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Review

Prec. Nanomed. 2019;2(1):230-245

Precision nanomedicine in atherosclerosis therapy: how far are we from reality?

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Submitted: November 14, 2018 Accepted: January 16, 2019 Published: January 21, 2019

Abstract

Atherosclerosis, characterized by the build-up of lipids and chronic inflammation of the arterial wall, is the primary cause of cardiovascular disease and is a leading cause of death worldwide. Currently available therapies are inadequate and warrant the demand for improved technologies for more effective treatment. Although primarily the domain of antitumor therapy, recent advances have shown the considerable potential of nanomedicine to advance atherosclerosis treatment. This review details the arsenal of nanocarriers and molecules available for selective targeting in atherosclerosis and emphasize the challenges in atherosclerosis treatment.

Keywords

Atherosclerosis, nanomedicine, nanoparticles, therapeutics

Purpose and Rationale

World Heart Day, 29 September, is a day to remind people around the globe that cardiovascular disease (CVD) is the world's leading cause of death claiming 17.5 million lives yearly, despite advances in cardiovascular research, and highlight the actions that individuals can take to prevent and control CVD [1]. Atherosclerosis, characterized by build-up of lipids and chronic inflammation of the arterial wall, is a critical component of the pathologies underlying CVD [2]. Dysfunctional endothelium is a crucial pathophysiological factor in atherosclerosis, causing increased permeation of cholesterol-containing low-density lipoprotein (LDL) particles into the arterial wall. Chemical reactions occurring within the damaged wall of arteries cause cholesterol molecules to oxidize. This initiates an inflammatory response to recruit monocytes and subsequently differentiate them into macrophages. The macrophages ingest cholesterol molecules and transform into foam cells, which further accumulate to form plaque. The smooth muscle cells migrate out of the smooth muscle layer and into the fatty plaque, and then secrete collagen and elastin to form a fibrous cap to seal the plaque from the blood stream. Atherosclerotic plaques can rupture as a result of the breakdown of the fibrous cap, which can consequently lead to catastrophic events such as thrombotic occlusion, stroke, myocardial infarction, and sudden cardiac death (Figure 1) [3].

On the currently available treatment options for atherosclerosis, statins, which are hydroxymethyl-glutaryl coenzyme A (HMG-CoA) reductase inhibitors, are the primary pharmacological approach to lowering LDL cholesterol level in the serum, a primary risk factor in coronary artery disease. When prevention of atherosclerosis fails, interventional therapies such as balloon angioplasty and stenting are required, but they are invasive and restenosis risks are significant in up to 16% for drug eluting stenting and 30% for bare metallic stenting [4, 5]. However, all of these treatment approaches unfortunately fail to address localized oxidative damage and inflammation governing atherosclerosis. These

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suboptimal atherosclerosis therapies warrant the need for developing effective therapeutic approaches to counter atherosclerosis. Therefore, the question for researchers is: what other options should we use in the battle against atherosclerosis?

Nanomedicine is one possible option to advance atherosclerosis treatment. Nanoparticles (NPs), with diameters ranging from 10 to 1000 nm, have unique properties such as large surface-to-volume ratio, small size, the ability to encapsulate various active therapeutic agents (APIs), and incorporate bio-recognition ligands for specific targeting. These properties make NPs an attractive drug delivery system potentially superior to conventional atherosclerosis therapies [6]. Moreover, in a similar vein to cancer nanomedicine, it is believed that nanoparticles offer opportunities to (1) modulate the pharmacokinetic and pharmacodynamics profiles of existing APIs, (2) lower drug toxicity, (3) improve drug solubility, (4) deliver their payload in a controllable manner, and (5) be multifunctional to allow selective targeting, imaging, and delivery with improved precision and specificity to the diseased sites [7-9].

Although still in its infancy, the concept of "atherosclerosis nanomedicine" has generated significant interest, based on the rapid increase in the number of publications on this topic over the past 10 years. In this review, it is an excellent time for us to reflect if atherosclerosis nanomedicine has lived up to expectations by presenting the current nanomedicine-based atherosclerosis therapeutic strategies, highlighting the challenges and clinical translation opportunities.

