adaptive immunity and play an essential function in wound healing and angiogenesis.[46] Research has shown that mast cells regulate the production of inflammatory mediators.[47] Stimulated mast cells can have deleterious consequences on their immediate microenvironment in the vessel wall, causing matrix degradation, cell death, and can also increase the infiltration of inflammatory cells, which are instrumental in atherosclerosis and plaque formation.[48] Upon stimulation, these cells release a host of growth factors, proteolytic enzymes, pro-inflammatory cytokines, and vasoactive mediators.[49]

Research into the function of inflammatory cells in the initiation and development of atherosclerosis and related ischemic diseases has recently intensified. Given this, nanomedicine research on CVDs has led to the discovery of new diagnostic and therapeutic regimens for patients. There is still much to learn about the roles of these inflammatory cells in atherosclerotic formation and progression. We strongly believe that further studies should be conducted on these cells to make them more reliable diagnostic and therapeutic targets.

Table 1. Nanomedicine-based regimens under clinical evaluation for atherosclerosis and related ischemic diseases.

Nanotechnology	Interventions	Indications	Sponsor	Phase	Status	Outcome	ClinicalTrials.gov identifier
Gold nanoparticles (Au NPs) with iron oxide-silica shells	Stenting and micro-infusion of nanoparticles	Coronary artery disease Atherosclerosis	Ural Medical University	Phase 1	Terminated	Data not available	NCT01436123
Silica-Au NPs	Transplantation of nanoparticles	Stable angina Heart failure Atherosclerosis Multivessel coronary artery disease	Ural Medical University	Not applicable	Completed	Efficiently targeting lesion revascularization with lower mortality and major adverse cardiovascular events at the long-term follow-up	NCT01270139
Cholesterol-rich non-protein nanoparticles	Delivery of paclitaxel	Coronary artery disease Atherosclerosis Inflammation	University of Sao Paulo General Hospital	Phase 2 Phase 3	Recruiting	Data not available	NCT04148833
Paclitaxel albumin-bound nanoparticles	Delivery of paclitaxel	Vascular disease	Celgene Corporation	Phase 2	Terminated	Data not available	NCT00518284
Paclitaxel albumin-bound nanoparticles	Delivery of paclitaxel	Angina pectoris Coronary artery disease	Celgene Corporation	Phase 2	Completed	Data not available	NCT00093223
Paclitaxel albumin-bound nanoparticles	Delivery of paclitaxel	Coronary restenosis	Celgene Corporation	Phase 1 Phase 2	Completed	Preventing in-stent restenosis with limited adverse events	NCT00124943
polyethylene glycol (PEG)-liposome	Delivery of prednisolone sodium phosphate	Atherosclerosis	Academisch Medisch Centrum-Universiteit van Amsterdam (AMC-UvA)	Phase 1 Phase 2	Unknown	Data not available	NCT01647685
PEG-liposome	Delivering prednisolone sodium phosphate to silence inflammatory activity	Atherosclerosis Inflammation	AMC-UvA	Phase 1 Phase 2	Unknown	Data not available	NCT01601106
Liposome	Delivery of alprostadil	CVDs	Guangzhou Yipinhong Pharmaceutical Co., Ltd.	Phase 1	Completed	Data not available	NCT02889822
Liposome	Delivery of alendronate	Coronary artery stenosis	BIOrest Ltd.	Phase 2	Completed	Demonstrated safety and effective modulation of monocyte behaviors	NCT00739466
Ultrasmall superparamagnetic particles of iron oxide (USPIO)	Ferumoxytol enhanced MRI	MI Inflammation	University of Edinburgh	Phase 2	Unknown	Data not available	NCT01995799
USPIO (ferumoxtran)	MRI	MI	University of Edinburgh	Not applicable	Completed	The infarcted and remote myocardium uptake USPIO following acute MI	NCT01323296
Iron oxide nanoparticles	Labeling human blood cells for MRI	MI Inflammation	University of Edinburgh	Not applicable	Suspended	Data not available	NCT01127113
^{64}Cu–CANF–comb nanoparticles	PET-MRI	Carotid atherosclerosis	Washington University School of Medicine	Phase 1	Recruiting	Data not available	NCT02417688
Magnetic nanoparticles coated with hydrophilic surfactants and bioprobes	Measuring the concentration of biomolecules with immunomagnetic reduction	Acute coronary syndrome	Far Eastern Memorial Hospital	Not applicable	Unknown	Data not available	NCT02226523

2.1.2. Inflammatory Mediators

Tumor necrosis factor (TNF) is produced and discharged by mast cells and M1 macrophage subtypes.[50] Mast cell-derived TNF can injure microvasculature and stimulate the protease-activated receptors, to cause an inflammatory reaction. TNF is a powerful pro-inflammatory cytokine that modulates the stimulation of T cells by the phosphorylation of nuclear factor kappa-light-chain-enhancer of activated B cells in inflammatory diseases.[51] Hyperlipidemia serves as stimuli for the synthesis and release of TNF, which may trigger the endothelial cells to participate in atherosclerosis initiation and progression.[52] Branen et al. evaluated the role of TNF-α in atherosclerosis formation in mice deficient in both Apolipoprotein E (ApoE) and TNF-α. After several weeks of experiments, the atherosclerotic mice deficient in TNF-α had a smaller lesion area relative to the control group.[53]

Platelet-derived growth factor (PDGF) is another cytokine most commonly derived from mast cells, platelets, smooth muscles, macrophages, and endothelial cells. It is responsible for smooth muscle migration and proliferation. PDGF is implicated in the pathogenesis of atherosclerotic lesions, and an increase in PDGF levels signifies clinical risk factors.[54] He and colleagues established the activity of PDGF receptor β (PDGFRβ) in atherosclerosis formation in hypercholesterolemic ApoE$^{-/-}$ or Ldlr$^{-/-}$ mice. It was evident that increased PDGFRβ signaling triggers plaque buildup at atherosclerotic-resistant areas and initiates the development of fibroatheroma plaques in the coronary arteries.[55]

Mast cells with C-C chemokine receptor 2 (CCR 2), CCR3, CCR5 receptors exist in the endothelium and they bind to eotaxin, chemokine (C-C motif) ligand 5, and MCP-1 which enable their infiltration in the arterial intima, and undergo degranulation to discharge chemical molecules.[56] During the degranulation process, the extracellular vesicle system permits signals to be transferred between mast cells, through uncertain mechanisms. When the mast cells are stimulated in the intima of the artery by oxidized lipoproteins, it discharges granules with numerous inflammatory compounds such as angiotensin I, tryptase, matrix metalloproteinases, TNF, and chymase. They generate the final inflammatory chemokines which form inflammatory plaques in the endothelium of the arterial wall.[57] The inhibition of these inflammatory mediators can decelerate atherosclerotic progression and also inhibit thrombus formation.

2.2. Cellular and Non-Cellular Components

Mitochondria mediate adenosine triphosphate (ATP) production which supports the contractile capacity of cardiomyocytes and regulation of Ca^{2+} fluxes to ensure cardiac activity. The dysfunction of mitochondria, therefore, generates pathogenic inflammatory responses.[58] Due to their crucial roles in the regulation of cardiovascular homeostasis, mitochondria are identified as one of the most significant targets for the treatment of atherosclerosis and related ischemic diseases.[59] Various strategies have been developed to manage cardiomyopathy (CM) and ischemic heart diseases from the perspective of mitochondrial dysfunction. For example, cofactors, antioxidants, dichloroacetate, and L-arginine are proposed as modulators of mitochondrial metabolism to manage CMs.[60] The future research of mitochondrial target therapy for CVDs should have a large focus on the connection between mitochondrial regulation and inflammatory responses.[58]

The ER is the organelle where protein synthesis, folding, and processing occur. ER stress is regarded as another critical pathological characteristic of CVDs, it occurs under specific conditions such as ischemia, hypoxia, and inflammation and leads to the unfolded protein response (UPR) for the preservation of ER homeostasis.[61] This underlies the feasibility of the treatment of CVDs by targeting UPR components and reducing ER stress. There are mainly two approaches studied regarding this treatment, one is to activate components of the adaptive pathway of the UPR, the other is invalidating the components of the pro-apoptotic pathways of UPR.[62] Since the correlative mechanisms currently known are limited, therapeutic intervention by targeting ER stress as the treatment option for CVDs is still at an early stage.

