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A new approach to the diagnosis and treatment of atherosclerosis: the era of the liposome

A new approach to the diagnosis and treatment of atherosclerosis: the era of the liposome

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Highlights

- Atherosclerotic cardiovascular disease (ASCVD) is a major cause of mortality and morbidity worldwide.
- Improved visualization of early atherosclerosis and better management of residual risk can reduce ASCVD burden.
- Recent research has investigated the potential of biomaterials and nanomedicines in meeting this demand.

- Liposomes have a wide range of applications in both imaging and treatment of atherosclerosis.
- We review the scientific and clinical evidence relating to the use of liposomes in the context of ASCVD.

Teaser: This review describes the experimental and clinical evidence for the use of liposomes in the diagnosis and management of atherosclerotic cardiovascular disease.

The consequences of atherosclerotic cardiovascular disease (ASCVD) include myocardial infarction, ischemic stroke, and angina pectoris, which are major causes of mortality and morbidity worldwide. Despite current therapeutic strategies to reduce risk, patients still experience the consequences of ASCVD. Consequently, a current goal is to enhance visualization of early atherosclerotic lesions to improve residual ASCVD risk. The uses of liposomes, in the context of ASCVD, can include as contrast agents for imaging techniques, as well as for the delivery of antiatherosclerotic drugs, genes, and cells to established sites of plaque. Additionally, liposomes have a role as vaccine adjuvants against mediators of atherosclerosis. Here. we review the scientific and clinical evidence relating to the use of liposomes in the diagnosis and management of ASCVD.

Keywords: atherosclerosis treatment; atherosclerosis diagnosis; liposome; nanoliposome; plaque.

Introduction

The consequences of ASCVD include myocardial infarction, ischemic stroke, and angina pectoris, which are major causes of mortality and morbidity worldwide [1]. One of the most important risk factors for the development of ASCVD is life-long exposure to elevated circulating concentrations of apolipoprotein-B (apoB)-containing lipoproteins, such as low-density lipoprotein (LDL) cholesterol [2]. Additional risk factors for ASCVD include smoking, hypertension, diabetes, abdominal obesity, and physical inactivity [1].

Atherosclerosis is a progressive lipid-driven, inflammatory disease that can be divided conceptually into various stages. The early stages of the

disease are characterized by vascular endothelial dysfunction. Leukocytes are recruited to the endothelium and express proinflammatory cytokines. This is followed by the infiltration of monocytes into the vascular wall and their maturation into macrophages, which incorporate lipids and become foam cells. The next step in the atherogenic cascade involves the infiltration of smooth muscle cells into the intima. As plaques further develop and mature, they ultimately restrict blood flow by reducing the diameter of the lumen and are prone to rupture. Subsequent rupture exposes the highly thrombogenic plaque contents to the blood, which results in intravascular thrombus formation [3,4].

Looking more closely at the series of steps in atheroma formation reveals that an initial step includes inflammation and the release of inflammatory cytokines, which results in the overexpression of cell adhesion molecules, such as selectin and vascular cell adhesion molecule-1 (VCAM-1, also known as CD106), on the vascular endothelial cell surface. This causes endothelial dysfunction, which predisposes the individual to the permeation of lipoproteins, as well as the recruitment of T cells and monocytes, into the vascular wall. Subsequently, the recruited monocytes differentiate into macrophages. These phagocytic cells not only promote further inflammation, but also activate the endothelium by releasing proteases, growth factors, interleukins, and prostaglandins. Plaques then form on the tunica intima, the innermost lining of the vessels. Thickening of the intimal layer of the vessel, which follows plaque formation, diminishes the diffusion of oxygen across the vessel wall. This leads to local hypoxia, which stimulates neovascularization and further promotes plaque progression. Furthermore, oxidative stress activates nuclear factor κB (NF-κB) within the endothelial cells. Activation of NF-κB increases the expression of cell adhesion molecules and leads to a vicious cycle of disease progression [2,5].

In advanced atherosclerotic lesions, a large number of lipids and necrotic cells are evident. The plaques contain a lipid core and a fibrous cap containing type 1 collagen. Measuring the collagen content of the plaques provides valuable information concerning plaque stability. Plaques that are susceptible to rupture are generally uncalcified and have a thin fibrous cap and a large lipid core [6]. Plaque rupture typically occurs following the accumulation of macrophages behind the fibrous cap and their subsequent conversion to foam cells, which are formed when macrophages engulf aggregated oxidized LDL in the core. Given that foam cells express collagenases that damage the fibrous cap, the plaques become swollen and inflamed, and subsequently rupture [7,8]. The rupture of atherosclerotic plaques in coronary and carotid arteries leads to myocardial infarction and ischemic stroke, respectively [9].

In recent years, impressive advances have been made in cardiovascular risk prediction, and risk factor management. However, risk calculators, such as Framingham [10], ASCVD [11], and QRISK-3 [12], do not always identify as 'high-risk' those individuals who later experience cardiovascular events despite the fact that atherosclerosis is a progressive pathology with early physical manifestations evident in the vasculature even in young adults. Furthermore, despite guideline-driven risk factor management, patients still experience the consequences of ASCVD, because of 'residual risk', which is not managed even by optimal use of current medication [13]. Consequently, improved visualization of the early stages of atherosclerosis, and better management of residual risk, have the potential to reduce the burden of ASCVD.

Liposomes are biocompatible and nontoxic structures, comprising phospholipids, which self-assemble in water and form layered vesicles. Multilamellar vesicles with a size range of 0.1–10 mm contain more than one concentric lamellar bilayer, whereas unilamellar vesicles contain one concentric lamellar bilayer. Unilamellar vesicles can be categorized by size into small unilamellar vesicles (SUVs) (<100 nm), large unilamellar vesicles (LUVs) (100–500 nm), and giant unilamellar vesicles (GUVs) (>1 mm) [14]. Multilamellar liposomes can be passed through a series of polycarbonate filter membranes to produce unilamellar liposomes [15]. Since their discovery during the 1960s, liposomes have proven useful in a variety of cardiovascular diseases and conditions and have potential use in both the diagnosis and treatment of atherosclerosis [16]. Combining liposomes with conventional diagnostic tools can improve the detection of plaques. Combining drugs, especially anti-inflammatory agents, with liposomes has been shown to result in improved therapeutic effects at significantly lower doses. Here, we review scientific and clinical evidence relating to the use of liposomes in the diagnosis and management of ASCVD.

Targeting liposomes to atherosclerosis

Applications of liposomes in atherosclerotic disease rely on the ability of liposomes to target early atherosclerotic lesions or advanced plaques, which permits the effective delivery of drugs, genes, cells, and contrast agents. Liposomes can be modified such that they have polyethylene glycol (PEG) on their surface, in a process called PEGylation, which is the attachment of PEG molecules to the liposome. This increases the time that liposomes reside in the circulation and increases their opportunities to target the plaques. A range of innovative strategies for the targeting of plaques by liposomes have been investigated. These strategies span the various stages in the progression of atherosclerosis, from targeting early endothelial dysfunction to lipid accumulation by macrophages and, lastly,

targeting of vulnerable plaques on the verge of rupture, which are a feature of advanced atherosclerotic disease. These techniques are described here and are summarized in Figure 1.

Targeting the endothelium

The simplest way to target atherosclerotic plaques is to utilize the enhanced permeability and retention (EPR) of the injured endothelium in atherosclerosis. This effect enables nanoliposome extravasation from the bloodstream at the site of plaques [17].

As mentioned earlier, damaged endothelium overexpresses specific molecules, which can be used as liposome targets. The luminal endothelium and newly formed microvessels overexpress cell adhesion molecules, such as VCAM1, intercellular adhesion molecule 1 (ICAM-1), P-selectin, E-selectin, and alpha V beta 3 ($\alpha_V\beta_3$) integrin. Even noncellular components of plaques, such as junctional adhesion molecules (JAMs), which serve to direct the inflammatory cells to the atherosclerotic site, could be a potential target for liposomes [18]. Antibodies against the above-mentioned molecules are attached to the surface of the liposome to target the dysfunctional endothelium. For example, liposomes containing an anti-inflammatory agent accumulated successfully at the site of the lesion when anti-VCAM-1 was attached to the surface of liposomes [19]. Additionally, anti-ICAM-1-decorated liposomes were found to bind strongly to endothelial cells [20]. Attaching antibodies against E-selectin also increased the accumulation of liposomes in endothelial cells in atherosclerotic lesions [21].

Lectin-like oxidized LDL receptor-1 (LOX-1) is another potential target for liposomes because of the upregulation of this receptor on the endothelium following the release of inflammatory cytokines and exposure to oxidative stress during atherosclerosis. In fact, oxidized LDL (oxLDL) exerts detrimental effects during plaque formation by binding to this receptor [22,23]. Liposomes functionalized with an anti-LOX-1 antibody successfully targeted LOX-1 receptors after intravenous injection in LDL receptor-deficient ($LDLR^{-/-}$) mice [24]. In plaques in which angiogenesis occurs, $\alpha_v\beta_3$ integrin (expressed in endothelial cells of neovessels), can be used as a target for liposomes. RGD (Arg-Gly-Asp) functionalized liposomes with the ability to target $\alpha_v\beta_3$ integrin have been developed to target tumor angiogenesis and, hence, could be utilized to target atherosclerotic plaques [25].

Although the attachment of antibodies to the surface of liposomes provides specific targeting, it creates a problem by increasing the immune recognition of the liposomes. This results in faster clearance from the circulation. Therefore, the density of antibodies on the liposome surface must be optimized to tackle this problem. A density of 70–80 mg

antibody/mmol phospholipid, approximately equal to 30–40 antibody molecules per a 100 nm diameter liposome, is thought to be optimal [26]. Where appropriate, multiple antibodies could be attached to a single liposome [27,28].