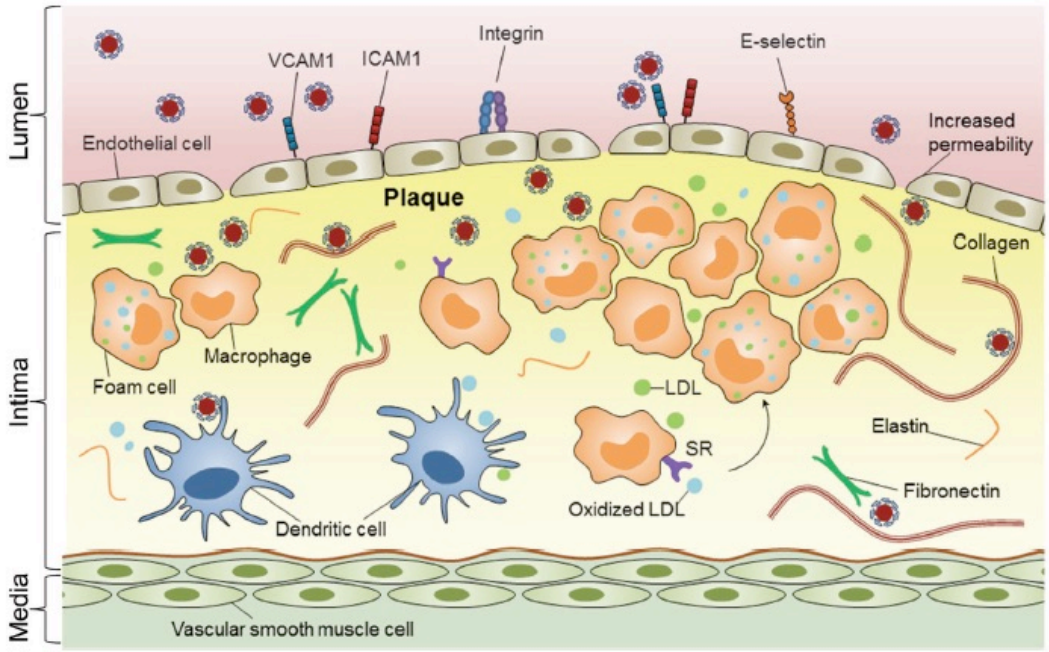

Figure 1. Pathogenesis of atherosclerosis. Dysfunctional endothelium permits low-density lipoproteins (LDLs) to enter the intima, where they are oxidized (oxLDLs). oxLDLs upregulate the expression of adhesion molecules such as E-selectin, intercellular adhesion molecule (ICAM1) and vascular adhesion molecule (VCAM1) to recruit monocytes. Recruited macrophages engulf oxLDL via scavenger receptors (SRs) and transform into foam cells. The smooth muscle cells migrate out of the smooth muscle layer and into the fatty plaque, and then secrete collagen and elastic to form a fibrous cap to seal the plaque from the blood stream. Atherosclerosis plaques can rupture as a result of the breakdown of the fibrous cap, which can consequently lead to catastrophic events such as myocardial infarction or stroke. Reprinted with permission. Chan et al 2018.[10]

Summary of relevant literature

Nanoparticle targeting in atherosclerosis

Owing to greater understanding of the underlying molecular mechanism of atherosclerosis, the key targeting strategies in atherosclerotic plaque can generally classified into nonspecific targeting (the enhanced permeability and retention [EPR] effect) and active targeting using functionalized nanoparticles. The EPR effect has been thoroughly investigated in cancer; the

characteristic features of tumors include leaky blood vessels, whose endothelium is fenestrated with gaps between 100 and 780 nm of size, and dysfunctional lymphatic drainage [11, 12]. The enhanced permeability of the abnormal tumor vasculature enables NPs to extravasate (escape) into the tumor interstitial space, while ineffective lymphatic drainage causes the NPs retention within the tissue and allows them to release drugs into the vicinity of the cancer cells [13, 14]. Although it has never been thoroughly investigated in atherosclerosis, nonspecific targeting based on the EPR effect can be exploited owing to the increased permeability of the dysfunctional endothelium, angiogenic expansion, and leakiness of the neovessels of the vasa vasorum [15]. In general, NPs with diameters less than 200 nm are considered the most effective for the EPR effect [12, 16].

For active targeting, dysfunctional endothelium is a key pathophysiological feature in atherosclerosis and it naturally serves as a candidate for targeting as there is a wide spectrum of vascular targets available for the site-specific targeted drug delivery of therapeutic agents to stabilize and regress the plaque (Figure 2). For instance, cell adhesion molecules (such as intercellular adhesion molecules 1 (ICAM 1) and vascular cell adhesion molecules 1 (VCAM 1), selectins or integrins such as $\alpha_v\beta_3$ integrin) represent an important class of attractive targets for delivery of atherosclerotic drugs [17, 18], as these adhesion molecules are typically overexpressed on the activated/angiogenic endothelium of the luminal wall.

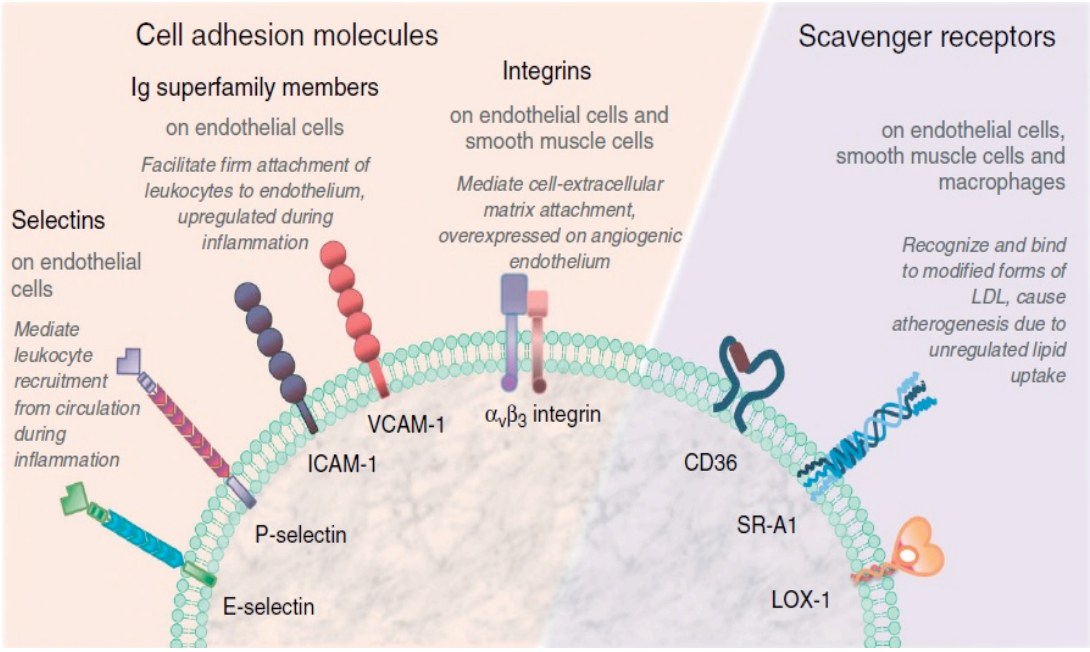

Figure 2. *Targetable cell-surface receptors for therapeutic applications of atherosclerosis. Lewis et al 2011.* [2]