The ECM accounts for more than 50% of the vessel wall mass in arteries and veins and forms the structural foundation for the exertion of cellular functions, which indicates that any defects of ECM could cause disorders in functional systems.[63] The lesion of ECM macromolecules is a prevalent phenomenon among CVDs. For example, a significant vascular wall alteration is caused by elevated collagen content.[64] Additionally, the increase of vessel stiffness can be ascribed to excessive fibrosis and remodeling of ECM macromolecules,[65] while elastin fiber fatigue has also been discovered in stiff vessels.[66] Consequently, targeting ECM macromolecules provides a different approach to the treatment of CVDs. Collagen IV (Col IV) takes up half of the vascular basement membrane and is exposed due to enhanced vascular permeability.[67] Thus, Chan and co-workers developed lipid-polymeric nanoparticles loaded with paclitaxel, whose surface were functionalized with Col IV -targeting peptide, and a 50% reduction in arterial stenosis was achieved compared with sham-injury groups.[68] Likewise, another Col IV-targeting peptide (KLWVLPKGGGCGC) was developed in Zhang's work and contributed to the accumulation of nanoparticles at injured carotid arteries.[69]

2.3. miRNAs

miRNAs are responsible for the post-transcriptional regulation of gene expression and the dysregulation of specific miRNA expression is associated with CVDs.[70] A variety of miRNAs participate in mouse atherosclerotic model pathways.[71] In a study of miRNA expression profiles, the level of miR-21, -34a, -146a, -146b-5p, and -210 revealed a significant upregulation in atherosclerotic arteries compared to non-atherosclerotic left internal thoracic arteries.[72] In previous works, the alteration of miRNA levels, including miR-15,[73] miR-21,[74] miR-320,[75] miR-208,[76] and miR-1,[77] have been found to exacerbate cardiac ischemia. miR-133 is broadly involved in heart disorders. It regulates the level of cardiac sarco/endoplasmic reticulum calcium ATPase-2 that supports myocardial contractility and is downregulated in MI patients.[76,78] The vital role of miRNA in cardiovascular biology makes it a promising target for the treatment of CVDs.

The major intention of miRNA-based therapies is to restore normal miRNA expression levels by downregulation of the overexpressed ones or upregulation of the suppressed ones. However, several obstacles are to be overcome to satisfy outcomes in clinical translation, including the stability of miRNA, inefficient endocytosis, and renal clearance. Above all, many miRNAs related to CVDs are also found to be extensively expressed in other tissues, leading to inevitable off-target effects.[79] Moreover, miRNAs targets for the treatment of CVDs can also be potential targets for cancer or other diseases. For instance, the inhibition of tumor-suppressive miR-34a leads to post-MI cardiac repair, so the clinical implications of miR-34a in both diseases remain to be determined.[80] Due to their extraordinary advantages, including prolonged blood circulation half-life, stable encapsulation of nucleic acid, enhanced cellular internalization, and targeted delivery of payloads, the application of nanocarriers in miRNA therapy has demonstrated tremendous potential to improve treatment of atherosclerosis and will likely facilitate the translation of miRNA therapeutics into clinical use.

2.4. Proteins

Proteins, more specifically, receptors and enzymes, are extensively involved in the developmental process of CVDs as mediators or functional components. For example, peroxisome proliferator-activated receptors (PPARs) regulate lipid and carbohydrate metabolism and inflammatory responses, and PPARs agonists show positive effects in the treatment of atherosclerosis.[81] In addition to that, proper modulation of the levels of cholesteryl ester transfer protein, cholesterol O-acyltransferase, microsomal triglycerides transfer protein, etc. have been shown to reduce the risks associated with atherosclerosis.[82] Among all of the potential protein targets, proprotein convertase subtilisin/kexin type 9 (PCSK9) has been one of the most promising targets for drug development. LDL receptors (LDLR) bind to LDL for its degradation in the lysosome and subsequently recycle to cell membrane. However, PCSK9 is capable of binding to LDLR on the cell surface, which leads to the degradation of LDLR as well as the interruption of LDL clearance from blood and therefore increases the risks of CVDs.[83] Therefore, the inhibition of PCSK9 function would be a potent approach for the management of atherosclerosis. Various strategies have been established for this purpose, including the inhibition of PCSK9-LDLR interaction with monoclonal antibodies and the suppression of PCSK9 expression via small molecule inhibitors or gene silencing techniques.[84] Small interfering RNA (siRNA) impedes protein synthesis by targeting mRNA and degrading it before translation, serving as a powerful tool for PCSK9 gene silencing therapy.[85] Inclisiran is a long-acting siRNA drug targeting PCSK9 co-developed by Alnylam Pharmaceuticals and The Medicines Company. In early-phase clinical trials, Inclisiran was found to sustainably downregulate PCSK9 and LDL cholesterol levels without serious adverse effects.[86] Notably, in 2019, The Medicines Company announced positive phase 3 study results of Inclisiran, indicating a giant step towards clinically available siRNA drug for atherosclerosis.

3. Advanced Diagnosis of Atherosclerosis and Related Ischemic Diseases via Nanomedicine

3.1. Nanoparticles for Noninvasive Imaging

Cardiovascular imaging enables the visualization of pathomorphology and tissue characteristics via technologies including CT, MRI, PET, SPECT, and fluorescence techniques. The applications of nanoparticles as contrast agents for available molecule imaging technologies are promising and potent tools for noninvasive visualization of diagnostic purposes. Nanoparticles can be designed and manipulated for the detection of typical pathological changes of atherosclerosis and related ischemic diseases, such as inflammation, calcification, cell apoptosis, and angiogenesis,[87] presenting enormous potential to enhance the efficiency and accuracy of diagnosis once developed in clinical applications.

3.1.1. Iron Oxide Nanoparticles

Among different catalogs of iron oxides, hematite (α-Fe_2O_3), magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3) exhibit excellent biochemical properties and are promising candidates for bioimaging applications.[88] Superparamagnetic iron oxide nanoparticles (SPIONs) are monodispersed Fe_3O_4 or γ-Fe_2O_3 particles with surface modifications comprised of dextran, carboxydextran, or PEG, which have been shown to enhance stability, water solubility and biocompatibility and extend circulation half-life for in vivo use.[89] Clinically, SPION formulations, such as ferumoxytol and ferucarbotran, have been utilized for liver, central nervous system, and cell imaging.[90]

Iron oxide nanoparticles have also been applied to visualize and evaluate atherosclerotic plaques and inflammation at ischemic sites after macrophage-particle phagocytosis. Furthermore, modifications of iron oxide nanoparticles with biological ligands, such as anti-human E selectin antibody fragments and VCAM1 targeting peptides, have made it possible for them to actively target pathological sites in CVDs.[91] In Ta and co-workers' work, monodisperse ultra-small magnetic dual contrast iron oxide nanoparticles (DCIONs) modified with single-chain antibodies against activated platelets were developed for targeted MRI of atherothrombosis.[92] A series of in vitro and in vivo experiments proved that functionalized DCIONs successfully targeted thrombosis for the simultaneous performance of T1 and T2-weighted imaging. Recently, multimodal iron oxide imaging systems are emerging for the diagnosis and treatment of CVDs. For instance, Bhabatosh et al. developed high-density lipoprotein (HDL)-mimicking nanoparticles with encapsulated iron oxide nanoparticles and mito-magneto, which was capable of targeting both mitochondria and macrophages and exhibits theranostic potential in the management of atherosclerosis.[93]

3.1.2. QDs

QDs are semiconductor nanocrystals with excellent optical properties which can be precisely adjusted through composition and size. Advanced desirable fluorescence features, such as photostability, high molar extinction coefficient, and narrow emission