The membrane fluidity of liposomes enables antibody mobility and improves binding and uptake of liposomes by inflammatory endothelial cells. Gunawan *et al.* tested differences in membrane fluidity by a method called fluorescence polarization. In this method, a hydrophobic fluorophore (BODIPY® FL) was positioned between phospholipid layers in the liposome structure, and the fluorescent intensity of the probe was measured. Probe motion within the bilayer demonstrated membrane fluidity and resulted in lower anisotropy values [29]. Increased expression of interleukin-10 (IL-10) receptors on the surface of the aortic endothelium in atherosclerosis is another potential liposome target. IL-10 has important regulatory roles during inflammation and can enter the plaque via its receptors located on injured vascular endothelium. However, the short *in vivo* half-life of IL-10 and its limited *in vivo* stability necessitate encapsulation into biocompatible carriers, such as liposomes. Almer *et al.* incorporated IL-10 into fluorescently labeled liposomes and, by measuring the intensity of fluorescence, were able to observe increased accumulation of IL-10 in the aorta [30].

Despite the theoretical and experimental promise of the above-mentioned strategies to target endothelium in atherosclerosis, introduction of these methods to the clinic has been problematic. For example, blood flow in the vessels applies shear stress to the endothelial wall. This can wash the particles away from the targeted site and decrease the time of interaction of the particles with their target in the plaque. However, there are some liposomal formulations that have been designed to release their 'cargo' or 'payload' at the plaque site despite abnormal blood flow and increased shear stress [31,32].

Targeting macrophages

Another approach to treating atherosclerosis using liposomes is to target macrophages in the plaque. Previous research demonstrated that the existence of phosphatidylserine (PS) on the surface of apoptotic cells leads to their recognition and engulfment by macrophages [13,33,34]. Therefore, it is possible to incorporate synthetic PS into liposomes during manufacture to improve recognition by macrophages. Uptake of liposomes by macrophages brings them to the macrophage-rich site of the plaque [33]. To test this idea, an *in vitro* study of liposomal uptake by macrophages was performed by encapsulating ^{111}In-nitrilotriacetic acid into liposomes and injecting the radiolabeled liposomes into Watanabe heritable hyperlipidemic rabbits. The investigators documented the

accumulation of radiolabeled liposomes in plaque via single-photon emission computed tomography (SPECT) images. In the resulting SPECT images of harvested aorta 48 h after injection of liposomes, atherosclerotic regions were easily identified. Given that this method relies on the engulfment of liposomes by macrophages, it is important to maintain the size of the liposomes at <100 nm to achieve successful uptake [35].

PS is recognized by the glycoprotein CD36 receptors expressed on the macrophage. There are other ligands specific to the CD36 receptor, such as Hexarelin, which could be bound to the surface of liposomes and aid macrophage uptake of the liposomes [36]. The C-terminal globular domain of adiponectin (gAd) could also have a role in targeting. It binds efficiently to atherosclerotic plaques because of the presence of adiponectin receptors on macrophages. In addition to being useful in targeting, gAd and adiponectin have antiatherosclerotic effects, because they stimulate nitric oxide (NO) production by endothelial cells and have been shown to prevent the expression of the mRNA for VCAM-1 and ICAM-1 in rabbit atherosclerotic plaques.

The apolipoprotein E-deficient ($ApoE^{-/-}$) mouse model has been used to study the effects of liposomes on atherosclerosis. These genetically engineered mice lack the *ApoE* gene, and develop severe hypercholesterolemia and atherosclerotic lesions when consuming a cholesterol-rich chow diet [37–40]. Fluorescence images of an aortic sample taken from atherosclerotic $ApoE^{-/-}$ mice revealed the accumulation of gAd-functionalized liposomes in plaques. Fluorescent signals were evident in the plaque regions of aorta incubated with the gAd-functionalized liposomes. The gAd liposomes demonstrated a significantly stronger signal compared with free gAd. This was thought to occur because the gAd-liposomes accumulated on the surface of plaque, whereas free gAd penetrated deeper into the plaque [41,42].

Folate receptor beta (FRß) on the surface of activated macrophages in vulnerable plaques is another agent that could be exploited for the targeting of liposomes [43]. Various other receptors on macrophages can also be used for the purpose of liposome targeting. For instance, coating liposomes with a peptide sequence such as GGPNLTGRW (GGP-peptide) results in selective association with monocytes. Karathanasis *et al.* showed a >30-fold increase in the association of GGP-peptide-coated liposomes with monocytes compared with noncoated liposomes [44].

Decorating the liposome surface with the RGD tripeptide improves its association with integrin receptors expressed by monocytes [44–49]. Fc receptors on the surface of macrophages, especially the FcγRI receptor, are involved in phagocytosis. Fc-receptor-mediated phagocytosis enhances the uptake of liposomes by monocytes and macrophages, because immunoglobulins (immune liposomes) are recognized by these receptors

[50]. Macrophages also express high levels of the mannose receptor (MR, CD206); therefore, attaching a MR ligand to the liposomes directs them towards the fibrous cap [51–56].

Targeting the fibrous cap of advanced plaques

The fibrous cap comprises collagen, proteoglycans, and smooth muscle cells. The fibrin in the cap can be used as a target for liposomes. Demos *et al.* performed an *in vitro* study in which a liposomal formulation comprising phosphatidylcholine (PC), 4-(p-maleimidophenyl) butyryl phosphatidylethanolamine (MPB-PE), phosphatidylglycerol (PG), and cholesterol was conjugated to rabbit antihuman fibrinogen. This liposomal formulation successfully targeted fibrin-coated filter paper and slides. Attaching immune-labeled liposomes to the fibrin was confirmed by scanning electron microscopy (SEM). Subsequently, the evaluation of the aforementioned liposomes in an animal model of atherosclerosis revealed that antibody-conjugated liposomes specifically attached to the atheroma and not to the normal arteries [57]. Two years later, the same team targeted fibrous sections of the atheroma in Yucatan mini-pigs by conjugating antifibrinogen to the surface of liposomes [58]. To optimize the targeting potential of antifibrinogen-conjugated liposomes and their retention at the atheroma site, binding was studied under a variety of flow conditions (i.e., various shear stresses and temperatures). It was shown that this attachment was stable under nearly all of the conditions mimicking *in vivo* blood flow [59].

Diagnostic applications of liposomes in atherosclerosis

Previously, the diagnosis of atherosclerosis was limited to methods such as assessment of electrocardiography at rest and during exercise, assessment of the ankle-brachial index, and invasive angiography. Today, visualizing plaques is possible through non-invasive imaging techniques [60]. Plaque imaging techniques in clinical use include ultrasound, magnetic resonance imaging (MRI), and computed tomography (CT), as well as nuclear imaging techniques, such as positron emission tomography (PET) and SPECT. In these techniques, liposomes act as diagnostic agents for the non-invasive early detection of atherosclerosis. Liposomal imaging agents provide signals directly from the lesion site. This allows the plaque to be detected and for inferences to be made about its size and composition. The most important role of liposomes in the diagnosis of atherosclerosis is to carry and deliver contrast agents, thereby improving image resolution. The liposomes can be made multifunctional by the simultaneous loading of multiple contrast agents.

A range of imaging techniques are available (Figures 2–5) and the choice of the specific approach depends upon the developmental stage of the plaque being visualized. Early endothelial dysfunction can be

diagnosed using functional measurements, such as peripheral artery tonometry, flow-mediated dilatation, and pulse wave velocity, and can be visualized using PET and CT. More advanced lesions with lipid accumulation can be observed using coronary intravascular ultrasound, MRI, and coronary CT angiography. Advanced plaques can be seen using electron-beam-CT [61] (Figure 6).

Nuclear imaging (PET and SPECT)

Nuclear imaging techniques rely upon a source of radiation within the body. Both SPECT and PET techniques construct images based upon the detection of gamma rays emanating from radioactive substances within tissues. ^{18}F-fluorodeoxyglucose (FDG) is a radio-labeled glucose analog that can be used in PET scanning. In the context of atherosclerosis, this technique is useful in identifying macrophages and inflammation [62]. ^{18}F-sodium fluoride is another PET radiotracer that is used for dynamic evaluation of coronary microcalcification [63]. SPECT can also be used with a variety of tracers to identify inflammation [62]. Liposome-based probes are currently under investigation to enhance SPECT and PET imaging in a range of diagnostic settings. For example, PS-containing liposomes of 100 or 200 nm (PS100 and PS200) were injected into Watanabe heritable hyperlipidemic rabbits, scanned with SPECT and compared with CT images 48 h after injection (Figure 2) [64].

CT

CT is an X-ray-based imaging technique that is fast and relatively inexpensive. However, in this method of detection, a bolus injection of contrast agent is required. CT angiography is suitable for the detection of calcification in atherosclerosis. With CT, liposomes have a similar role to that in MRI. Liposomes (either simple or PEGylated) typically carry a CT contrast agent ,such as iopromide, gold, or bismuth [65–68].

Danila *et al.* encapsulated a contrast agent called 5-[*N*-acetyl- (2,3-dihydroxypropyl)-amino)-*N*,*N*′-bis(2,3-dihydroxypropyl)-2,4,6-triiodo-benzene-1,3dicarboxamide (iohexol) into PEGylated liposomes with the aim of overcoming the short residence time and renal toxicity of free iohexol. The liposomes comprised dipalmitoyl phosphatidylcholine (DPPC), cholesterol, and a linker (molar ratio of 3:1:0.3) and were then conjugated to an antibody against ICAM-1 to target the plaque (Figure 3) [69].