Likewise, lectin-like oxidized low-density lipoprotein receptor (LOX-1) present in endothelial cells is also a targeted site for atherosclerosis. Oxidized low-density lipoprotein (oxLDL) contributes to the atherosclerotic plaque formation and progression via the induction of endothelial dysfunction, macrophage/foam cell formation, SMC migration and proliferation. LOX-1 is the main oxidized LDL (oxLDL) receptor of endothelial cells, and it is also expressed in macrophages and SMCs [19]. Nanoparticles targeted to these vascular endothelial sites not only provide site-specific targeted drug delivery, but can also attenuate the leukocyte-endothelium adhesion and consequently reduce atheroinflammation by competitive binding to the sites.

In addition, macrophages represent another important component for specific targeting of plaques with functionalized nanoparticles. For example, SR-A1 and CD36 scavenger

receptors, predominately expressed on macrophages, are both desirable targets for NPs. Moreover, lipoproteins such as LDL and high-density lipoprotein (HDL), are potential candidates for atherosclerotic targeting. These lipoproteins interact with plaques through a natural conduit, thus engineering nanoparticles that possess the lipoprotein properties can accumulate in the plaque via inherent targeting [20, 21]. Table 1 summarizes the most frequently used targets in atherosclerosis.

Table 1 Examples of targeting moieties in atherosclerosis

Processes	Target	Targeting moiety	References
Endothelial cell activation	Cell adhesion molecules, such as VCAM-1, ICAM-1, E-selectin, P-selectin	VINP-28 peptide, VCAM-1 targeting cyclic peptide CVHSPNKKC, VCAM-1 targeting linear peptide VHPKQHR, PECAM-1 antibody	[22],[23],[24],[25]
Vascular basement membrane	Extracellular matrix, collagen, elastin	Collagen-IV targeting peptide KLWVLPK MMP12	[26],[27]
Cell accumulation	Lipoproteins such as HDL, oxLDL	PolyG for targeting SR-A1, SR-A1 antibody	[28],[29],[30]
Inflammation	Macrophages-surface receptors such as SR-1, CD36, LOX-1 and dextran receptor SIGNR-1	PS residues, Dextran modification, Anti-CD36, Anti-LOX-1	[31],[32],[33]
Angiogenesis	αVβ3 integrin	αVβ3 antagonist	[34],[35],[36]
Thrombosis	Fibrin	tPA, CREKA	[37],[38]

Abbreviations: CREKA = cysteine-arginine-glutamic acid lysine-alanine; IV = intravenous; PECAM-1 = platelet-endothelial call adhesion molecule; PolyG = polyguanylic acid; PS = phosphatidylserine; tPA = tissue plasminogen activator; VINP-28 = peptide ligand for VCAM-1.

Nanoparticle therapeutics in atherosclerosis

Various types of nanocarriers have been employed in atherosclerosis therapy including natural or synthetic polymeric nanoparticles, micelles, liposomes and HDL nanoparticles, and many of these nanocarriers are coupled with targeting ligands for specific targeting. Currently, the most common approaches to encapsulate and deliver therapeutic compounds for atherosclerosis treatment have focused on developing liposomal and HDL nanoparticle formulations. Table 2 summarizes the nanoparticle systems for atherosclerosis therapy.

Since the discovery in the 1960s, liposomes have a long history of use as a drug delivery system with a few clinically approved therapeutics in market for cancer application [7], making them industrially favorable due to available large-scale production infrastructures and the accessibility of the composition. Liposomes are structurally spherical vesicles, consisting of one or more lipid bilayers enclosing an aqueous core. This structure

allows the incorporation and delivery of therapeutic and/or diagnostic cargos into the lipid layer or the core, depending on their hydrophilicities. It also allows the encapsulation of polyanions such as nucleic acids (DNA and siRNA) by using cationic lipids. Liposomes can be further modified to improve blood circulation and cell-specific targeting by incorporating polyethylene glycol (PEG) and targeting moieties, respectively. The hydrophilic surface of the PEGylated liposomes can hinder the recognition by the mononuclear phagocytes and prevent carriers from opsonization, thereby avoiding blood clearance and prolonging blood circulation half-life. However, the circulation half-life of the nanoparticles also depends on other factors, such as size, shape, chemical composition, and surface charge. Furthermore, the use of liposomes for atherosclerosis therapy is attractive as they have displayed anti-atherogenic properties based on the ability of a cholesterol-free phospholipid bilayer to pick up cholesterol from lipoproteins or the atherosclerotic vessel wall [15].