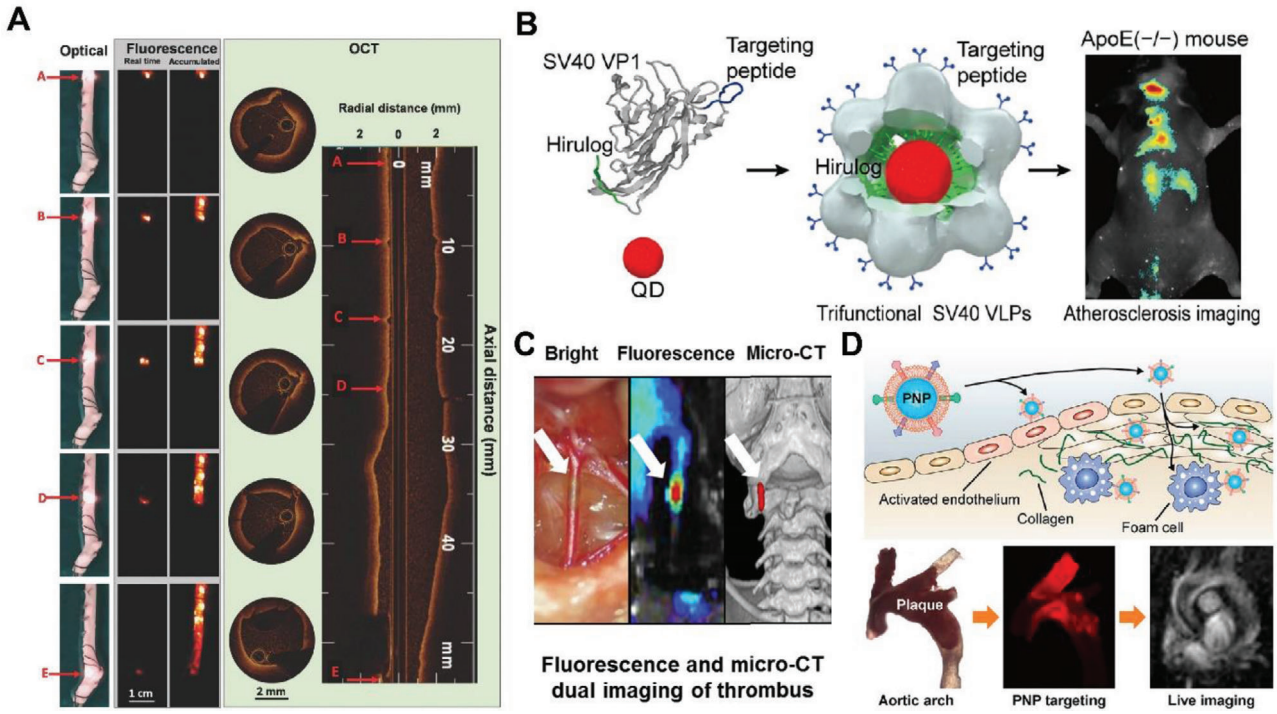

Figure 3. Recent advances in nanoparticle-based imaging technologies for atherosclerosis and related ischemic diseases. A) Optical images, real-time and accumulative infrared fluorescence images, and optical coherence tomography cross-sectional images of the aorta excised from a sacrificed rabbit generated by the QDs. Reproduced with permission.[96] Copyright 2017, Wiley-VCH. B) Schematic representation of SV40-based nanoparticles and fluorescence imaging of atherosclerosis in ApoE$^{-/-}$ mice injected with QDs. Reproduced with permission.[97] Copyright 2016, American Chemical Society. C) NIRF and micro-CT imaging using TAPSiO$_2$@AuNPs. Reproduced with permission.[99] Copyright 2018, Elsevier. D) A schematic presentation of PNP targeting atherosclerotic plaques. Macroscopic fluorescent imaging proved the targeting ability of PNP and PNP allowed MRI live detection in an animal model of atherosclerosis. Reproduced with permission.[107] Copyright 2018, American Chemical Society.

spectrum, have made them particularly suitable for deep tissue imaging.[94] Generally, QDs exert rapid and comprehensive distribution in the bloodstream after intravenous injection so that effective visualization of vasculature can be implemented.[95] Consequently, QDs have become one of the optimum contrast agents for near-infrared fluorescence (NIRF) imaging applied to vascular visualization. Hu et al. used semiconductor QDs, which emit in the third infrared biological window (1.55–1.87 µm), to serve as contrast agents of optical coherence tomography for intracoronary multimodal imaging and acquired high penetrating fluorescence images (**Figure 3**A).[96] Sun et al. encapsulated QDs in Simian virus 40 (SV40)-based nanoparticles for noninvasive imaging of atherosclerotic plaques (Figure 3B).[97] Through modifications with different targeting peptides, the imaging of atherosclerosis progression was achieved with desired photostability and detection sensitivity in ApoE$^{-/-}$ mice. Despite emerging positive research outcomes, the clinical translation of near-infrared imaging by QDs is still faced with challenges. Namely, inconclusive in-vivo targeting and inevitable short- and long-term toxicity are issues that must be overcome.

3.1.3. Other Inorganic Nanoparticles

Silica nanoparticles have gained continuous attention for diagnostic purposes as hybrid imaging probes in the management of CVDs. Jeong et al. took advantage of the homing properties of macrophages to atherosclerotic plaques and synthesized aza-dibenzocyclooctyne-tethered PEGylated mesoporous silica nanoparticles labeled with F-18 azide-radiotracer via covalent reaction inside macrophage cells, making it a PET imaging tool in tracking RAW 264.7 at atherosclerotic plaque sites in ApoE$^{-/-}$ mouse models.[98] This strategy could also be applied to other cell tracking studies using PET imaging with F-18, including tracking of stem cells. AuNPs bear a variety of advantageous properties for imaging applications and are one of the most widely used inorganic nanoparticles. Bare AuNPs are of low stability and minimal functionality. To ameliorate this, Kwon and co-workers developed silica-coated AuNPs, which were modified with thrombin-activatable fluorescent peptide probes, to image thrombi by dual NIRF and micro-CT imaging (Figure 3C).[99] The nanoparticles simultaneously allowed rapid, direct, and high-resolution imaging of thrombin activity and anatomical information of thrombotic lesions in live thrombotic animal models. Such dual-imaging nanoplatforms might be able to provide image-guided therapy for thrombosis. Various categories of inorganic nanoparticles, such as MnO nanoparticles[100] and upconversion nanoparticles[101] have also been widely studied in a preclinical setting. Nevertheless, the biggest concern about the clinical translation of inorganic imaging nanoparticles is focused on the toxicity issue associated with long-term safety. Researchers should emphasize minimizing the toxicity profile when developing

new systems for cardiovascular imaging to rectify safety concerns.

3.1.4. Conjugated/Encapsulated Nanoparticles

Except from inorganic nanoparticles with inherent characteristics for imaging applications, other nanoparticles, including polymeric nanoparticles,[102] liposomes,[103] micelles,[104] and dendrimers[105] can also be applied to cardiovascular imaging through chemical conjugation or encapsulation of fluorescent molecular, radioisotope, or other contrast agents. To improve homing accuracy, targeting carriers have been designed by attaching specific ligands to nanoparticles. Nanoparticles could be further coated with biocompatible polymers such as PEG to prolong blood circulation half-life and better accumulate in pathological areas with leaky endothelia. Oumzil et al. synthesized nucleoside-lipids-based solid lipid nanoparticles (SLN) loaded with iron oxide particles and prostacyclin.[106] The SLNs performed better relaxivity in MRI compared to the clinically used Feridex, and this work provided critical insights into the development of theranostic nanomedicine for atherosclerotic management. Another example is shown in platelet membrane-coated poly(lactic-*co*-glycolic acid) (PLGA) nanoparticles (PNP) that encapsulate an MRI contrast agent, gadolinium, for live imaging of atherosclerosis (Figure 3D).[107] The results demonstrated that the biomimetic nanoparticles efficiently localized to atherosclerotic plaques, preatherosclerotic areas, and areas that tend to form plaques. This strategy can be adapted to a wide range of imaging modalities as a result of the universality of the cell membrane-coating technology. Recently, Liu et al. developed ^{64}Cu–CANF–comb nanoparticles through self-assembly of a comb-co-polymer, followed by conjugation of c-atrial natriuretic factor (CANF) peptide and radiolabeling with ^{64}Cu.[108] In a rabbit double-injury atherosclerosis model, the nanoparticle provided sensitive and specific PET imaging of plaque progression by targeting natriuretic peptide clearance receptor overexpressed in atherosclerotic lesions. More significantly, ^{64}Cu–CANF–combs were also seen to bind to human carotid endarterectomy specimens, which revealed their potential for the translation of human plaque imaging. Notably, the phase 1 clinical trial of PET/MR imaging with ^{64}Cu–CANF–comb nanoparticles is currently recruiting participants (**Table 1**).

In summary, nanoparticles present impressive prospects for cardiovascular imaging applications with excellent properties of specificity and sensitivity. With the continuous alteration and endothelial remodeling present in atherosclerosis, a diversity of targets and biomarkers are provided to develop novel nanoparticle imaging systems that integrate detection, treatment, and tracking functionalities. Nevertheless, in most cases, complicated designs of nanoparticles with subtle moieties are required for multifunctional imaging techniques, which increase the difficulty in ensuring their safety and reproducibility for clinical translation.

3.2. Nanodevices for Biomarker Detection

Cardiac troponins, particularly troponin I (cTnI) and troponin T (cTnT) are important biomarkers for the detection of atherosclerosis and related ischemic diseases because they are released into the bloodstream when cardiomyocytes are damaged.[109] The conventional diagnosis of CVDs is mainly grounded on a physician's evaluation in collaboration with laboratory examinations. Unfortunately, these laboratory tests cannot detect CVDs at an early stage, making it impossible to reverse tissue damage at the time the traditional tests confirm the lesion.[110] The development of nanoparticle-based diagnostics is improving the detection of molecular events of CVDs at an early stage. The following section will highlight some examples that demonstrate current trends in nanobiosensors. Each project will be discussed from the perspective of its implications for CVDs detection.

3.2.1. Electrochemical Techniques

Electrochemical immunosensors are made of bioreceptor components used in the recognition of an antigen. The attached transducer converts the biochemical signals into electrical signals.[111] The various types of electrochemical methods adopted include amperometry, conductometry, impedimetry, potentiometry, and voltammetry.[112] These biosensors employ biomolecules as diagnostic probes, like nucleic acids, enzymes, and other metabolites. Electrochemical sensors possess high selectivity, reliability, and sensitivity.