MRI

MRI is a useful approach for the detection of various plaque components, including the fibrous cap and lipid core. It provides information on plaque volume, composition, endothelial permeability, and plaque neovascularization, as well as 3D images of the plaque at near-cellular resolution. Thus, MRI has proven useful for the detection of macrophages

and macrophage-rich areas in atherosclerotic plaques. Targeting liposomes to the plaque enables increased accumulation of loaded contrast agent at the plaque site, which results in improved signal strength (Figure 4).

An example of liposomes that contain an MRI contrast agent are PEGylated liposomes into which gadopentetate dimeglumine (Gd-DTPA) has been incorporated [70,71]. In addition, as mentioned earlier, PS-enriched liposomes are recognized by macrophages located in plaques. Thus, loading gadolinium into such liposomes increases its concentration at the site of the plaque [72]. The mechanism responsible for the accumulation of gadolinium-loaded liposomes in plaque has been attributed to EPR [73]. Gadolinium has also been loaded into liposomes functionalized with antibodies against LOX-1 receptors in the dysfunctional endothelium of plaque [24].

Other examples of liposomes containing gadolinium and detected with the use of MRI include work by Paulis *et al.*, in which Gd-tetraxetan (Gd-DOTA) and distearoyl phosphatidylethanolamine (DSPE) were used to facilitate ICAM-1 targeting [20]. In another study using MRI, an antagonist of integrin α4β1 (THI0567) was loaded into Gd-containing liposomes. This liposomal formulation binds to the integrin α4β1 (very late antigen-4, VLA-4) receptors on monocytes and was used to identify plaque in the aortas of atherosclerosis-prone ApoE$^{-/-}$ mice [74]. In summary, targeted liposomes containing gadolinium-based contrast agents produce low background enhancement and are suitable for MRI of endothelial markers that are abundant inside plaque deposits [75].

Ultrasonography

Ultrasound imaging techniques are beneficial for the detection of vulnerable atherosclerotic plaques. Using ultrasound, it is possible to perform a catheter-based, real-time measurement of carotid intima-media thickness. Emerging techniques, such as intravascular photoacoustic-ultrasound (IVPA-US) imaging, can provide more precise information concerning the morphology of the arterial wall. The major advantage of IVPA-US over conventional ultrasound methods is that it can provide information on the composition of the plaque [76,77].

The layered structure of liposomes allows for the entrapment of gas bubbles, which efficiently reflect sound waves and produce ‘acoustically reflective’ liposomes. Such acoustically reflective liposomes can be attached to antibodies (antifibrinogen or anti-ICAM-1) to enable the recognition and targeting of plaque. Multilamellar acoustic liposomes have been prepared that comprise phosphatidylcholine, phosphatidylethanolamine, phosphatidylglycerol, and cholesterol, with entrapped gas bubbles between the lipid layers. This resulted in a

significant acoustic enhancement during ultrasound imaging when evaluated in the Yucatan mini-swine model of induced atherosclerosis (Figure 5) [58,78]. An alternative approach to the IVPA-US method is to include a contrast agent, such as indocyanine green (ICG) J-aggregate (IJA), into liposomes. Such liposomes have been targeted towards FRß, which is overexpressed on activated macrophages in atheromatous plaque. Increased uptake of FRß-targeted liposomes was evident *in vivo* using an ApoE$^{-/-}$ mouse model of atherosclerosis [43].

Roles for liposomes in the treatment of atherosclerosis

Liposomes as cholesterol-lowering agents

Given that the deposition of cholesterol results in plaque progression, reducing the concentration of circulating cholesterol is a routine therapeutic approach in the treatment of atherosclerosis. In one study of the expression of genes involved in the efflux of cholesterol in an LDLR$^{-/-}$ mouse model in which the mice consumed a cholesterol-rich diet showed that nanoliposomes upregulated ATP binding cassette subfamily A member 1 (*ABCA1*) and subfamily G member 1 (*ABCG1*) genes. These genes encode cholesterol transporter proteins. Liposomes containing anionic phospholipids, such as hydrogenated soy phosphatidylcholine (HSPC) and distearoyl phosphatidylglycerol (DSPG), have the ability to enhance reverse cholesterol transport. This means that liposomes can take up cholesterol and return it to the liver for reuse or excretion in the bile. Reverse cholesterol transport decreases the availability of cholesterol at the vascular wall and thereby decreases the production of foam cells [79–83]. ABC transporters act as a mediator of the cholesterol efflux pathways and result in antiatherogenic effects. Additionally, ABC transporters control the proliferation and mobilization of hematopoietic stem and progenitor cells in the bone marrow. Hence, activation of cholesterol efflux pathways suppresses the mobilization of stem cells and hematopoiesis, which consequently lowers the production of monocytes and macrophages. Thus, inflammation, an important contributor to the pathogenesis of atherosclerosis, is attenuated [84].

In another interesting study, high-density lipoprotein (HDL) was adsorbed onto PS-containing liposomes (synthetic dimyristoylphosphatidylcholine). After 5 weeks of infusion of such liposomes into cholesterol-fed rabbits, aortic cholesterol content was significantly reduced. This was attributed to the high affinity of macrophages for PS, leading to the accumulation of liposomes containing HDL at the macrophage-rich plaque site, and then HDL subsequently carrying cholesterol away from the plaque site [85].

Liposomes as drug delivery carriers

Liposomes and nanoliposomes are interesting candidates for drug delivery applications because of their ability to entrap both hydrophilic and hydrophobic drugs. Biocompatibility, low toxicity, and high loading capacity make liposomes useful as drug carriers. Typically, the surface of liposomes is modified with PEG to prevent aggregation and rapid systemic clearance [71].

Liposomes were the first drug delivery system with clinical applications. The benefits of combining drugs with liposomes include; increased biodistribution of the entrapped drug, improved therapeutic index (achieved by altering the pharmacokinetics and pharmacodynamics of the drug), and prevention of drug degradation by components of plasma. Some clinically approved liposomal formulations include Doxil® for the treatment of ovarian cancer, DaunoXome® for the treatment of advanced HIV-associated Kaposi's sarcoma, Depocyt®, Myocet®, Mepact®, and Marqibo® for cancer management, Onivyde™ for metastatic adenocarcinoma of the pancreas, Amphotec® and Ambisome® for the treatment of fungal infections, Visudyne® for photodynamic light therapy, DepoDur™ for pain management and for use as an anesthetic agent, and Epaxal® and Inflexal® for vaccination against hepatitis and influenza, respectively. Although Liprostin™ for the treatment of restenosis subsequent to angioplasty is in Phase III clinical trials, no liposomal formulations for atherosclerosis are currently available on the market [86].

In the context of treating atherosclerosis, liposomes can be used to improve the solubility of drugs, increase their half-life in the circulation, and enable selective or targeted delivery of the drug to the site of atherosclerotic plaque. The surface of liposomes can be modified with antibodies or ligands to target the endothelium of the atherosclerotic plaque or the macrophages recruited therein, such that healthy cells are not affected [87].

Liposomes release drug when they interact with cells. Given that the cell membrane, similar to liposomes, comprises phospholipids, liposomes fuse with the cell membrane easily. After attachment, the fused liposomes are degraded by lipase enzymes and release their contents. Physicochemical characteristics of nanoliposomes and liposomes, including lipid composition, size, fluidity, net surface charge, steric stabilization, dose, and route of administration, influence the interaction of cells and liposomes and, consequently, affect the pharmacokinetics of the drugs they carry. Incorporation of cholesterol within liposomes could influence their rigidity and uptake by cells. As mentioned earlier, the physicochemical properties of the encapsulated drug also affects how it is released. The value of the octanol/buffer partition coefficient of drugs determines whether prolonged or rapid release of the drug occurs. The

drug:lipid ratio is another factor that determines the rate of release of the incorporated drug [71,87].

Liposomes have been investigated as potential carriers for a range of drugs with potential therapeutic benefits in atherosclerosis, including anti-inflammatory and antiangiogenic drugs. Some of these studies are described below.

Anti-inflammatory drugs Glucocorticoids are widely studied anti-inflammatory agents for the treatment of atherosclerosis and can be loaded into liposomes. Loading glucocorticoids into liposomal carriers reduces the adverse effects when dosed by the oral route, which include diabetes mellitus, osteoporosis, and hypertension. Additionally, loading glucocorticoids into liposomes and administering them via the oral route prevents problems associated with the systemic injection of glucocorticoids, which include a very short circulating half-life and suboptimal disposition of the incorporated glucocorticoids *in vivo* (i.e., the associated pharmacokinetic parameters). Thus, these limitations often necessitate the frequent intravenous administration of glucocorticoids at high doses. Loading glucocorticoids into PEGylated liposomes increases the circulation time and improves their accumulation in atherosclerotic lesions [88]. In a study by Lobatto *et al.*, glucocorticoids were loaded into PEGylated nanoliposomes and tested in a rabbit model of atherosclerosis. The accumulation of drug in the plaque and its anti-inflammatory effects were evaluated using MRI, because some of the ingredients in the nonliposomal formulation contained gadolinium to serve as the contrast agent for MRI analysis. It was demonstrated that, 2 days after administration, gadolinium-labeled liposomes accumulated in the atherosclerotic lesions and increased the intensity of the MRI signal [89]. It was suggested that the EPR effect had a significant role in the targeting of plaque by the glucocorticoid-containing PEGylated nanoliposomes used in this study [89].