To this end, our group has developed injectable 100-nm cholesterol-free liposomal NPs encapsulated with a glucocorticoid, fluocinolone acetonide (FA). We demonstrated the anti-inflammatory effect and cholesterol efflux capability of FA-liposomal NPs in vitro and liposomal NPs accumulation and colocalization in plaque macrophages using Apo$^{-/-}$ mice model (Figure 3). Moreover, Lobatto et al. [39] developed a PEGylated liposomal formulation containing prednisolone phosphate and i.v. injected to a rabbit model of atherosclerosis at a dose of 15 mg/kg. The liposomal NPs exhibited a prolonged half-life of 39.9 hours and substantial anti-inflammatory effects at 2 days and up to at least 7 days post injection as compared to the drug-free animals that were tested. In a follow-up study, the group fabricated the GMP-grade liposomal nanoparticles with drug incorporation efficiency of 3–5% and further examined them in a randomized clinical trial in 2015 [40, 41]. This first clinical study showed the prolonged circulation half-life of prednisolone to 45–63 hours administered as a liposomal NP in human and observed intravenously administered liposomal NPs to successfully accumulate in 75% of macrophages isolated from atherosclerotic plaques of patients with symptomatic iliofemoral atherosclerosis. However, the short-term liposomal NP treatment (2 doses of 1.5 mg/kg at day 0 and 7) did not reduce arterial wall permeability or arterial wall inflammation in patients with atherosclerotic disease.

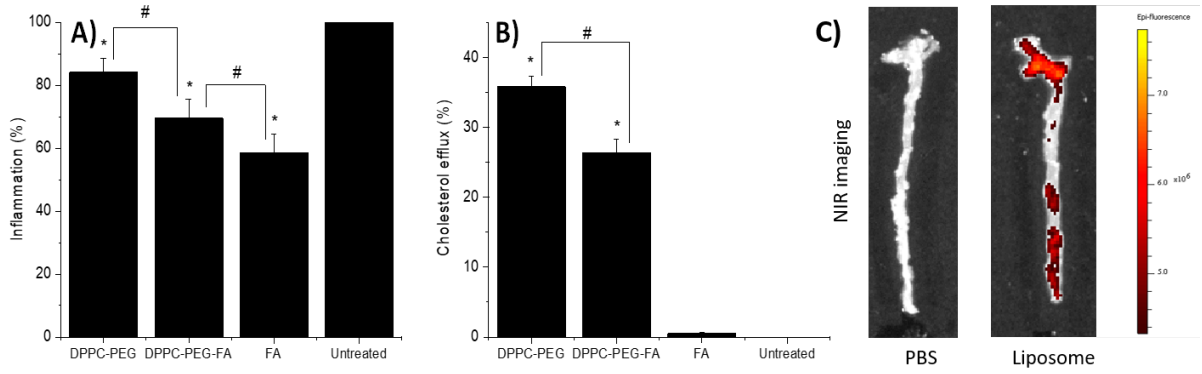

*Figure 3. (A) Anti-inflammation and (B) cholesterol efflux capacities of FA-loaded liposomes in vitro; * $p < 0.05$ vs. untreated, # $p < 0.05$, $n = 3$. (C) Near-Infrared Flourescence imaging of excised aortas from ApoE-/- mice 24 hours after injection.*

In addition to liposome, HDL nanoparticle (7–20 nm in diameter) is an attractive platform for atherosclerosis therapy as there are clinical observations correlating higher plasma HDL levels with decreased incidence of CVD. HDL is believed to protect against atherosclerosis primarily through reverse cholesterol transport, removing cholesterol from lipid-laden macrophage "foam cells" in atherosclerotic lesions via efflux through their ATP-binding cassette transporter A1 (ABCA1) and scavenger SR-B1 receptors [42-44]. With the advent of nanotechnology, biomimetic HDLs can be readily synthesized by facile bottom-up

method via self-assembly of apolipoprotein A-1 (Apo A1), cholesterol, phospholipids, oligo-amino acids, and/or inorganic core [45]. Apo A1 is the main component of HDL and is responsible for scaffolding the size and shape of HDL. Along with Apo A1, cholesterol and phospholipids, particularly phosphatidyl-cholines, make up the bulk of HDL nanoparticle mass. Towards this end, Duivenvoorden et al. [42] synthesized simvastatin-loaded reconstituted HDL nanoparticles and demonstrated that they can target macrophages in the plaque upon i.v. injection and show to exhibit anti-inflammatory effects using an apolipoprotein E-knockout $ApoE^{-/-}$ mice model of atherosclerosis. In a 3-month low-dose treatment (15 mg/kg statin), statin-HDL nanoparticles inhibited plaque inflammation progression while they substantially decreased inflammation in a 1-week high-dose (60 mg/kg statin) treatment in advanced atherosclerotic plaques.

Polymeric nanocarriers, including natural and synthetic, are another promising tool for atherosclerotic treatment. Because of their chemical versatility, polymeric nanocarriers can be formed into different structures including micelles, vesicles and solid nanoparticles. Furthermore, different moieties can be decorated on the surface of polymeric nanocarriers through active functional groups of polymers. Among them, poly (lactic-co-glycolic acid) (PLGA) is a widely used biodegradable and biocompatible polymer for nanocarriers. As an example, Nakashiro et al. [46] delivered PLGA solid nanoparticles loaded with pioglitazone, a PPARγ agonist shown to enhance M2 (anti-inflammatory) macrophage polarity, to inflammatory monocytes and macrophages in the $ApoE^{-/-}$ mice model (7 mg/kg weekly for 4 weeks). By reducing $Ly6C^{high}$ inflammatory monocytes and inducing M2 macrophage-associated genes in the aorta, pioglitazone-loaded PLGA NPs stabilized atherosclerotic plaques assessed by a reduction in the number of buried fibrous cap and increased the fibrous cap thickness in the plaque. In another study, Katsuki et al. [47] used pitavastatin-incorporated PLGA nanoparticles and observed inhibited plaque destabilization and rupture in $ApoE^{-/-}$ mice model. It was associated with inhibited MCP-1 induced monocyte chemotaxis and the secretion of MCP-1, thus decreasing monocyte infiltration and gelatinase activity in the plaque.