Amperometric immunosensors require a redox probe which is used to detect any changes in the current produced at the electrode, thus supplying data according to the binding activity of the antigen and the detecting antibody after a constant potential has been applied to the electrode. The change in current is calculated in comparison or relative to the reference electrode. The amperometric sensors are more sensitive than the potentiometric counterparts.[113] Moreover, a specific temperature during titration is not required and the analyte to be analyzed does not have to be reactive at the electrode.[114] Gomes-Filho et al. developed an amperometric nanosensor made up of polyethyleneimine (PEI) and carbon nanotubes (CNT) for effective detection of cTnT.[115] PEI and CNTs were linked at the surface of the electrode based on a carboxyl-to-amine cross-linkage. The nanosensor could detect cTnT concentrations as low as 0.033 mg L^{-1}. However, this particular sensor is unstable due to the multiple binding sites resulted from a variety of amino groups on the surfaces of antibodies. The random nature of these binding sites leads to the loss of antigen-binding interaction due to steric hindrance.

Conductometric immunosensors measure a change in the value of the ionic strength of a solution. This technique is preferred among researchers owing to the absence of a specialized reference electrode thereby making them simple and easy to use. In addition, these immunosensors can analyze a wide range of molecules. Chua et al. designed a silicon nanowire (SiNW)-based biosensor, which operates on the principle stated above.[116] The sensor was used to detect cTnT in human serum in a linear range of 1 ng mL^{-1} to 1 fg mL^{-1} with high specificity and selectivity. It demonstrated a platform able to obtain multiple confirmatory diagnoses from a single medical analysis and provide a precise indication of cellular necrosis at the early stages of cardiac diseases.

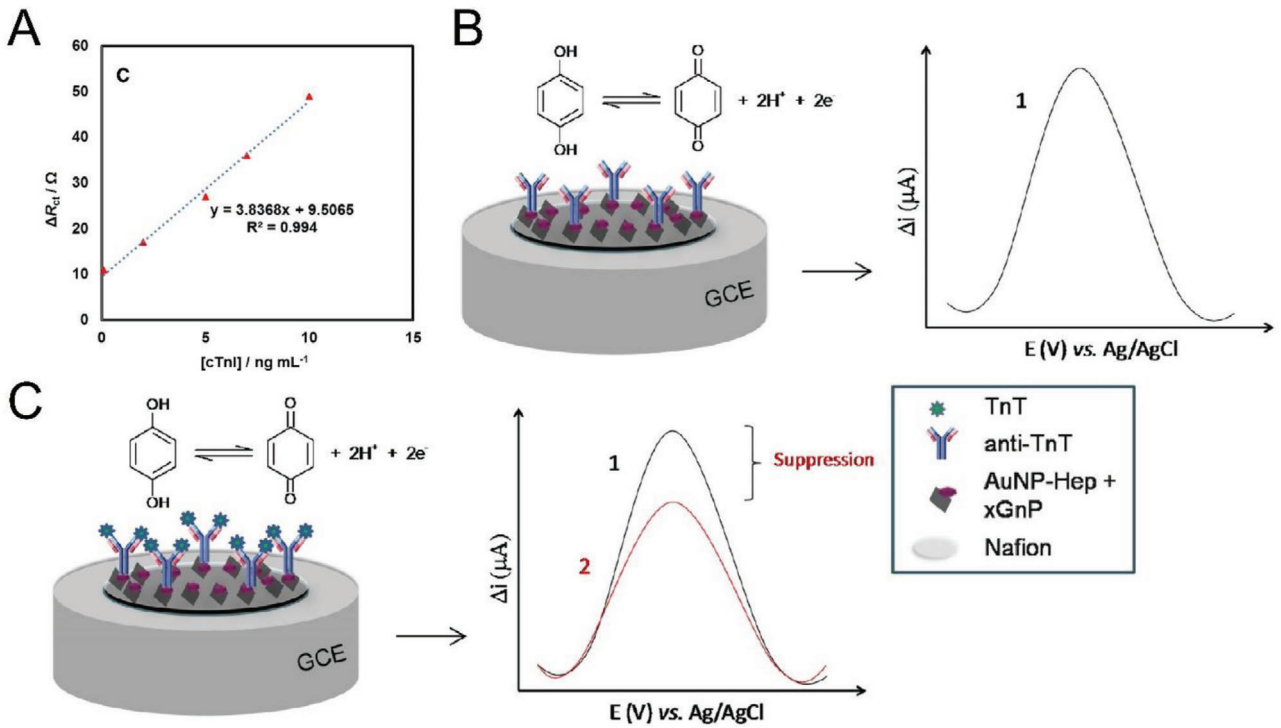

Figure 4. Electrochemical immunosensors for the detection of MI biomarkers. A) A graph of impedimetric biosensor response toward cTnI biomarker. Reproduced with permission.[117] Copyright 2016, Elsevier. B,C) A graphical presentation of the principle of the label-free voltammetric immunosensor for cTnT determination. The respective voltammograms generated by the immunosensor before B) and after C) incubation in human blood samples containing cTnT. Reproduced with permission.[120] Copyright 2017, Wiley-VCH.

The impedimetric device records any changes in the impedance value after potential is applied to the electrode. The devices are designed to track unwanted substances affecting the capacitance of an electrochemical system. Kazemi et al. designed novel nanostructures comprised of label-free porous graphene oxide for the detection of cTnI to investigate MI.[117] The immunosensor showed good sensitivity and selectivity for human-cTnI detection in clinical samples due to an excellent limit of detection (LOD) (0.07 ng mL^{-1}) and reasonable linearity within a wide concentration range (0.1–10 ng mL^{-1}) (**Figure 4**A).

Potentiometric sensors detect analyte activity by measuring the potential difference between two electrodes, which have also been designed for CVD diagnosis. They are grouped into three categories: light-addressable potentiometric sensors, pH electrode-based glass ion-selective electrodes (ISEs), and solid-state ion-selective field-effect transistors. Field-effect transistor devices are improved versions of ISEs and the preferred sensor for troponin detection. In one example, Kim et al. constructed a robust and sensitive honeycomb structure of SiNW that used a field-effect transistor mode to detect cTnI, where a LOD of 5pg mL^{-1} was obtained.[118] The achievement of high sensitivity by the device was due to the large effective area of the honeycomb structure of SiNW.

Voltammetric biosensors obtain information based on a change in potential and measure the resultant current arising from the varied potential. There are different voltammetric methods due to the various means to change the potential. These methods include cyclic voltammetry, linear sweep voltammetry, differential staircase voltammetry, and differential pulse voltammetry.[119] Irrespective of the method utilized, a potential must be applied to generate a redox reaction of the electroactive molecules at the surface of the electrode, this process leads to a variety of electron flow across the electrode interface. Zanato et al. developed a voltammetric immunosensor by synthesizing exfoliated graphite nanoplatelets coated with AuNPs.[120] This sensor utilized hydroquinone as a redox probe that oxidized at its surface in the absence of an antigen thereby generating an electrical signal termed as the base peak known as "peak 1" (Figure 4B). After the incubation of the sensor with the sample containing the cTnT antigen, a new differential pulse voltammetry was then measured as "peak 2" (Figure 4C). This platform exhibited good selectivity, reproducibility, and sensitivity for the measurement of cTnT concentration below the cutoff and held enormous potential for clinical applications.

3.2.2. Optical Techniques

Optical methods detect biomarkers through the reaction between bioanalytes and the optical field. There are mainly two categories of optical biosensors: label-free optical sensing and label-based optical sensing techniques. With label-free optical sensing, a label is not required in the detection process. This can be seen in surface-enhanced Raman spectroscopy (SERS). The reverse

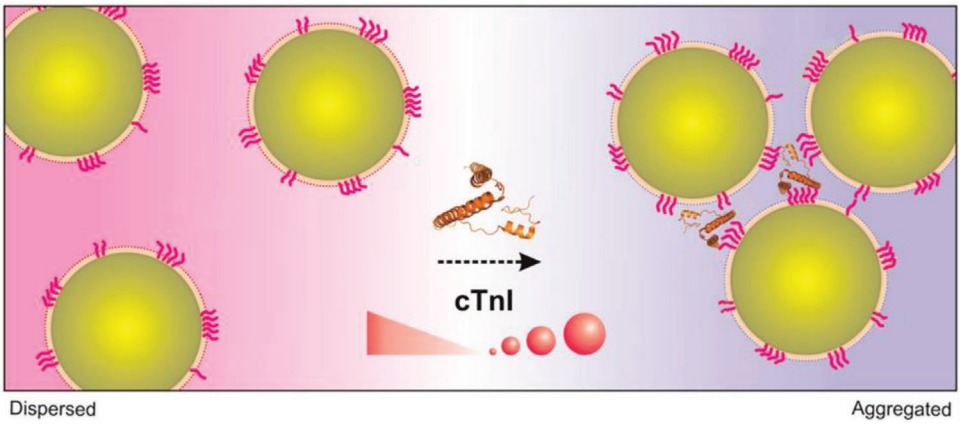

Figure 5. Graphical presentation of a functionalized AuNPs-based colorimetric sensor that exhibits tremendous colloidal stability and remains dispersed in aqueous solution but aggregates in the presence of the biomarker cTnI, resulting in a colorimetric change that is concentration-dependent. Reproduced with permission.[130] Copyright 2016, American Chemical Society.

is true for label-based optical sensing and examples include colorimetry, fluorescence, and luminescence imaging.[121]

SERS was introduced to overcome the major drawback of traditional Raman spectroscopy, low signal intensity.[122] Both methods are based on the inelastic emission of incident photons by a molecule exposed to a light source. Surfaces are enhanced with metals like copper, gold, and silver to resolve their weak signaling outputs.[123] Bai et al. designed SERS-based lateral flow assay strips for the detection of cTnI.[124] Four different citrate-capped nanoparticles were synthesized and coated with Nile blue A (NBA). The nanoparticles were bound to a detection antibody. The test line (T) of the strip was coated with a capture antibody before the serum-containing cTnI was then added to the sample pad, then the analyte was conjugated to the NBA labeled nanoparticles which led to the formation of an immunocomplex, and this complex can be captured in T. The presence of the nanoparticles on the test line displayed a colored band and the migrant of excess immunocomplex to control line resulted in a second colored band, while the control line (C) demonstrated a single colored band in the absence of cTnI. Consequently, the SERS intensity peak is directly proportional to cTnI concentration, and this is determined using a Raman spectrometer. SERS-based nanosensors can detect other substances like glucose,[125] vasopressin,[126] and oligonucleotides[127] due to its wide excitation wavelength range.