Prednisolone phosphate is a synthetic glucocorticoid that has beneficial effects against atherosclerosis when incorporated into liposomes. PEGylated liposomes containing prednisolone phosphate were successfully used to target atherosclerotic lesions in a rabbit model of atherosclerosis [89]. Joner *et al.* encapsulated the same drug into PEGylated liposomes comprising HSPC, cholesterol, and TRX-20 (3,5-dipentadecyloxybenzamidine hydrochloride) at a molar ratio of 50:42:8. This liposome showed high affinity for subendothelial proteoglycans. The cationic lipid component of TRX-20 facilitates endocytosis of the liposome and subsequent release of the drug inside the cell. Intravenous administration of this formulation in a rabbit model of atherosclerosis reduced the percentage of the ‘in-stent’ stenosis area, the inflammation

score, infiltration of giant cells, and the percentage of the neo-intima occupied by macrophages [90].

Despite these promising therapeutic effects of prednisolone-loaded liposomes, lipotoxicity can be generated by liposomes, which has the potential to exacerbate the degree of atherosclerosis. It was reported that, 2 weeks after infusion of liposomes loaded with prednisolone, the number of inflammatory cells and the area of the necrotic core had increased. Moreover, existing plaques had progressed to a more advanced stage. In this case, increased recruitment of monocytes to the site of the plaque, as well as the increased expression of ER-MP58, a characteristic of circulating immature myeloid cells, were observed. Prednisolone-loaded liposomes might confer a benefit to counteracting inflammation at the site of atherosclerotic lesions, but only if the dose and duration of therapy can be optimized [91]. The first clinical study of prednisolone-loaded PEGylated liposomes in humans was conducted in 2015. Administration of this formulation (1.5 mg/kg) resulted in an increased plasma half-life of prednisolone of 63 h. In this clinical study, liposomes successfully accumulated in atherosclerotic plaque, but inflammation of the arterial wall was not reduced. This was suggested to result from either an insufficient dose of prednisolone at the plaque site or from an inadequate duration (i.e., 10 day duration) of the prednisolone at the plaque site to elicit optimal anti-inflammatory effects. Interestingly, it has been shown that even empty liposomes can absorb intracellular cholesterol and facilitate the regression of atherosclerosis [92].

In addition to prednisolone, other drugs can be incorporated into liposomes for the treatment of atherosclerosis. As an example, anti-inflammatory corticosteroids can be targeted locally to the site of inflammation through the use of liposomes. A local anti-inflammatory effect is advantageous, because it negates the need for the induction of systemic immunosuppression and the associated adverse off-target effects that result. A liposome formulation comprising a mixture of DPPC, cholesterol, and PEG 2000 DSPE was used to load a water-soluble corticosteroid derivative into the aqueous interior of the structure [88,89]. Other attempts to utilize anti-inflammatory compounds in liposomes to treat the inflammation associated with atherosclerosis include the use of anti-inflammatory cyclopentenone prostaglandins. The prostaglandins were loaded into liposomes and were shown to be beneficial in a mouse model of atherosclerotic vascular injury [19]. Dexamethasone is another drug that has been incorporated into liposomes for the treatment of atherosclerosis. Liposomes in this study were formed from egg yolk phosphatidylcholine, cholesterol, and dicetylphosphate. Administration of this formulation to atherogenic mice once a week for 8–14 weeks reduced the level of aortic cholesterol ester, which was suggested to result from

the antiatherosclerotic effects of the dexamethasone-loaded liposomes [93].

Given the potential of antagonists of chemokines involved in the development of chronic inflammation to reduce inflammation and atherosclerosis, PEGylated liposomes have been loaded with chemokine receptor antagonists (Teijin compound 1) and targeted against activated endothelium. When released, the antagonists occupied the binding sites for chemokines and, consequently, blocked the pathway responsible for cytokine-mediated inflammation. Targeting was achieved by binding a specific peptide to the liposomes that subsequently bound to endothelial VCAM-1. A mixture of dioleoyl phosphatidylethanolamine (DOPE), dioleoyl phosphatidic acid (DOPA), and maleimide-PEG-DSPE was used to prepare the liposomes. Upon reaching the target, liposomes fused with the cell membrane, collapsed, and released their contents. In experiments with ApoE$^{-/-}$ mice, this approach resulted in a reduction in the size of atherosclerotic lesions [94]. Other bioactive compounds and/or drugs considered for liposomal plaque targeting include the liver X receptor (LXR) agonist T09, simvastatin, and pterostilbene. These compounds significantly inhibit NF-κB activity, suppress the proliferation of macrophages, and confer antioxidant activity [95]. Delivery of the compound T0901317, an agonist of LXR receptors found on macrophages, using cationic liposomes has been shown to exert atheroprotective effects by increasing the efflux of cholesterol from macrophages. This protective effect results from a threefold increase in the expression of cholesterol efflux genes; specifically, *ABCA1* and *ABCG1*, when liposomes containing LXR receptors antagonists were evaluated in ApoE$^{-/-}$ mice [96].

Antiangiogenic drugs Drug-loaded liposomes also have the potential to reduce angiogenesis within plaques. Incorporation of bevacizumab (BEV), a US Food and Drug Administration (FDA)-approved monoclonal antibody to vascular endothelial growth factor (VEGF), into liposomes has the potential to reduce atherosclerosis. This antibody inhibits the expression of VEGF, neovascularization, and the progression of atherosclerosis. Given that systemic delivery of BEV is accompanied by adverse effects, such as bleeding, arterial thrombosis, and hypertension, the use of BEV-containing liposomes as a carrier would appear to be a logical approach. Interestingly, a 5-min exposure of HUVECs to 15-MHz ultrasound accelerated the release of BEV from the liposomes. As a result, VEGF expression was reduced by 90% [97]. Lastly, fumagillin, a selective inhibitor of endothelial cell proliferation and migration, which is able to prevent the expansion of the vascular network, has been incorporated into liposomes and targeted to plaque [98].

Liposomes as vaccines

Given that components of the immune system are involved in atherosclerosis, the course of the disease can be altered by modulating immune responses. The immune system can be deliberately activated in response to specific antigens given as vaccines. The goal of vaccination is to enhance the response of the immune system to pathogens. Liposomes have the potential to be immunogenic, either by targeting antigens toward the immune system, or by acting as antigens themselves. The release of antigens from circulating liposomes produces a long-lasting humoral immune response. After administration of liposomes into the body, either through systemic delivery or using other routes of administration, such as nasal or transcutaneous, the body immunizes itself against the liposome-loaded antigen [99–101]. In fact, phagocytosis by macrophages of antigen incorporated into liposomes leads to the accumulation of antigens in the lysosome. In lysosomes, the peptide is degraded and presented to the major histocompatibility complex class II (MHCII) on the surface of the macrophage. As a result, T helper cells and specific B cells are activated and antibodies are secreted [102]. Administering high doses of plaque-specific antigens both stimulates and initiates a regulatory T cell (Treg) response. The Tregs exert protective effects in atherosclerosis by reducing chronic inflammation via secretion of anti-inflammatory cytokines, such as IL-10 and transforming growth factor (TGF)-ß. Liposomes could be used to stimulate cells to increase the secretion of immune suppressive agents, such as immunoglobulin (Ig)M. There is some evidence that the IgG-producing B2 cell subset increases plaque progression and atherosclerosis. However, by contrast, it has been demonstrated that B cells are antiatherogenic because of the production of IgM antibody [81]. A variety of plaque specific antigens have been investigated and these are described below.

Phosphatidylserine plaque-specific antigen The first plaque-specific antigen incorporated into liposomes was PS. Hosseini *et al.* developed PS liposomes that mimic apoptotic cells, which are known to be immunogenic and anti-inflammatory. Similar to apoptotic cells that express double-stranded (ds) DNA, which activates toll-like receptor 9 (TLR9) on B1a, PS activates TIM1/4 (a molecule that recognizes PS). This activation results in the mobilization and expansion of atheroprotective B1a cells in the peritoneum, as well as an elevation in the production of IgM. Intraperitoneal injection of synthetic PS liposomes in $ApoE^{-/-}$ mice leads to a reduction in overall atherosclerotic plaque size, as well as the size of the necrotic core, plaque macrophage and T cell content, and cytokine expression. The precise mode of action of PS liposomes in atherosclerosis is not clear [103]. PS liposomes have some advantages over the use of human primary or immortalized cells utilized for other chronic inflammatory or autoimmune diseases. First, liposomes can be produced

in a more controlled manner and on a larger scale. Second, they are associated with lower health risks. PC head groups in the liposome structure, which also exist on the surface of apoptotic cells, are another plaque-specific antigen. It has been reported that production of anti-PC antibodies increases levels of IgM and reduces atherosclerosis [104].

Lipoprotein plaque-specific antigens LDL and apoB-100 (the apolipoprotein of LDL) have been used as plaque-specific antigens and incorporated into liposomes. ApoB100 is a protein surrounding LDL and contains several potential CD4+ T cell epitopes. LDL itself is a large and heterogeneous molecule; therefore, antigenic epitopes in LDL include ~102 peptides. As an alternative, a peptide derived from apoB-100 can be used [104]. In one study, liposomes containing the anionic phospholipid DSPG were loaded with apoB-100. The first step involves attachment of complement component 1q (C1q) to the surface of the anionic liposomes, which can then be recognized by scavenger receptors on phagocytic cells and subsequently taken up by the phagocytic cells. Immunization with these peptides initiates CD4+ T cell responses and, thereby, vaccinates the body against atherosclerosis. In a study by Benne *et al.*, 10 nmol of this vaccine was shown to decrease plaque formation by 50% and reduce serum cholesterol concentrations in $LDLR^{-/-}$ mice. Importantly, administration of the vaccine resulted in increased antigen-specific CD4+ T cell proliferation after 7 days [105].