In addition, amphiphilic copolymers are also used as the starting block for polymeric nanocarriers in atherosclerotic treatment. For instance, polymeric micelles (5–100 nm) are self-assembling colloids based on amphiphilic block copolymers, forming a hydrophobic core and hydrophilic shell. The hydrophobic core region serves as a reservoir for hydrophobic drugs, whereas the hydrophilic shell region stabilizes the hydrophobic core for prolonged circulation. In one study, Peter et al. [48] investigated the use of micelle with clot-binding peptide cysteine-arginine-glutamic acid-lysine-alanine (CREKA) for targeted delivery of anticoagulant drug Hirulog. In the $ApoE^{-/-}$ mice model, it was found that the CREKA/hirulog mixed micelles accumulated in the rupture-prone shoulder regions of plaques and significantly increased anti-thrombin activity, thus representing a potential strategy to reduce the risk of thrombus formation on plaque rupture at the late stage of atherosclerosis. Furthermore, amphiphilic copolymers can also self-assemble into a myriad of aggregate structures such as vesicles or polymersomes. Polymersomes have an architecture similar to that of liposomes, but they are composed of amphiphilic block copolymers. As an example, Broz et al. [28] have developed pravastatin-loaded triblock copolymer vesicles consisting of poly(dimethylsiloxane) (PDMS) middle block and poly(2-methyloxazoline) (PMOXA) side chains with targeting biotinylayed polyG ligand, which has an affinity for inflammatory macrophages via scavenger receptor binding. It demonstrated specific uptake by macrophages but no other cell types and slow intracellular controlled release of pravastatin, thus reducing systemic toxicity.

Besides synthetic polymer, natural polymers have been employed in the fabrication of polymeric nanocarriers. In this concept, siRNA containing dextran NPs were developed and injected into $ApoE^{-/-}$ mice to silence the monocyte-recruiting receptor, C-C chemokine receptor type 2 (CCR-2) [49]. With combined PET/MRI imaging, it showed dextran NPs uptake predominantly in monocytes and macrophages (76.7%) and specific targeting of monocyte recruitment with siRNA dampened

inflammation gene expression in plaques. Interestingly, a research group in China recently developed multifunctional virus-based nanoparticles (SV-40) for atherosclerotic theranostics [50]. In the design of their NPs, quantum dots and Hirulog are encapsulated within SV-40 nanoparticles with different targeting peptides for VCAM-1, macrophages and fibrin in order to monitor different stages of atherosclerosis. In ApoE$^{-/-}$ mice model, it showed the local accumulation of NPs and an increased drug amount in the plaque.

Discussion

Though the NP therapy approach in atherosclerosis has shown favorable results in vitro and in vivo, plenty of questions remain to be answered before we can actually contribute to clinically feasible therapeutic approaches.

Table 2. Nanoparticle systems for atherosclerosis therapy

Nanocarrier	Drug	Targeting moiety	Animal model	Half-life	Plaque biodistribution	Ref.
Lipid-based NPs						
Reconstituted HDL NP (DMPC, MHPC, ApoA-1)	Simvastatin		ApoE$^{-/-}$ mice	21.9 h	1.0% ID/g at 24 h	[44],[42]
HDL NP (DMPC, MHPC, ApoA-1)	Simvastatin (60 ± 7% EE)		ApoE$^{-/-}$ mice		1.5-2% ID/g at 24 h	[51]
HDL NP (POPC, PHPC, ApoA-1)	Liver X receptor agonist (GW3965)		ApoE$^{-/-}$ mice	10.5 h	0.07% ID/g at 24 h	[52]
Lipid NP (C12-200, PC, Chol, PEG-DMG)	siRNA	Chemokine receptor (CCR2)/ Target: macrophage	ApoE$^{-/-}$ mice	8.1 min		[53]
Liposomes						
Liposome with coated cationic lipoparticle core (DOTAP, HSPC, Chol, DSPE-PEG2k)	Anti-miR-712	VHPK peptide/ Target: VCAM1	ApoE$^{-/-}$ mice	167.7 sec		[54]
Liposome (DPPC, Chol, DSPE-PEG2k)	Prednisolone phosphate		New Zealand white rabbits, Sprague-Dawley rats, Human atherosclerotic plaque	39.9 hours 39.8 hours 63 hours		[39],[40],[41]
Liposome (DPPC, Chol, DSPE-PEG2k)	Simvastatin (71 ± 3% EE)		ApoE$^{-/-}$ mice		1.5-2% ID/g at 24 h	[51]
Micelles						

Micelle (mPEG-*b*-p (HPMAm-Bz) copolymer)	Simvastatin (65 ± 8% EE)		ApoE$^{-/-}$ mice	1.5-2% ID/g at 24 h	[51]
Other NPs					
PLGA NP	Pitavastatin		ApoE$^{-/-}$ mice		[55]
PLGA NP	Pioglitazone		ApoE$^{-/-}$ mice		[46]
Polymeric NP (NH_2-PLGA-NH_2, PDLA-PEG-OMe)	Interleukin 10 (IL-10)	PLGA-PEG-Collagen IV/ Target: collagen IV	LDLr$^{-/-}$ mice		[56]

Table 2. Nanoparticle systems for atherosclerosis therapy (Cont.)

Nanocarrier	Drug	Targeting moiety	Animal model	Half-life	Plaque biodistribution	Ref.
Sugar-based amphiphilic macromolecules		Target: macrophage	ApoE$^{-/-}$ mice	28 hours	-	[58]
Dextran-based NP	siRNA	Chemokine receptor (CCR2)/ Target: macrophage	ApoE$^{-/-}$ mice	3.73 hours	1.2% ID/g at 48 hours	[49]

Abbreviations: HDL = high-density lipoproteins; NP = nanoparticles; PLGA = poly (lactic-co-glycolic acid).