Colorimetric detection is the visible color change that occurs when an analyte reacts with a colorimetric probe and this can be a chemical or biochemical reaction.[128] The most widely used nanoparticles for detection include Au and silver nanoparticles. Park et al. designed a 12-mer synthetic peptide receptor with a high binding affinity for cTnI, and a polyvalent phage-displayed library was immobilized onto AuNPs to detect cTnI in serum samples.[129] The AuNPs naturally aggregate when the peptide binds to the cTnI, while in the absence of cTnI, the AuNPs remain in their dispersed state (**Figure 5**).[130] AuNPs of different concentrations and sizes were utilized to optimize the sensitivity and LOD. The main advantages of this technique are its low cost and ease of operation. Comparatively, it is less sensitive than other optical sensors. Analyte detection can be evaluated with the naked eye or with unsophisticated software.[128]

In fluorescence-based biosensors, the target analyte is labeled with a fluorescent dye and the intensity of the fluorescence indicates the conjugation of the target molecule. Kar et al. developed an advanced TiO_2 nanotube-based fluorescence biosensor capable of detecting human cardiac troponin biomarkers at a very low concentration without the use of enzymatic amplification.[131] In another example, amine-functionalized graphene quantum dots (afGQD) conjugated with anti-cTnI (anti-cTnI/afGQD) showed high sensitivity and selectivity for cTnI detection with a linear range from 0.001 to 1000 ng mL^{-1} and a LOD of 0.192 pg mL^{-1}.[132] The anti-cTnI/afGQD nanoplatform exhibited strong fluorescence which was quenched in the presence of graphene and restored by the addition of targeting antigen cTnI. Fluorescence-based biosensors have a higher sensitivity than colorimetric biosensors, but their main disadvantage is based on the laborious labeling process. Future research can be focused on the design of label-free biosensors for the detection of CVD biomarkers by tuning the surface chemistry of nanomaterials and nanocomposites.

Luminescence detects chemical or biological reactions based on the emission of electromagnetic radiation, its measurement can either be direct or indirect by using an enzyme label. Chemiluminescence-based sensors offer multiple benefits over the fluorescence-based biosensors. For example, no external light source is required and they are cheap and easy to use.[133] Like other optical techniques, AuNPs are widely used to increase the sensitivity of chemiluminescence-based sensors for the detection of CVD biomarkers.

This section gave an overview of the different types of biosensors used for the detection of cardiac biomarker troponin and their design features. A few techniques have found their way into the market, and novel techniques are under development. Some of the disadvantages associated with optical-based immunosensors such as colorimetric and fluorescence-based sensors are their low sensitivity and the need for bulky apparatus. On the other hand, electrochemical immunosensors are preferred due to their portability, ease of operation, and high efficiency. An optimal CVD nanosensor would be portable, easy to use, and provide high detection efficiency.

4. Nanotechnology for the Treatment of Atherosclerosis and Related Ischemic Diseases

4.1. Nanostructured Materials for Cardiac Tissue Repair

4.1.1. Cell Therapy

In recent times, many efforts in myocardial regeneration have been geared towards cell therapy-based cardiac tissue repair.[134,135] Various cells including endothelial progenitors, bone-marrow-derived mononuclear stem cells, induced pluripotent stem cells, and mesenchymal stem cells have been utilized to remuscularize the damaged heart.[136] Nonetheless, clinical translation has yet to be achieved due to four cardinal drawbacks: a) arrhythmic complications[137]; b) inability to generate sufficient patient-specific mature and functional cardiomyocytes to restore cells which are lost through infarction; c) poor engraftment[135]; and d) lack of accurate, robust, and safe methodologies to monitor in vivo therapeutic cells.[138] Even with these limitations, several groups have enlightened our knowledge about the fate of these therapeutic cells in the myocardium of diseased and healthy hearts.[139] There have been several reports about bone marrow stem cells differentiating into other cell types including cardiomyocytes.[140] Research has shown that therapeutic cells implanted into animal models can improve the contractility of the heart muscle of the organism.[141] The mechanism of action by which this physiological improvement is attained is yet to be known, and to date, there has not been any resounding demonstration of either transplanted myoblastic or hematopoietic adult stem cells taking on the cardiac phenotype.[142] Since 80% of the cell suspension injected into the myocardium ends up not engrafting due to the stated drawbacks above, there is ongoing clinical research in humans employing adult stem cells to probe the safety and feasibility of cell therapy into the myocardium.[143] The initial clinical interventions lacked a clear understanding of stem cell biology and techniques to effectively transplant therapeutic cells in diseased heart tissue. Hence, most of the efforts are now channeled into the development of tissue engineering approaches utilizing nanomaterials to efficiently transplant new therapeutic cells into diseased cardiac tissue. This technology does not only introduce adhesion molecules on the cell surface but also various techniques are used to increase homing to the heart tissue. For example, Wang et al. engineered a cardiac patch containing polypyrrole nanoparticles. The nanoparticles enhanced the expressions of α-actinin and connexin-43, leading to a harmonious contraction of the cardiac muscle in myocardial infarcted rat models.[144]

4.1.2. Platelet-Mimetic Nanotechnologies

Platelet Mimetics: The role of platelets in CVD and their interactions with the related disease substrates cannot be understated, thus, bioinspired approaches have been recently introduced to overcome the drawbacks of conventional nanocarriers. Platelets bind to the injured endothelial cells through P-selectin which are overexpressed in these cells. Inspired by this, platelet glycoprotein (GP Iba)-conjugated PLGA nanoparticles and carboxylated polystyrene nanoparticles have been developed for targeted delivery to damaged endothelial cells.[145] The biomimetic system enhanced the targeting, adhesion, and cellular uptake of platelet-like nanoplatforms in inflamed endothelial cells. Multimodal platelet-like nanoplatforms are rapidly emerging to improve upon the single-ligand targeting nanosystems. By modifying the surfaces of multimodal platelet-like nanoparticles with different binding peptides, the biophysical structure and the biochemical interactions of naturally occurring platelets can be duplicated.[146] Another example involves the alteration of the stem cell membranes with platelet-derived nanovesicles, offering platelet-like properties to live stem cells. For instance, platelet vesicles were obtained by a process involving freeze-thaw lysis and sonication. Subsequently, they were integrated onto the surfaces of cardiac stem cells in the presence of PEG, the modified cells were able to successfully target the injured vasculatures.[147]

Platelet Membrane Coated Nanoparticles: The aforementioned platelet mimetic platforms modify nanoparticle surface using the membrane of platelets, which requires complex chemical alterations to accomplish the functionalization and might disfigure the proteins' structure. Also, integrating a limited variety of proteins would not result in full mimicking of the biological function of the platelets since an immense array of proteins are required for apt protein activity.[148] The cell membranes play a vital role in various biological processes including cell to cell communication, intercellular recognition, and adhesion.[149] Concerning these characteristics, cell membranes were inducted onto nanoparticle surfaces to design biomimetic nanomaterials. Zhang and co-workers were the first to implement the idea using red blood cell membranes to encapsulate nanoparticles.[150] Subsequently, different cell membranes from cancer cells, stem cells, and platelets have been used to construct membrane-structured nanosystems for the treatment of diseases.[151] Platelet membrane-cloaked nanoplatforms have great potential for the management of atherosclerosis because they can enhance immunocompatibility and effectively bind to cardiovascular damaged sites.[152] Song et al. developed a platelet membrane-cloaked nanoparticle which served as a targeted drug delivery system to treat atherosclerosis.[152c] In ApoE$^{-/-}$ mice, the nanoplatform significantly reduced atherosclerotic progression and also stabilized atherosclerotic plaques.