Peptide plaque-specific antigens The peptides ovalbumin-derived $CD4^{+}$ T-cell-specific peptide (OVA323) and proprotein convertase subtilisin/kexin type 9 (PCSK9) are plaque-specific antigens that have also been incorporated into liposomes. Production of antibodies against these peptides increased after administration of peptide-loaded liposomes. Liposome-loaded OVA323, in comparison to free OVA323, resulted in an increase in the proliferation of Tregs [106]. Additionally, PCSK9-loaded liposomes induced anti-PCSK9 production. This antibody disrupts the interaction between PCSK9 and LDLR, which leads to metabolism and, thus, a lowering of plasma LDL cholesterol. Forty-eight weeks after subcutaneous inoculation of this formulation into BALB/c mice, plasma PCSK9 concentration were decreased by nearly 50% and the PCSK9–LDLR interaction was inhibited. Anti-inflammatory T cells (CD4+ and IL-4+) increased, whereas the number of proinflammatory cells remained unchanged. The negative surface charge of liposomes enhances the humoral immune response to surface-exposed peptide antigens, because these antigens are better recognized by B cell receptors and give rise to more efficient antibody production [34].

Cholesterol plaque-specific antigens Immunization against cholesterol is another strategy against atherosclerosis. Liposomes containing 71% cholesterol have been shown to induce anticholesterol antibodies. These

antibodies capture the cholesterol in circulating lipoproteins, which results in a mean decrease in serum cholesterol of 35%. In fact, immunization of hypercholesterolemic rabbits using this technique resulted in a reduction of atherosclerosis [107]. In another similar study, liposomes containing cholesterol were injected into rabbits that had been fed 2 g/day of cholesterol, 5 days a week for a duration of 18 weeks. At autopsy, the extent of atherosclerotic plaques in the heart and aorta was measured. It was shown that even after cholesterol feeding, the reactivity of anticholesterol antibodies increased in the serum after liposome injection and this resulted in a dramatic reduction in serum cholesterol. Additionally, no atherosclerotic plaques were formed in the aortic intima of rabbits immunized with these liposomes [108].

Liposomes as stem cell carriers

In atherosclerosis, damaged tissue repair is prevented by apoptosis and the loss of vascular stem cells. Therefore, transplantation of exogenous stem cells helps the vascular tissue to repair and prevents plaque rupture. Stem cells can differentiate into neo-intima smooth muscle cells or endothelial cells and enable the recovery of vessel function. Direct injection of stem cells to the lesion site has poor therapeutic results. However, liposomes have been used as a carrier for stem cells. For this purpose, liposomes have been modified with two antibodies, one for CD34 antigens on the surface of stem cells and the other for ICAM-1 (Figure 7). After liposomes containing stem cells have reached the atheromatous plaque, the cells can be conveniently separated from the liposomes using a sonoporation technique. In this method, a 1-MHz low-amplitude continuous ultrasonic wave is used to break the gas bubbles between the lipid bilayers of the liposomes, which creates cavities in the liposomes and allows the release of the stem cells [109,110].

Liposomes as nonviral gene delivery vectors

Delivery of specific genes to the site of plaque formation could stop the progression of atherosclerosis. Nanoliposomes can be used as nonviral vectors for the delivery of therapeutic mRNA to cells, which enables the transfection of cells with specific genes. This overcomes the problem of the short half-life of mRNA, as well as to protect the sensitive structure of siRNA from degradation by serum RNases [111]. In one study, liposomes were used as a small interfering (si)RNA delivery system to achieve a reduction in the expression of fatty acid binding protein 4 (FABP4). The liposomal carriers were labeled with quantum dots to enable their biodistribution to be mapped in the aorta after intravenous injection in mice. Immunofluorescent staining demonstrated successful uptake of liposomal siRNA into atherosclerotic plaque and the silencing of FABP4 [112]. Additionally, targeted delivery of siRNA of endothelial gene VE-

cadherin to activated endothelial cells has been investigated. Given the negative charge of siRNA, cationic liposomes from amphiphilic SAINT-C18 [1-methyl-4-(*cis*-9-dioleyl) methyl-pyridinium-chloride] were formulated and their surface was modified with anti-VCAM-1 and anti-E-selectin for more efficient targeting of endothelial plaque, as well as for upregulation of the VE-cadherin gene (Figure 8) [113].

Gene delivery using liposomes could prevent graft atherosclerosis, a common problem limiting the long-term success of cardiac transplantation. Graft atherosclerosis is induced because of a decrease in the level of tissue plasminogen activator (tPA) in the arteriolar smooth muscle cells. Gene delivery, with the goal of overexpressing tPA, could reduce the risk of atherosclerosis. Cationic liposomes comprising (+/–)-*N*-(3-aminopropyl)-*N*,*N*-dimethyl-2,3-bis(dodecyloxy)-1-propanaminium bromide (GAP: DLRIE) have been used for the incorporation and subsequent delivery of tPA cDNA. Intracoronary infusion of this complex in a rabbit heterotopic cardiac transplant model resulted in increased expression of the *tPA* gene in the vascular wall and inhibited the progression of atherosclerosis [114].

As it pertains to gene delivery, physicochemical parameters, such as the size and charge of liposomes, determine the rate of uptake by cells at the plaque site. Some evidence suggests that liposomes <100 nm have longer circulating half-lives and, therefore, better accumulation in plaque. Uptake of such liposomes by the mononuclear phagocytic system (MPS) has been shown to be improved. Conversely, other studies have found improved MPS uptake when the size of the liposomes was increased. Negatively charged liposomes internalize more readily, because they are more easily recognized by macrophages [115,116]. In an atherosclerotic plaque, macrophages are the metabolically active regions. One study investigating the uptake of anionic liposomes into atheromas using Watanabe heritable hyperlipidemic rabbits showed that the uptake of anionic liposomes was improved in the metabolically active sites of the plaque [117,118]. In general, anionic liposomes cannot pass through the endothelial barrier because of electrostatic repulsion of like charges. However, because the endothelium of atherosclerotic plaques is dysfunctional, the permeability is increased. Injecting fluorescently labeled liposomes into ApoE$^{-/-}$ mice led to accumulation of the liposomes in lipid-rich areas of atherosclerotic plaques and, subsequently, into macrophages. A study of human coronary artery endothelial cells pretreated with an inhibitor of clathrin-mediated endocytosis demonstrated that clathrin-mediated endocytosis is the pathway responsible for the uptake [40]. One difference between anionic and cationic liposomes is that cationic liposomes can induce cytotoxicity, cytokine activation, and proinflammatory effects. Despite these

limitations, cationic liposomes are more suitable for gene delivery applications. The positive charge on these liposomes facilitates electrostatic interaction with negatively charged proteoglycans on the cell membrane. Therefore, delivery of a gene to the cell is enhanced [105,119].

Concluding remarks and future direction

We have reviewed the potential role of liposomes and nanoliposomes in the diagnosis of atherosclerosis. The research to date suggests that liposomes are suitable for carrying and concentrating CT and MRI contrast agents into the site of plaques to sufficiently enable high-quality imaging. To increase the precision and resolution of plaque imaging, a combination of different imaging techniques can be used. Liposomes are a good candidate for this purpose because of their multilayered structure, which enables the simultaneous incorporation of different hydrophobic and hydrophilic contrast agents. Additionally, this feature of liposomes makes them attractive for the codelivery of drugs and contrast agents by a single liposomal formulation. This strategy, referred to as theranostics, is an emerging field in which the effects of a therapeutic agent can be monitored after it has been administered to the patient.

The potential application of liposomes to the management of atherosclerosis was also reviewed. Liposomes have been used successfully to deliver genes, stem cells, and anti-inflammatory or antiangiogenic drugs to the plaque site. Administration of liposomes has been shown to lower LDL cholesterol, and liposomes have been used to produce vaccines against atherosclerotic mediators. Targeting liposomes to the plaque site has been achieved using various strategies, which are discussed in this review and are shown in XXX. The targeting of liposomes to macrophages has been extensively studied, but no study to date has investigated the targeting of liposomes to enzymes or proteins that are oversecreted by macrophages at the plaque site. A future approach might be to use matrix metalloproteinases (MMPs) produced by macrophages to target liposomes to macrophages. The increased concentration of MMPs at the site of atheromatous plaques makes the plaque more vulnerable to rupture [120]. Therefore, loading MMP inhibitors into liposomes would not only target them toward MMP-rich regions in the vessel (i.e., plaque), but also reduce MMP levels and potentially prevent plaque rupture.

Liposomes have many advantages over other materials used in nanomedicine. They have the potential for the clinical management of atherosclerosis. However, much of the research to date has been conducted in laboratory settings, or in small clinical studies. Therefore, the potential to draw major inferences relating to the clinical treatment of patients is limited. Proof-of-concept studies, which demonstrate improved

delivery of therapeutic agents to specific sites of atherosclerotic activity, do not necessarily imply improved clinical effectiveness and provide no guarantee against unexpected harm that might result from any new drug or therapeutic technology. Ultimately, similar to all novel therapies, the benefits need to be evaluated in terms of both safety and cost. Nevertheless, based upon available data, it would be prudent to be cautiously optimistic about the potential uses of liposomes in the diagnosis and treatment of atherosclerosis at this time. However, it would appear to be an appropriate time to move to large-scale trials to determine whether liposome-based imaging techniques can improve the prediction of cardiovascular risk when used in conjunction with the advanced imaging techniques that have now been developed and described in this review [121,122]. Moreover, it would seem logical to determine whether liposome-based therapeutics are more effective than currently available drugs for the treatment of atherosclerosis. Finally, the large-scale production of targeted liposomes with different ligands attached to their surface is another issue that urgently needs to be addressed.