Status and what is missing

Somewhat in parallel with targeted delivery of chemotherapy for cancer, atherosclerotic lesion targeting still awaits meaningful clinical translation. So far, the predominant approach is by using stealth particles with increased circulation half-lives, then reach the targeted site (in smaller proportions than the liver and spleen) and deliver its payload. What is lacking is an understanding of how various nanoparticle parameters affect target lesion accumulation. It is well known that the physiochemical properties of nanoparticles (composition, size, shape, surface charge, hydrophilicity, and ligand type and density) impact the payload properties (loading and release kinetic) and in vivo performance (pharmacokinetics, tissue distribution, atherosclerotic plaque targeting and efficacy). One such attempt is by Tang et al. [52] who constructed a combinational library of 15 HDL-mimicking NP formulations varying with respect to size, shape, core payload and lipid composition for in vivo screening studies using ApoE$^{-/-}$ mice model to identify the optimal NP characteristics for atherosclerotic plaque targeting and compared it with 2 extensively studied nanocarriers, such as PEGylated micelle and liposome.

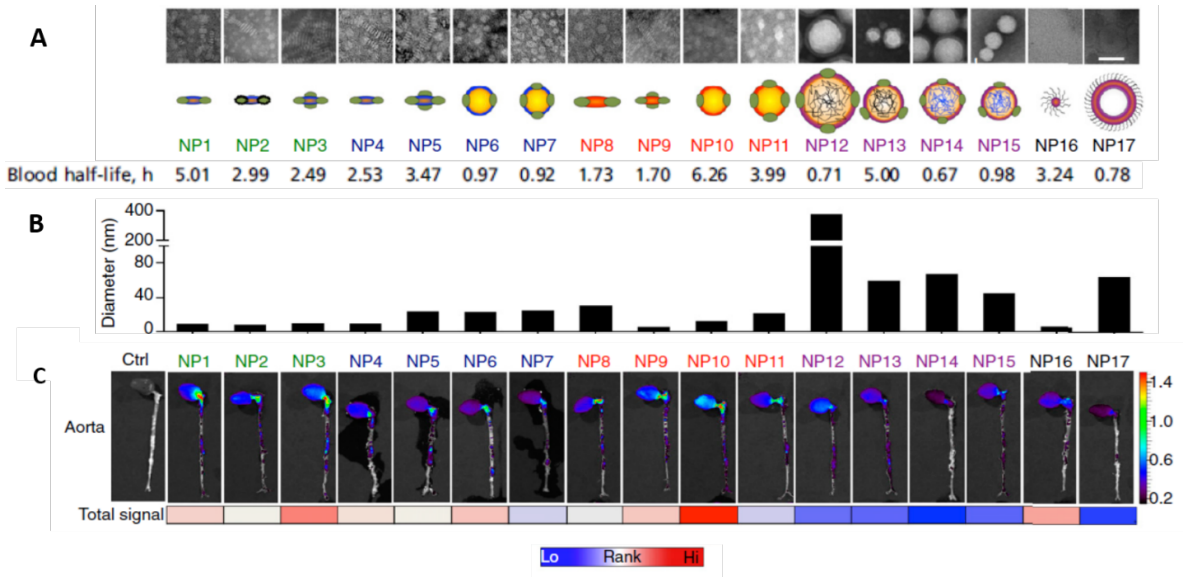

Figure 4. *The effect of high-density lipoprotein (HDL)-mimicking nanoparticle (NP) properties on atherosclerotic plaque targeting.* ***(A)*** *Representative transmission electron microscopy images and schematic diagrams of a library of HDL-mimicking NPs with their blood circulation half-lives.* ***(B)*** *Size of HDL-mimicking NPs as measured by dynamic laser scattering.* ***(C)*** *Representative Near-Infrared Fluorescence (NIRF) images of nanoparticle accumulation in the aorta. The heat map presented below the NIRF images shows the mean total fluorescene signals in the aorta with red indicating a high and blue a low ratio. Tang et al.*[52]

Based on the results of the library screening (Figure 4), the authors identified the favorable lipid composition (POPC-based), pharmacokinetics (long circulation half-life), size (~ 30nm), and morphology (spherical) to achieve optimal plaque macrophage-specific targeting delivery.

Furthermore, another comparative study of 3 established nanoparticles (HDL, polymeric micelle and liposome, targeting simvastatin to atherosclerotic plaque was conducted [51]. The group systemically investigated and compared the performance of the 3 distinct nanocarriers in term of drug loading and release, biodistribution, cellular uptake, and therapeutic efficacy (Figure 5). It was found that while both polymeric micelle and liposome possessed longer circulation half-lives than HDL, these 3 nanoparticles had similar levels of accumulation in atherosclerotic plaques (~ 1.5-2% ID/g). Despite their similar plaque accumulations, polymeric micelle demonstrated a higher plaque macrophage uptake and efficacy in reducing macrophage burden in the advanced ApoE$^{-/-}$ mice model. It is worth noting that this finding does not correlate with the observation made by Tang et al. that HDL nanoparticles demonstrated better in vivo performance [52]. Once again, this highlights the importance of the properties of the NPs; even a slight twist in the nano-formulation will have thorough impact on the overall therapeutic outcome.

Reality of precision targeting?