4.1.3. Stent Devices

Percutaneous coronary intervention is the most frequently implemented therapeutic technology in the modern treatment of coronary disease.[153] The management of obstructive coronary disease was transformed when Andreas Gruntzig engineered coronary balloon angioplasty in 1977, providing an alternative to surgical therapy. Regrettably, this procedure leads to the high occurrence of restenosis,[154] elastic recoil, abrupt vessel closure,[153] neointimal proliferation, and late vascular remodeling. Bare metal stents (BMS) were first employed by Sigwart in 1986 to avert acute vessel closure caused by balloon angioplasty.[155] Unfortunately, the novel technology resulted in neointimal hyperplasia. Drug-eluting stents (DES) were designed to overcome the shortfalls of the BMS and its coun-

terpart. First-generation DES were made of sirolimus-eluting stents and paclitaxel-eluting stents[156] while second-generation DES are zotarolimus-eluting stents and everolimus-eluting stents, respectively. Though DES has had some level of success, there are some drawbacks associated with these devices like restenosis (though the level of occurrence is lower than BMS, it is prevalent),[157] ST elevation and late-stage thrombosis.

Given these drawbacks regarding DES, nanotechnology has rapidly emerged to revolutionize stent therapeutic outcomes. Nanoporous systems have shown the capability to offer controlled drug-release behavior over a scheduled duration, which have been used for cancer studies.[158] Researchers are considering nanoporous stent surfaces with regard to titanium oxide,[159] aluminum oxide,[160] gold,[161] and carbon-carbon nanoparticle matrices[162] for the delivery of several payloads. One major challenge associated with stent procedures is compromised revascularization of blood vessels induced by the actual presence of stents. In light of this, nano-texturing is being explored to improve the interaction of endothelial cells with stent surfaces. Reports have shown that nanoscale topography on hydroxyapatite[163] and titanium[164] could enhance cell adhesion on the endothelium and reduce thrombosis.[165] The next line of technology would include nanoporous stents with nanotextured properties to further reduce the occurrence of restenosis and the incidence of late-stage thrombosis.

This section discussed the making of clinically viable implants using nanotechnology for cardiovascular tissue regeneration. These nanostructured scaffolds and drug delivery systems enhance the remuscularization of the damaged heart, increase cell adhesion, and improve the homing activities of therapeutic payloads in the diseased heart tissue. Tissue engineering can be applied to achieve controlled cell delivery. The controlled release of these therapeutic payloads also enhances the tissue engineering potency. From a pharmaceutical point of view, both the drug-delivery systems and scaffolds need to be biocompatible and biodegradable. The biological functions of encapsulated drugs and cells can be used to effectively manage atherosclerosis and related ischemic heart diseases.

4.2. Drug Delivery

4.2.1. Chemical Drugs

In addition to surgery, the administration of chemical drugs serves as the first-line approach to control atherosclerosis and related ischemic diseases. These small-molecule agents mainly include statins, fibrates, niacin, and β-blockers. To overcome the drawbacks of small molecule-based drugs, such as low water solubility and rapid metabolism, nanoparticle delivery systems have been developed to improve their pharmacokinetic profiles. Duivenvoorden et al. reported an injectable reconstituted HDL nanoparticle to deliver statins to atherosclerotic plaques, resulting in the enhanced systemic bioavailability and anti-inflammatory effects of statins.[166] By virtue of nanomedicine-based drug delivery systems, emerging chemicals also pose exciting potential opportunities for clinical translation. LXR agonists, such as GW3965, have been proven to inhibit pro-inflammatory signals in macrophages and attenuate atherosclerosis.[167] However, severe adverse effects of LXR agonists, including hepatic lipid biosynthesis and hyperlipidemia, have impeded their approval by the Food and Drug Administration. Given this, a series of nanoscale delivery systems have been developed to relieve the side effects of LXR agonists and accelerate their applications in clinical practice. For instance, self-assembly PLGA-*b*-PEG nanoparticles have been fabricated to deliver GW3965 to atherosclerotic plaque macrophages.[42] Compared to free GW3965, the nanoparticles exhibited strikingly higher efficiency in inducing the expression of LXR-target genes and inhibiting inflammatory factors in macrophages (**Figure 6**A).[168] Likewise, in a work of Yu et al., lipid–polymer hybrid nanoparticles modified with Col IV targeting peptide were developed to deliver GW3965, which enhanced the accumulation of GW3965 at atherosclerotic lesion sites and resulted in significantly elevated curative effects (Figure 6B).[169] Tang et al. developed a combinatorial library of HDL-based nanoparticles to deliver GW3965 to pathological macrophages and assessed the library's immune cell specificity via immunological techniques and in vivo PET imaging (**Figure 7**).[170] This study provided an approach to screen nanoparticles with distinct organ distribution and immune cell targeting specificities, which allowed the conversion of immunomodulatory small molecules into precise medicines for atherosclerosis.

4.2.2. Therapeutic Proteins and Peptides

With the continued understanding of pathologic mechanisms of CVDs, diverse therapeutic options spring up and more research focus is laid on the development of protein drugs. Interleukin 10 (IL-10), an anti-inflammatory cytokine that inhibits the generation of inflammatory mediators from activated macrophages and dendritic cells,[171] is a promising drug candidate for the treatment of atherosclerosis. To improve the pharmacokinetic characteristics of IL-10, pluronic-based nanocarriers modified with cRGD (IL-10-NC) was developed for targeted delivery of IL-10 to atherosclerotic plaques.[172] The systemic injection of IL-10-NC generated remarkably prolonged residence of IL-10 in serum compared to free IL-10 in an ApoE$^{-/-}$ C57BL/6 mouse model. This indicates the anti-inflammatory potential of this engineered nanocarrier to treat atherosclerosis by targeted delivery of IL-10.

Peptides exist in the "sweet spot" between chemical drugs and macromolecular biopharmaceuticals in terms of their properties.[173] Peptides such as apolipoprotein A-I (apoA-I) and ApoE mimetic peptides, which imitate the function of mediators associated with pathologic processes during CVDs, present giant potential for therapeutic intervention of atherosclerosis and MI.[174] Nevertheless, disadvantages of peptides, including poor stability, short circulating half-life, and inadequate delivery, are still to be solved for the development of therapeutic peptides. For example, amino acids 2–26 (Ac2-26), the therapeutic peptide that mimics the pro-resolving actions of annexin A1, is rapidly eliminated from plasma and inappropriate for the treatment of chronic diseases like atherosclerosis. Thus, Fredman et al. developed Col IV-targeted nanoparticles to deliver Ac2-26 to atherosclerotic lesions and stabilized vulnerable plaques in fat-fed Ldlr$^{-/-}$ mice

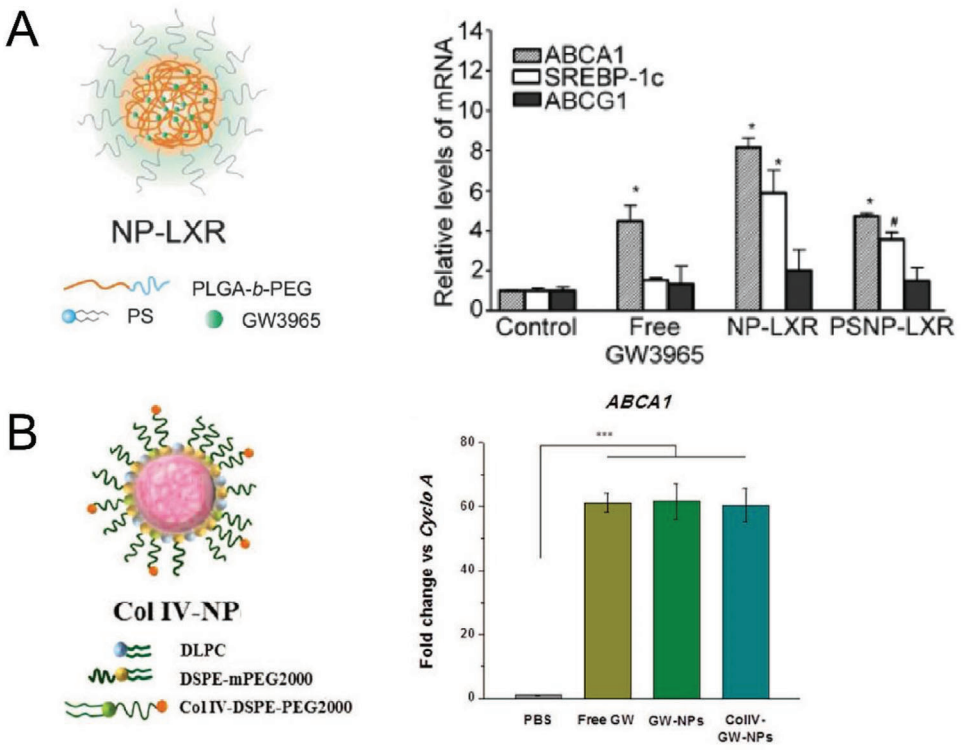

Figure 6. Nanoparticles delivering GW3965 for the treatment of atherosclerosis. A) Structure of GW3965-encapsulated nanoparticles and regulation of LXR-target gene expression by the free GW3965 or the indicated nanoparticles in the aortic arch. Reproduced with permission.[168] Copyright 2015, Wiley-VCH. B) Schematics of the lipid–polymer hybrid nanoparticles and total mRNA isolated and retrotranscribed to determine the expression levels of the ATP-binding cassette A1 (ABCA1). Reproduced with permission.[169] Copyright 2017, Wiley-VCH.

with advanced atherosclerosis.[175] Another example includes the inhalation of peptide-loaded calcium phosphate nanoparticles (CaPs) that restored cardiac functions and treated heart failure in a mouse model of streptozotocin-induced CM (**Figure 8**).[176] Notably, further data in a porcine model with the disposal of nebulization of CaP-hemagglutinin peptides also indicated effective delivery to the heart. For the first time, this study revealed the potential of inhaled peptide delivered via nanoplatforms to effectively treat cardiac diseases, which provides significant insights into the clinical translation through studies in large animals.