Conflict of interest

P.E.P. owns four shares in Astra Zeneca PLC and has received travel and/or speaker's fees from Amgen Inc. G.W. has received honoraria for advisory boards and lectures from Amgen, Sanofi, Regeneron, Kowa, and MSD. M.B. has presented at a speakers bureau for Abbott/Mylan, Abbott Vascular, Actavis, Akcea, Amgen, Biofarm, KRKA, MSD, Sanofi-Aventis, and Valeant; as a consultant to Abbott Vascular, Akcea, Amgen, Daichii Sankyo, Esperion, Lilly, MSD, Resverlogix, and Sanofi-Aventis; and has received grants from Sanofi and Valeant.

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References

1 Yusuf, S. *et al.* (2004) Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the interheart study): case-control study. *Lancet* 364, 937–952

2 Ference, B.A. *et al.* (2017) Low-density lipoproteins cause atherosclerotic cardiovascular disease. 1. Evidence from genetic, epidemiologic, and clinical studies. A consensus statement from the European Atherosclerosis Society Consensus Panel. *Eur. Heart J.* 38, 2459–2472

3 Libby, P. (2002) Inflammation in atherosclerosis. *Nature* 420, 868–874

4 Libby, P. *et al.* (2011) Progress and challenges in translating the biology of atherosclerosis. *Nature* 473, 317

5 Pamukcu, B. *et al.* (2011) The nuclear factor-kappa B pathway in atherosclerosis: a potential therapeutic target for atherothrombotic vascular disease. *Thromb. Res.* 128, 117–123

6 Naghavi, M. *et al.* (2003) From vulnerable plaque to vulnerable patient: a call for new definitions and risk assessment strategies: part I. *Circulation* 108, 1664–1672

7 Narula, J. *et al.* (2008) Arithmetic of vulnerable plaques for noninvasive imaging. *Nat. Clin. Pract. Cardiovasc. Med.* 5 (Suppl. 2), S2–S10

8 Virmani, R. *et al.* (2006) Pathology of the vulnerable plaque. *J. Am. Coll. Cardiol.* 47 (8 Suppl.), C13–C18

9 Braganza, D.M. and Bennett, M.R. (2001) New insights into atherosclerotic plaque rupture. *Postgrad. Med. J.* 77, 94–98

10 D'Agostino, R.B. *et al.* (2008) General cardiovascular risk profile for use in primary care: the Framingham Heart Study. *Circulation.*117, 743–753

11 Goff, D.C., Jr. *et al.*. (2014) 2013 ACC/AHA guideline on the assessment of cardiovascular risk: a report of the American College of Cardiology/American Heart Association Task Force on practice guidelines. *Circulation* 129 (25 Suppl. 2), S49–S73

12 Hippisley-Cox, J. *et al.* (2017) Development and validation of QRISK3 risk prediction algorithms to estimate future risk of cardiovascular disease: prospective cohort study. *BMJ* 357, j2099

13 Ridker, P.M. *et al.* (2017) Antiinflammatory therapy with Canakinumab for atherosclerotic disease. *N. Engl. J. Med.* 377, 1119–1131

14 Ulrich, A.S. (2002) Biophysical aspects of using liposomes as delivery vehicles. *Biosci .Rep.* 22, 129–150

15 Rasoulianboroujeni, M. *et al.* (2017) Development of a DNA-liposome complex for gene delivery applications. *Mater. Sci. Eng. C* 75, 191–197
16 Levchenko, T.S. *et al.* (2012) Liposomes in diagnosis and treatment of cardiovascular disorders. *Methodist Debakey Cardiovasc J.* 8, 36–41
17 Mulder, W.J.M. *et al.* (2004) A liposomal system for contrast-enhanced magnetic resonance imaging of molecular targets. *Bioconjugate Chem.* 15, 799–806
18 Nakhlband, A. *et al.* (2018) Combating atherosclerosis with targeted nanomedicines: recent advances and future prospective. *Bioimpacts* 8, 59–75
19 Homem de Bittencourt, P.I., Jr *et al.* (2007) LipoCardium: endothelium-directed cyclopentenone prostaglandin-based liposome formulation that completely reverses atherosclerotic lesions. *Atherosclerosis.* 193, 245–258
20 Paulis, L.E.M. *et al.* (2012) Targeting of ICAM-1 on vascular endothelium under static and shear stress conditions using a liposomal Gd-based MRI contrast agent. *J. Nanobiotechnol.* 10, 25
21 Yu, G. *et al.* (2005) Endothelial expression of E-selectin is induced by the platelet-specific chemokine platelet factor 4 through LRP in an NF-kappaB-dependent manner. *Blood* 105, 3545–3551
22 Pirillo, A. *et al.* (2013) LOX-1, OxLDL, and atherosclerosis. *Mediat. Inflamm.* 2013, 12
23 Chen, M. *et al.* (2002) LOX-1, the receptor for oxidized low-density lipoprotein identified from endothelial cells: implications in endothelial dysfunction and atherosclerosis. *Pharmacol. Ther.* 95, 89–100
24 Li, D. *et al.* (2009) Molecular imaging of atherosclerotic plaque targeted to oxidized LDL receptor LOX-1 using magnetic resonance. *J. Cardiovasc. Magn. Reson.* 11, O76
25 van Tilborg, G.A.F. *et al.* (2008) Improved magnetic resonance molecular imaging of tumor angiogenesis by avidin-induced clearance of nonbound bimodal liposomes. *Neoplasia* 10, 1459–1469
26 Almeda, D. *et al.* (2015) Minimizing antibody surface density on liposomes while sustaining cytokine-activated EC targeting. *Biomaterials* 41, 37–44
27 Laginha, K. *et al.* (2005) Liposomes targeted via two different antibodies: Assay, B-cell binding and cytotoxicity. *Biochim. Biophys. Acta* 1711, 25–32
28 Saul, J.M. *et al.* (2006) A dual-ligand approach for enhancing targeting selectivity of therapeutic nanocarriers. *J. Control. Release* 114, 277–287

29 Gunawan, R.C. *et al.* (2011) Complementary targeting of liposomes to IL-1alpha and TNF-alpha activated endothelial cells via the transient expression of VCAM1 and E-selectin. *Biomaterials* 32, 9848–9853
30 Almer, G. *et al.* (2013) Interleukin-10: an anti-inflammatory marker to target atherosclerotic lesions via PEGylated liposomes. *Mol. Pharm.* 10, 175–186
31 Epshtein, M. and Korin, N. (2017) Shear targeted drug delivery to stenotic blood vessels. *J. Biomech.* 50, 217–221
32 Calderon, A.J. *et al.* (2009) Flow dynamics, binding and detachment of spherical carriers targeted to ICAM-1 on endothelial cells. *Biorheology* 46, 323–341
33 Henson, P.M. *et al.* (2001) Apoptotic cell removal. *Curr. Biol.* 11, R795–R805
34 Momtazi-Borojeni, A.A. *et al.* (2019) Long-term generation of antiPCSK9 antibody using a nanoliposome-based vaccine delivery system. *Atherosclerosis* XX, YYY–ZZZ
35 Ogawa, M. *et al.* (2014) Development of 111In-labeled liposomes for vulnerable atherosclerotic plaque imaging. *J. Nucl. Med.* 55, 115–120
36 Tait, J.F. and Smith, C. (1999) Phosphatidylserine receptors: role of CD36 in binding of anionic phospholipid vesicles to monocytic cells. *J. Biol. Chem.* 274, 3048–3054
37 Nakashima, Y. *et al.* (1994) ApoE-deficient mice develop lesions of all phases of atherosclerosis throughout the arterial tree. *Arterioscler. Thromb.* 14, 133–140
38 Plump, A.S. *et al.* (1992) Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* 71, 343–353
39 Jawien, J. (2012) The role of an experimental model of atherosclerosis: apoE-knockout mice in developing new drugs against atherogenesis. *Curr. Pharm. Biotechnol.* 13, 2435–2439
40 Zhaorigetu, S. *et al.* (2014) Delivery of negatively charged liposomes into the atherosclerotic plaque of apolipoprotein E-deficient mouse aortic tissue. *Liposome Res.* 24, 182–190
41 Almer, G. *et al.* (2011) Adiponectin-coated nanoparticles for enhanced imaging of atherosclerotic plaques. *Int. J. Nanomedicine* 6, 1279–1290
42 Barseghian, A. *et al.* (2011) Adiponectin and vulnerable atherosclerotic plaques. *J. Am. Coll. Cardiol.* 57, 761–770
43 Harris, J.T. *et al.* (2017) Assessment of plaque vulnerability in atherosclerosis via intravascular photoacoustic imaging of targeted liposomal ICG J-aggregates. *SPIE BiOS* 10064, 1006413