In the perspective published by Wilhelm et al in 2016 [59], it is found by performing a multivariate analysis based on over 100 cancer nanomedicine-related publications, that only 0.7% of the administered NP dose (0.7% ID) is delivered to a solid tumor. In the context of atherosclerosis nanomedicine, many studies did not report the circulation half-life and delivery efficiency of NPs to plaque. Despite the broad range of the circulation half-lives, the delivery efficiency of NPs is typically between 0.07 and 1.5% ID/g, even with the incorporation of targeting moieties (Table 2). So, is specific targeting really happening?

Furthermore, we would like to highlight that most of the reported biodistribution data (i.e., circulation half-life and delivery efficiency) are based on tracking the imaging agents such as a fluorescent and radiolabel probe, not on the NPs as a "whole" nor the actual APIs. The experiment conducted by Li et al. [60] demonstrated the diverse difference in the distribution of a fluorescent probe and an anticancer drug in vivo, in which both of them were incorporated within the nanocarrier. Clearly, the fluorescence imaging data does not

reflect the actual distribution of APIs at the plaque nor does it govern regulatory approval of nano-formulations [61, 62]. Therefore, pharmacological evaluation of the nanoparticle's API is still desirable and relevant compared with the therapeutic outcomes.

Too much reliance on the mouse model?

Currently, the most widely used animal models in atherosclerosis research are genetically engineered mice. Specifically, these are the ApoE$^{-/-}$ mice generated by knocking out ApoE to prevent clearance of very low-density lipoprotein, and the Ldlr$^{-/-}$ mice generated by knocking out the LDL receptor responsible for the clearance of LDL, resulting in elevated plasma cholesterol levels for triggering the formation of atheroma [63]. Though these mouse models provide a convenient platform for nanoparticle screening, it remains controversial as to whether the observations made with these models are directly relevant to the human disease. Other animal models, including rabbit, pig, and non-human primates, have also been utilized for atherosclerosis research [64, 65]. In a study by Perez-Medina et al. [64, 66], the team investigated the biodistribution and plaque targeting of radiolabeled HDL in 3 different animal models of atherosclerosis; an ApoE$^{-/-}$ murine model, a New Zealand white rabbit model with a double balloon injury of thoracic and abdominal aorta, and a familial hypercholesterolemia porcine model with balloon injuries on the deep and superficial femoral arteries. In all the animal models, studies showed the same patterns in radioactivity distribution with kidneys as the main accumulation site, with consistent localization in atherosclerotic plaque (0.019 ± 0.04% %ID/g for mice vs. 0.013 %ID/g for rabbit, no data for pig).

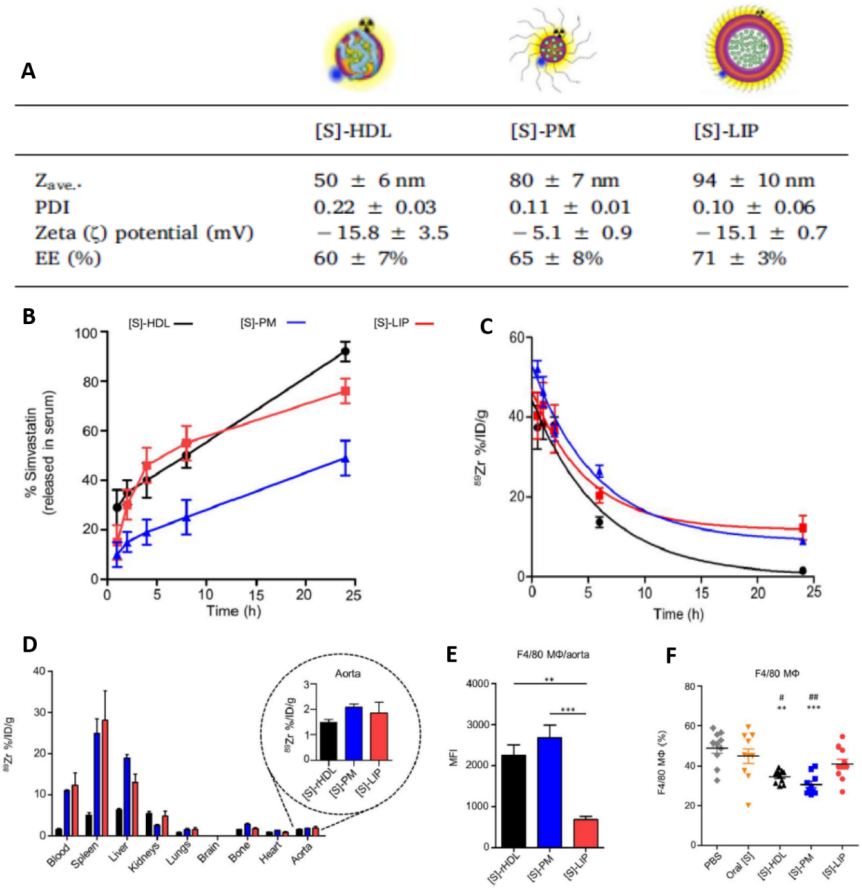

Figure 5. The effects of nanocarrier type on atherosclerotic plaque targeting. (A) Schematic diagrams of the 3 nanoparticle platforms and their physical characteristics. (B) In vitro release of simvastatin in fetal bovine serum as measured by high-performance liquid chromatography. (C) Blood circulation half-lives of the different 89Zr-labeled nanocarriers as determined by gamma counting. (D) Quantification of the radioactivity of the different nanocarriers in the selected tissues. (E) Quantification of mean fluorescence intensity of macrophages in the atherosclerotic aortas. (F) Quantification of macrophages in the atherosclerotic aortas using flow cytometry. Alaarg et al.[51]

However, there are no therapeutic efficiency study comparing the 3 animal models. Indeed, the metabolism and pathophysiological condition could be different between the animal species and some are closer to humans than others [67]. For example, it is well known that rat and mouse will not develop atherosclerotic plaque and are resistant to atherogenesis if they are nor genetically modified.