4.2.3. Gene Delivery

As mentioned in Section 2.3, many genes, especially miRNAs, are implicated in pivotal processes in the pathological progression of CVDs and are regarded as potential targets for treatment. However, the administration of naked nucleic acid is hard to realize due to the existence of negatively charged cell membranes and nucleases in serum. Nanotechnology-based RNA interference (RNAi) platforms hold great promise for safe, efficient, and tissue-specific delivery of therapeutic genes, while cationic lipid-based nanoparticles have already been applied to systemic delivery of siRNA in clinical practice, such as Patisiran.[177] Nguyen et al. reported chitosan nanoparticles (chNPs) to deliver functional miRNAs to macrophages. A series of in vitro and in vivo experiments have shown that chNPs were able to deliver miR-33 to naïve macrophages and inhibit the expression of ABCA1 (**Figure 9**).[178] Further in-vivo data revealed that mice administered with miR-33 chNPs generated decreased RCT to the plasma, liver, and feces, while those injected with chNPs that loaded efflux-promoting miRNAs, including miR-223 and miR-206, showed significantly improved levels of plasma cholesterol. This chitosan-based miRNA delivery platform can also be applied to other RNAi-based therapies for macrophages-dominated CVDs and shows potential for clinical use due to the favorable biocompatibility of chitosan derivatives. In a work of Nie et al., an unlockable nanoplatform comprised of disulfide-bridged heparin nanoparticle core and poly (glycidyl methacrylate) (PGEA) cationic shell (Hep@PGEA) was proposed for self-accelerating release of nucleic acid (**Figure 10**).[179] Based on this, miR-499 suppressing cardiomyocyte apoptosis and pDNA encoding vascular endothelial growth factor were sequentially delivered for staged gene therapy of MI, which attained the restoration in cardiac functions and the decrease of cardiac fibrosis and hypertrophy without apparent toxic effects. Since redox agents distribute widely in cells, the delivery system can be fit into a variety of applicable situations for the management of CVDs. For example, Zhao et al. designed a dual-targeting core-shell nanocomplex, which consisted of a lipid bilayer, an intermediate layer of apoA-I for macrophage targeting, and an outermost layer of hyaluronic acid DOPE for endothelial cell targeting, to deliver siRNA against

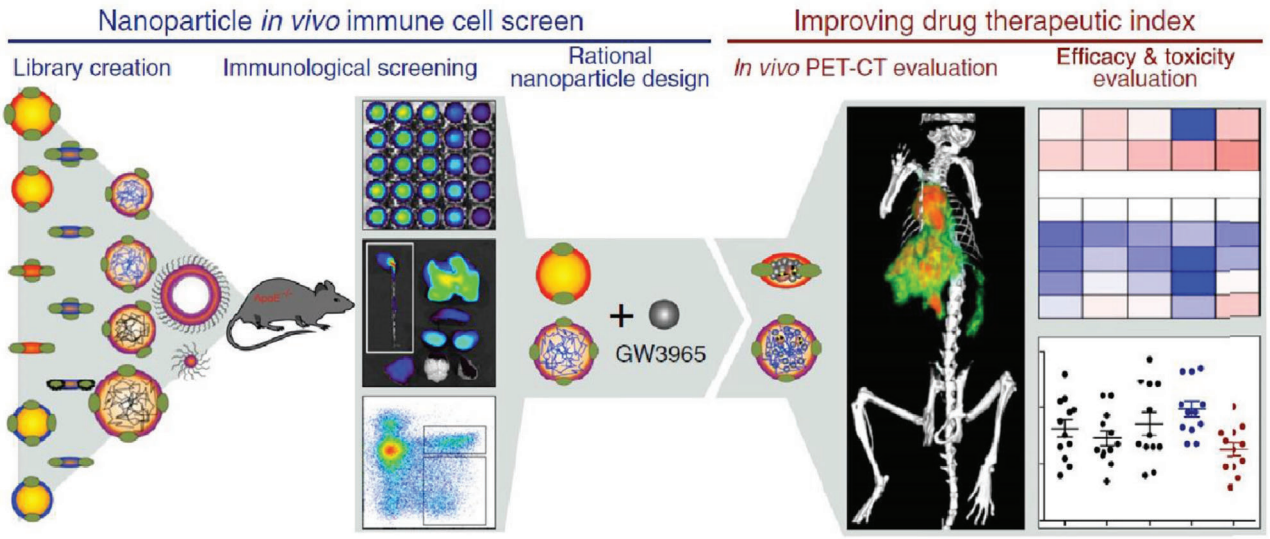

Figure 7. Schematic representation of the nanoparticle library. Reproduced with permission.[170] Copyright 2016, United States National Academy of Sciences (NAS).

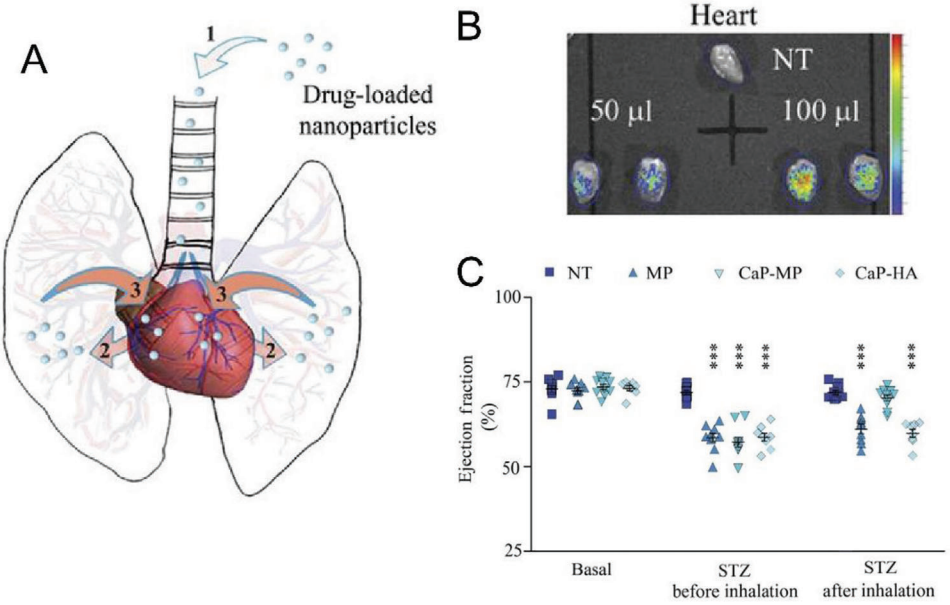

Figure 8. Inhalation of peptide-loaded nanoparticles improves heart failure. A) Schematic illustration of CaPs heart targeting after inhalation. B) Fluorescence imaging of hearts from nontreated mouse (NT) and mice inhaling 50 and 100 µL of CaP-Cyanine-7. C) Cardiac contractile function (indicated as ejection fraction) of streptozotocin-treated mice determined by echocardiographic analysis. Reproduced with permission.[176] Copyright 2018, American Association for the Advancement of Science (AAAS).

lectin-like oxidized LDLR-1 (LOX-1 siRNA) and atorvastatin.[180] Following a 12-week biweekly dosing regimen, the nanoparticles presented potent anti-atherosclerotic activities reflected by a prominent decrease in plaque size, lipid accumulation, $CD68^+$ macrophage content, and MCP-1 content. This work indicated a synergistic therapy of gene regulation and chemical interruption for enhanced atheroprotective efficacy.