44 Karathanasis, E. *et al.* (2009) Selective targeting of nanocarriers to neutrophils and monocytes. *Ann. Biomed. Eng.* 37, 1984–1992
45 Holig, P. *et al.* (2004) Novel RGD lipopeptides for the targeting of liposomes to integrin-expressing endothelial and melanoma cells. *Protein Eng. Des. Sel.* 17, 433–441
46 Jain, S. *et al.* (2003) RGD-anchored magnetic liposomes for monocytes/neutrophils-mediated brain targeting. *Int. J. Pharm.* 261, 43–55
47 Kelly, C. *et al.* (2011) Targeted liposomal drug delivery to monocytes and macrophages. *J. Drug Deliv.* 2011, 727241–727241
48 Misra, S.K. *et al.* (2014) Co-liposomes comprising a lipidated multivalent RGD-peptide and a cationic gemini cholesterol induce selective gene transfection in αvβ3 and αvβ5 integrin receptor-rich cancer cells. *J. Mater. Chem. B* 2, 5758–5767
49 Song, Z. *et al.* (2017) Cyclic RGD peptide-modified liposomal drug delivery system for targeted oral apatinib administration: enhanced cellular uptake and improved therapeutic effects. *Int. J. Nanomed.* 12, 1941–1958
50 Koning, G.A. *et al.* (2002) Interference of macrophages with immunotargeting of liposomes. *J. Liposome Res.* 12, 107–119
51 Barratt, G.M. *et al.* (1987) Use of mannosylated liposomes for *in vivo* targeting of a macrophage activator and control of artificial pulmonary metastases. *Ann. Inst. Pasteur Immunol.* 138, 437–450
52 Jeong, H-S. *et al.* (2014) Effect of mannose ligand of manosylated liposomes on targeting for macrophage cell. *J. Nucl. Med.* 55 (Suppl. 1), 1204
53 Kawakami, S. *et al.* (2000) Mannose receptor-mediated gene transfer into macrophages using novel mannosylated cationic liposomes. *Gene Ther.* 7, 292
54 Wijagkanalan, W. *et al.* (2008) Efficient targeting to alveolar macrophages by intratracheal administration of mannosylated liposomes in rats. *J. Control. Release* 125, 121–130
55 Kelly, C. *et al.* (2011) Targeted liposomal drug delivery to monocytes and macrophages. *J. Drug Deliv.* 2011, 11
56 Varasteh, Z. *et al.* (2019) Targeting mannose receptor expression on macrophages in atherosclerotic plaques of apolipoprotein E-knockout mice using 68Ga-NOTA-anti-MMR nanobody: non-invasive imaging of atherosclerotic plaques. *EJNMMI Res.* 9, 5
57 Demos, S.M. *et al.* (1997) *In vitro* targeting of antibody-conjugated echogenic liposomes for site-specific ultrasonic image enhancement. *J. Pharm. Sci.* 86, 167–171

58 Demos, S.M. *et al.* (1999) *In vivo* targeting of acoustically reflective liposomes for intravascular and transvascular ultrasonic enhancement. *J. Am. Coll. Cardiol.* 33, 867–875
59 Demos, S.M. *et al.* (1998) *In vitro* targeting of acoustically reflective immunoliposomes to fibrin under various flow conditions. *J. Drug Target* 5, 507–518
60 Sanz, J. and Fayad, Z.A. (2008) Imaging of atherosclerotic cardiovascular disease. *Nature* 451, 953–957
61 Groenendyk, J.W. and Mehta, N.N. (2018) Applying the ordinal model of atherosclerosis to imaging science: a brief review. *Open Heart.* 5, e000861
62 Tarkin, J.M. *et al.* (2016) Imaging atherosclerosis. *Circ. Res.* 118, 750–769
63 Joshi, N.V. *et al.* (2014) 18F-fluoride positron emission tomography for identification of ruptured and high-risk coronary atherosclerotic plaques: a prospective clinical trial. *Lancet* 383, 705–713
64 Xia, Y. *et al.* (2019) Liposome-based probes for molecular imaging: from basic research to the bedside. *Nanoscale* 11, 5822–5838
65 Xu, H. *et al.* (2017) Nanoliposomes for photodynamic therapy guided by fluorescence and computed tomography imaging. In *International Conference on Photonics and Imaging in Biology and Medicine*, pp. W3A.25, Optical Society of America
66 Leike, J.U. *et al.* (2001) Characterization of continuously extruded iopromide-carrying liposomes for computed tomography blood-pool imaging. *Invest. Radiol.* 36, 303–308
67 Krause, W. *et al.* (2010) CT imaging with iopromide liposomes in a rabbit model. *J. Liposome Res.* 21, 229–236
68 In Wee, T. *et al.* (2013) Preparation of gold coated liposomes for CT contrast medium. *J. Korean Chem. Soc.* 57, XXX–YYY
69 Danila, D. *et al.* (2009) Antibody-labeled liposomes for CT imaging of atherosclerotic plaques: *in vitro* investigation of an anti-ICAM antibody-labeled liposome containing iohexol for molecular imaging of atherosclerotic plaques via computed tomography. *Tex. Heart Inst. J.* 36, 393–403
70 Binderup, T. *et al.* (2014) PET/MRI to predict and quantify the uptake of 89Zr-labeled nanoparticles in the aortic wall of atherosclerotic rabbits. *J. Nucl. Med.* 55 (Suppl. 1), 186
71 Abu Lila, A.S. and Ishida, T. (2017) Liposomal delivery systems: design optimization and current applications. *Biol. Pharm. Bull.* 40, 1–10
72 Maiseyeu, A. *et al.* (2009) Gadolinium-containing phosphatidylserine liposomes for molecular imaging of atherosclerosis. *J. Lipid Res.* 50, 2157–2163

73 Mulder, W.J. *et al.* (2006) Liposome-enhanced MRI of neointimal lesions in the ApoE-KO mouse. *Magn. Reson. Med.* 55, 1170–1174
74 Woodside, D.G. *et al.* (2018) Magnetic resonance imaging of atherosclerotic plaque at clinically relevant field strengths (1T) by targeting the integrin α4β1. *Sci. Rep.* 8, 3733
75 van Bochove, G.S. *et al.* (2011) Contrast enhancement by differently sized paramagnetic MRI contrast agents in mice with two phenotypes of atherosclerotic plaque. *Contrast Media Mol. Imaging* 6, 35–45
76 Hui, J. *et al.* (2017) Real-time intravascular photoacoustic-ultrasound imaging of lipid-laden plaque in human coronary artery at 16 frames per second. *Sci. Rep.* 7, 1417
77 Jansen, K. *et al.* (2014) Intravascular photoacoustic imaging: a new tool for vulnerable plaque identification. *Ultrasound Med. Biol.* 40, 1037–1048
78 Alkan-Onyuksel, H. *et al.* (1996) Development of inherently echogenic liposomes as an ultrasonic contrast agent. *J. Pharm. Sci.* 85, 486–490
79 Hill, S.A. and McQueen, M.J. (1997) Reverse cholesterol transport--a review of the process and its clinical implications. *Clin. Biochem.* 30, 517–525
80 Soumian, S. *et al.* (2005) ABCA1 and atherosclerosis. *Vasc. Med.* 10, 109–119
81 Krishna, S.M. *et al.* (2018) Anionic nanoliposomes reduced atherosclerosis progression in low density lipoprotein receptor (LDLR) deficient mice fed a high fat diet. *J. Cell Physiol.* 233, 6951–6964
82 Sahebkar, A. *et al.* (2014) A simple and effective approach for the treatment of dyslipidemia using anionic nanoliposomes. *Colloids Surf. B Biointerfaces* 122, 645–652
83 Sahebkar, A. *et al.* (2015) Apolipoprotein B-100-targeted negatively charged nanoliposomes for the treatment of dyslipidemia. *Colloids Surf. B Biointerfaces* 129, 71–78
84 Westerterp, M. *et al.* (2014) ATP-binding cassette transporters, atherosclerosis, and inflammation. *Circ. Res.* 114, 157–170
85 Cho, B.H. *et al.* (2010) Synthetic dimyristoylphosphatidylcholine liposomes assimilating into high-density lipoprotein promote regression of atherosclerotic lesions in cholesterol-fed rabbits. *Exp. Biol. Med.* 235, 1194–1203
86 Bulbake, U. *et al.* (2017) Liposomal formulations in clinical use: an updated review. *Pharmaceutics* 9, 12

87 Cheraghi, M. *et al.* (2017) Heart targeted nanoliposomal/nanoparticles drug delivery: an updated review. *Biomed. Pharmacother.* 86, 316–323
88 Lobatto, M.E. *et al.* Novel nanomedicine for anti-inflammatory therapy of atherosclerosis monitored by multimodality imaging. *Proc. Intl. Soc. Mag. Reson. Med.* 16, 518
89 Lobatto, M.E. *et al.* (2010) Multimodal clinical imaging to longitudinally assess a nanomedical anti-inflammatory treatment in experimental atherosclerosis. *Mol. Pharm.* 7, 2020–2029
90 Joner, M. *et al.* (2008) Site-specific targeting of nanoparticle prednisolone reduces in-stent restenosis in a rabbit model of established atheroma. *Arterioscler. Thromb. Vasc. Biol.* 28, 1960–1966
91 van der Valk, F.M. *et al.* (2016) Liposomal prednisolone promotes macrophage lipotoxicity in experimental atherosclerosis. *Nanomedicine* 12, 1463–1470
92 van der Valk, F.M. *et al.* (2015) Prednisolone-containing liposomes accumulate in human atherosclerotic macrophages upon intravenous administration. *Nanomedicine* 11, 1039–1046
93 Chono, S. *et al.* (2005) Efficient drug delivery to atherosclerotic lesions and the antiatherosclerotic effect by dexamethasone incorporated into liposomes in atherogenic mice. *J. Drug Target.* 13, 267–276
94 Calin, M. *et al.* (2015) VCAM-1 directed target-sensitive liposomes carrying CCR2 antagonists bind to activated endothelium and reduce adhesion and transmigration of monocytes. *Eur. J. Pharm. Biopharm.* 89, 18–29
95 Alaarg, A. *et al.* (2015) Targeted liposomal drug delivery to inhibit atherosclerotic plaque inflammation. *Atherosclerosis* 241, e87
96 Hoekstra, M. *et al.* (2018) The anti-atherogenic potential of liposome-mediated delivery of LXR agonists to macrophages: a preclinical validation study. *Atherosclerosis* 32, 7
97 Klegerman, M.E. *et al.* (2016) Ultrasound-enhanced bevacizumab release from echogenic liposomes for inhibition of atheroma progression. *J. Liposome Res.* 26, 47–56
98 Pont, I. *et al.* (2018) Anti-angiogenic drug loaded liposomes: nanotherapy for early atherosclerotic lesions in mice. *PLoS ONE* 13, e0190540
99 Steers, N.J. *et al.* (2009) Liposome-encapsulated HIV-1 Gag p24 containing lipid A induces effector CD4+ T-cells, memory CD8+ T-cells, and pro-inflammatory cytokines. *Vaccine* 27, 6939–6949