This is because the majority of plasma cholesterol are HDL particles in mice, rather than LDL in humans [68, 69]. Leong et al. described the difference and similarity of atherosclerosis in different animal models and showed that rabbits, pigs and non-human primates [70-72] are the closer model to humans for atherosclerosis in terms of anatomical and physiological components; but it was noted that fewer lesions developed in rabbit models [73]. Despite that, there are no perfect animal models that completely replicate the condition of human atherosclerosis. For instance, Valk et al. [41] have showed reduced inflammation in atherosclerotic lesions using liposomal prednisolone in rabbit models; this treatment did not induce measurable anti-inflammatory effects in humans. To explain this discrepancy in the therapeutic efficacy between the preclinical rabbit study and human study, the team hypothesized that the drug payload may not be accumulated sufficiently in the plaques. Alternatively, the therapeutic inefficacy in humans could come from the fact that plaque development in rabbits occurs in weeks' time [64], whereas humans generally develop atherosclerotic plaques over the course of decades, making an acute inflammatory reaction in rabbits and a chronic low-grade inflammatory response in humans.

The genetically modified mice will still remain as the model of choice in the first instance to perform the mechanistic study as the facility to knock out or over express specific target has permitted pinpointing the relative functional importance of the specific gene products, facilitating the formulation of new strategies for future intervention in atherosclerosis. In any case, the mouse model can be used as validation of treatment due to the multifactorial condition of atherosclerosis. Today, there is no single animal model with the same pathophysiological and metabolism condition as humans in term of development, location, and progression [67]. While it is critical to screen the nanoparticle formulation across multiple animal species and verify if the results obtained with one animal species is translatable across different species, it does not guarantee successful clinical translation in human, Bind Therapeutics failure for cancer therapy is an example [74].

Complement activation

Most of the nanoparticles developed for the treatment of atherosclerosis are intended for intravenous (IV) application. Once introduced in the bloodstream, NPs interact with complement proteins and their surfaces are immediately modified by the adsorption of a "protein corona." These proteins prime the particles for removal by immune cells and may contribute toward infusion-related adverse effects such as allergic responses. There are several reviews detailing how nanoparticles with various physiochemical properties interact with the immune system [75-77]. In a recent review by Halamoda-Kenzaoui [78], 61% of the reported lipid-based nanoparticles showed an effect on the immune system, mainly the activation of the complement system. The complement system is a critical component of the innate immunity, generating immediate unspecific defense reactions against pathogenic infections. The complement system can be activated via 3 distinct pathways (classical, lectin, and alternative) that converge to generate the same set of effector molecules at the third component of complement (C3) [79]. The cascade of reactions can lead to (1) proinflammatory process with the release of anaphylatoxins, (2) opsonization of vesicles, which in turn, triggers their rapid clearance, and (3) membrane lysis [78]. All 3 pathways can be activated in contact with NPs, leading to inflammatory process or hypersensitivity reactions called complement activation-related pseudoallergy (CARPA). Research over the past years reported that PEGylated lipid-based NPs can induce CARPA effects in patients, often following their first IV administration [80, 81]. Although CAPRA resolves most of the time after stopping the infusion, the reaction may be life-threatening or fatal in a minority of patients (≤ 0.01%) [81, 82]. Hence, it is critical to wholly evaluate the impact of nanoparticles on their cytotoxicity and in vivo CAPRA

response before further investigation in human [75, 76, 78].

Currently, there is no standardized regulation guidelines in nanomedicine on how to test the physiochemical properties and toxicity of the NP formulations. In this respect, the Nanotechnology Characterization Laboratory in the United States and the recently formed European Nanomedicine Characterization Laboratory in Europe provide a platform for preclinical validated characterizations of NPs (physiochemical, toxicology, immunology, efficacy, sterility, and endotoxin) to facilitate the regulatory review of nanomedicine. Furthermore, in order to predict CAPRA, the frequent side effect of IV administrated nanosystems, in vitro complement assays could be first performed before subsequently testing those passed candidates on the in vivo porcine model of CARPA to evaluate the risk of hypersensitivity reaction induced by the nanoparticles [76, 81, 82].

Conclusions

Upon reflection, we have advanced quite far in a relatively short period of time thanks to the lesson learned from cancer nanomedicine. However, despite these advances, there is a great deal more work to be done to effectively combat this disease. In order to benefit from the parallel situation in cancer chemotherapy, we emphasize the importance of establishing a thorough understanding of nanoparticles' biological performance as well as drug distribution, ranging from the whole body to the target cells, would allow rational design and applications of nanoparticles. At the same time, regulatory reform is required for nanomedicine to establish "nano" regulatory guidelines and standardization in the matters of particle characterization and nanotoxicity testing in order to facilitate the clinical translation into commercial products in atherosclerosis nanomedicine.

Conflict of Interests

The authors declare no conflicts of interest. For signed statements, please contact the journal office: editor@precisionnanomedicine.com

Quote as: Wong YS, Czarny B, Venkatraman SS, Precision nanomedicine in atherosclerosis therapy: how far are we from reality? Precis. Nanomed. 2019;2(1):230-244.
https://doi.org/10.33218/prnano2(1).181114.1

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