The rational design and application of nanocarriers for drug delivery have strikingly improved pharmacokinetic parameters and bioavailability of currently available therapeutic agents for CVDs. Meanwhile, nanoparticle delivery systems also accelerate the clinical translation of emerging drugs which currently present serious adverse effects or poor tolerance to the physiological environment. Despite all the mentioned advances, nanoparticle delivery systems are still confronted with various challenges, including storage instability, extensive burst release of payloads, immunogenicity, and complicated fabrication procedures. Additionally, few researchers explore circulation half-lives and

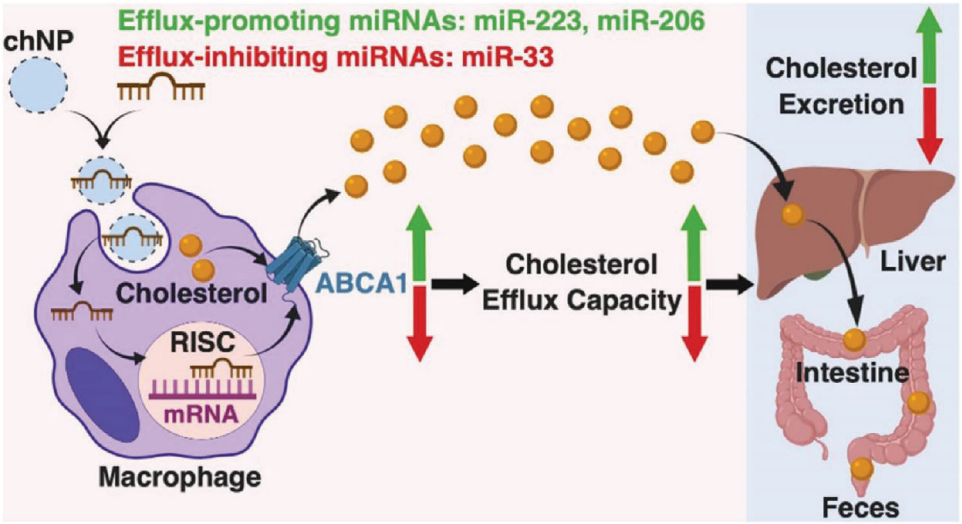

Figure 9. The treatment with miR33-chNPs decreased reverse cholesterol transport (RCT) to the plasma, liver, and feces, while efflux-promoting miRNAs delivered via chNPs improved ABCA1 expression and cholesterol efflux capacity into the RCT pathway. Reproduced with permission.[178] Copyright 2019, American Chemical Society.

long-term toxicity of nanomaterials to provide a reliable safety profile, which should also be stressed in future works.

5. Conclusions and Future Perspectives

Herein, we have extensively reviewed nanotechnology-based strategies to diagnose and combat atherosclerosis, and the associated ischemic diseases including restenosis, MI, and ischemic heart injury. In the discussion of nanomedicine we included four main strategies: nanoparticle-based cardiovascular imaging, nanosensors, nanoscale delivery of therapeutics, and nanostructured materials for cardiac tissue repair. We also highlighted the exceptional properties of nanomaterials; their size characteristics, physicochemical properties, and biocompatibility have made them an attractive and clinically important modality in improving the efficacy of CVD treatment and diagnosis. Newly developed nanomaterials provide a nanostructured microenvironment and binding sites for biological recognition and even improve the activity of biological molecules, enhancing therapeutic effects and diagnosis. Building upon these strategies, several nanotechnology-based treatments are in different stages of clinical development and hold great promise for successful translation (**Table 1**).

However, only a few nanomedicine-based technologies have been approved for the management of CVDs, and major challenges and limitations still remain to be solved. For example, nanostructured scaffolds are widely studied for tissue regeneration, yet engineered tissue with tensile strength and anisotropy similar to those of natural tissues are still to be developed. Designing nanomaterial-embedded scaffolds with biomimetic properties may represent a new trend for cardiovascular tissue regeneration. In terms of nanostructured biosensors, further investigation is required to improve poor reproducibility and complex electron-transfer pathways of electrochemical methods. Despite all the positive outcomes in lab research, nanoformulations present far more difficulties in clinical translation compared to conventional pharmaceuticals. The sophisticated construction of nanoformulations, including diverse surface modifications and delivery of multiple payloads, has led to elevated cost, complicated manufacturing and quality control processes, poor batch-to-batch reproducibility, and instability in storage, which impedes large-scale manufacturing. Moreover, it is far too often overlooked that nanomaterials present with intrinsically heterogeneous physicochemical properties which may result in differing interactions with subcellular components after entering cells. Therefore, the interactions between nanoformulations and biological systems, especially pathological tissues, need to be fully elucidated. Additionally, more attention needs to be paid to the possible mechanisms of short-term and long-term toxicity effects resulting from these interactions. In order to accelerate clinical translation, future studies should focus on reducing formulation complexity and simplifying the administration of nanomedicine. Furthermore, evaluation of the stability of nanoformulations should be conducted under both physiological and storage conditions (i.e., lyophilized form). In addition to improving therapeutic effect, researchers should strive to elucidate the biodistribution and bioaccumulation of these nanomaterials, the vitro/in vivo efficacy correlation, possible long term adverse effects, and mechanisms of action.

Despite the existing challenges, previous studies have demonstrated the tremendous potential of nanomedicine as one of the most promising approaches for managing CVDs. A comprehensive understanding of CVD pathogenesis may result in the discovery of novel biomarkers and therapeutic targets, providing new insight toward further innovation in nanomedicine. Nanomedicine also extends the range of applications for gene editing and chimeric antigen receptor T cell techniques, which shows the intricate intertwining of nanomedicine with other advanced technologies, even combining them for synergetic effects. These techniques, in turn, endow nanomaterials with new characteristics, such as immunomodulatory functions, further

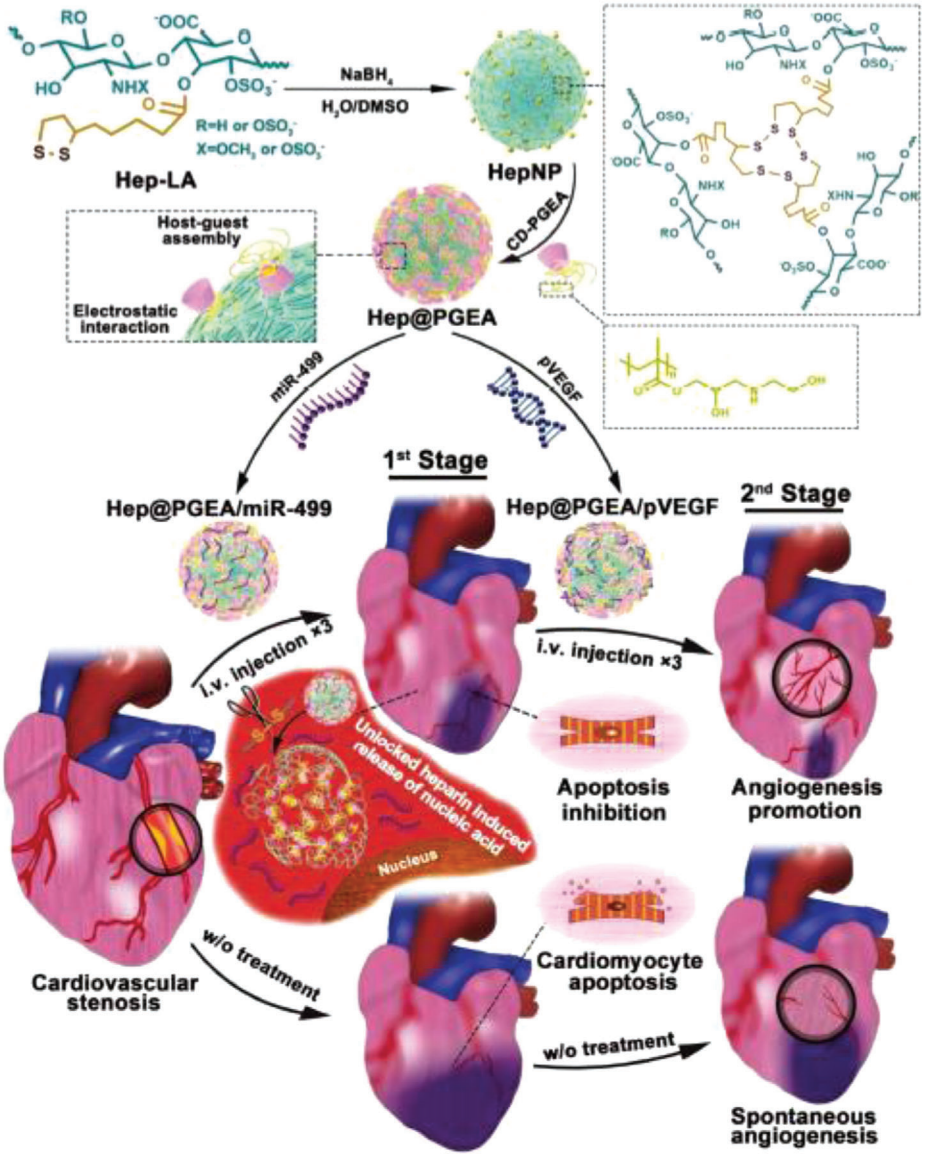

Figure 10. Schematic illustration of the preparation processes of Hep@PGEA and their applications in staged delivery of miRNA–plasmid deoxyribonucleic acid (pDNA) for the management of MI. Reproduced with permission.[179] Copyright 2018, Wiley-VCH.

enhancing therapies. In conclusion, the rapidly developing field of nanomedicine may very well signal a revolution in the management of atherosclerosis and associated ischemic diseases.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords

atherosclerosis, drug delivery, myocardial infarction, nanomedicines, nanosensors, noninvasive imaging, tissue engineering

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