100 Khademi, F. *et al.* (2018) Potential of cationic liposomes as adjuvants/delivery systems for tuberculosis subunit vaccines. *Rev. Physiol. Biochem. Pharmacol.* 175, 47–69
101 Zamani, P. *et al.* (2018) Nanoliposomes as the adjuvant delivery systems in cancer immunotherapy. *J. Cell Physiol.* 233, 5189–5199
102 Raposo, G. *et al.* (1997) Accumulation of major histocompatibility complex class II molecules in mast cell secretory granules and their release upon degranulation. *Mol. Biol. Cell* 8, 2631–2645
103 Hosseini, H. *et al.* (2015) Phosphatidylserine liposomes mimic apoptotic cells to attenuate atherosclerosis by expanding polyreactive IgM producing B1a lymphocytes. *Cardiovasc. Res.* 106, 443–452
104 Chyu, K.Y. *et al.* (2017) Vaccine against arteriosclerosis: an update. *Ther. Adv. Vaccines* 5, 39–47
105 Benne, N. *et al.* (2018) Anionic 1,2-distearoyl-sn-glycero-3-phosphoglycerol (DSPG) liposomes induce antigen-specific regulatory T cells and prevent atherosclerosis in mice. *J. Control. Release* 291, 135–146
106 Benne, N. *et al.* (2016) Stimulating antigen-specific regulatory T cells for an atherosclerosis vaccine. *J. Immunol.* 196 (1 Suppl.), 70.18–70.18
107 Alving, C.R. *et al.* (1996) Immunization with cholesterol-rich liposomes induces anti-cholesterol antibodies and reduces diet-induced hypercholesterolemia and plaque formation. *J. Lab. Clin. Med.* 127, 40–49
108 Horva, I. *et al.* (1998) Influence of cholesterol liposome immunisation and immunostimulation on rabbits atherosclerosis induced by a high cholesterol diet. *Med. Sci. Monit.* 4, 403–407
109 Herbst, S.M. *et al.* (2010) Delivery of stem cells to porcine arterial wall with echogenic liposomes conjugated to antibodies against CD34 and intercellular adhesion molecule-1. *Mol. Pharm.* 7, 3–11
110 Hitchcock, K.E. *et al.* (2010) Ultrasound-enhanced delivery of targeted echogenic liposomes in a novel ex vivo mouse aorta model. *J. Control. Release* 144, 288–295
111 Xiaowei, W. *et al.* (2018) Nanoliposomes for safe and efficient therapeutic CD39 mRNA delivery: prevention and treatment of inflammatory and cardiovascular diseases. *Atherosclerosis* 32, 143
112 Zhaorigetu, S. *et al.* (2014) Delivery of liposomal fatty acid binding protein 4 siRNA for the treatment of atherosclerosis in apolipoprotein E-deficient mice. *J. Am. Coll. Cardiol.* 63, A2118
113 Kowalski, P.S. *et al.* A(2013) nti-VCAM-1 and anti-E-selectin SAINT-O-Somes for selective delivery of siRNA into inflammation-activated primary endothelial cells. *Mol. Pharm.* 10, 3033–3044

114 Scholl, F.G. *et al.* (2001) Effects of human tissue plasminogen gene transfer on allograft coronary atherosclerosis. *J. Heart Lung Transplant.* 20, 322–329
115 Bozzuto, G. and Molinari, A. (2015) Liposomes as nanomedical devices. *Int. J. Nanomedicine* 10, 975–999
116 Kang, J.H. *et al.* (2017) The effect of surface charges on the cellular uptake of liposomes investigated by live cell imaging. *Pharm. Res.* 34, 704–717
117 Walton, B.L. *et al.* (2010) Delivery of negatively charged liposomes into the atheromas of Watanabe heritable hyperlipidemic rabbits. *Vasc. Med.* 15, 307–313
118 Bhargava, P. and Lee, C.H. (2012) Role and function of macrophages in the metabolic syndrome. *Biochem. J.* 442, 253–262
119 de Lima, M.C. *et al.* (1999) Gene delivery mediated by cationic liposomes: from biophysical aspects to enhancement of transfection. *Mol. Membr. Biol.* 16, 103–109
120 Te Boekhorst, B.C. *et al.* (2012) Molecular MRI of inflammation in atherosclerosis. *Curr. Cardiovasc. Imaging Rep.* 5, 60–68
121 Andrews, J.P.M. *et al.* (2018) New methods to image unstable atherosclerotic plaques. *Atherosclerosis* 272: 118–128
122 Doris, M.K. *et al.* (2016) The future of imaging in cardiovascular disease intervention trials:. (2017 and beyond. *Curr. Opin. Lipidol.* 27, 605–614
123 Kee, P. *et al.* (2015) Noninvasive detection of macrophages in atheroma using a radiocontrast-loaded phosphatidylserine-containing liposomal contrast agent for computed tomography. *Mol. Imaging Biol.* 17, 328–336

Figure 1. A schematic representing strategies for targeting liposomes to atherosclerosis. Abbreviations: CAMs, cell adhesion molecules; EPR, enhanced permeability and retention; FRβ, Folate receptor beta; gAd, globular domain of adiponectin; IL-10, Interleukin-10; JAMs, junctional adhesion molecules; LOX-1, lectin-like oxidized LDL receptor-1; PS, phosphatidylserine;.

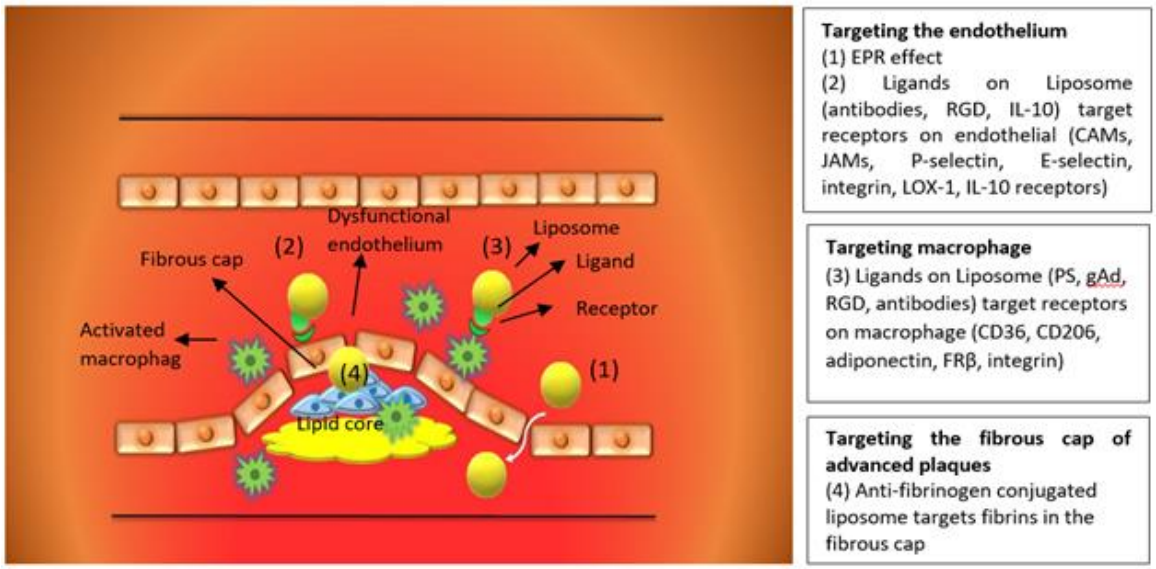


Figure 2. Single-photon emission computed tomography (SPECT) and computer tomography (CT) images, *ex vivo* autoradiography, and histological images of ^{111}In-PS100 **(a)** and ^{111}In-PS200 **(b)** in Watanabe heritable hyperlipidemic (WHHL) rabbits and ^{111}In-PS200 in normal rabbits **(c)**. Arrows indicate the position of aorta. L indicates the liver. Magnified images of Azan-Mallory staining show macrophage foam cell-rich regions with fewer smooth muscle cells (dashed red circle) and a more fibrotic region with dead macrophages (dashed yellow circle). Atherosclerotic regions were successfully visualized with ^{111}In-PS200 and ^{111}In-PS100 in WHHL rabbits. Radioactivity accumulated in macrophage foam cell areas and was low in fibrotic areas. No aortic accumulation was seen in normal rabbits. ^{111}In-PS100: Indium 111-labled liposome with 100 nm size. Reproduced from [35].

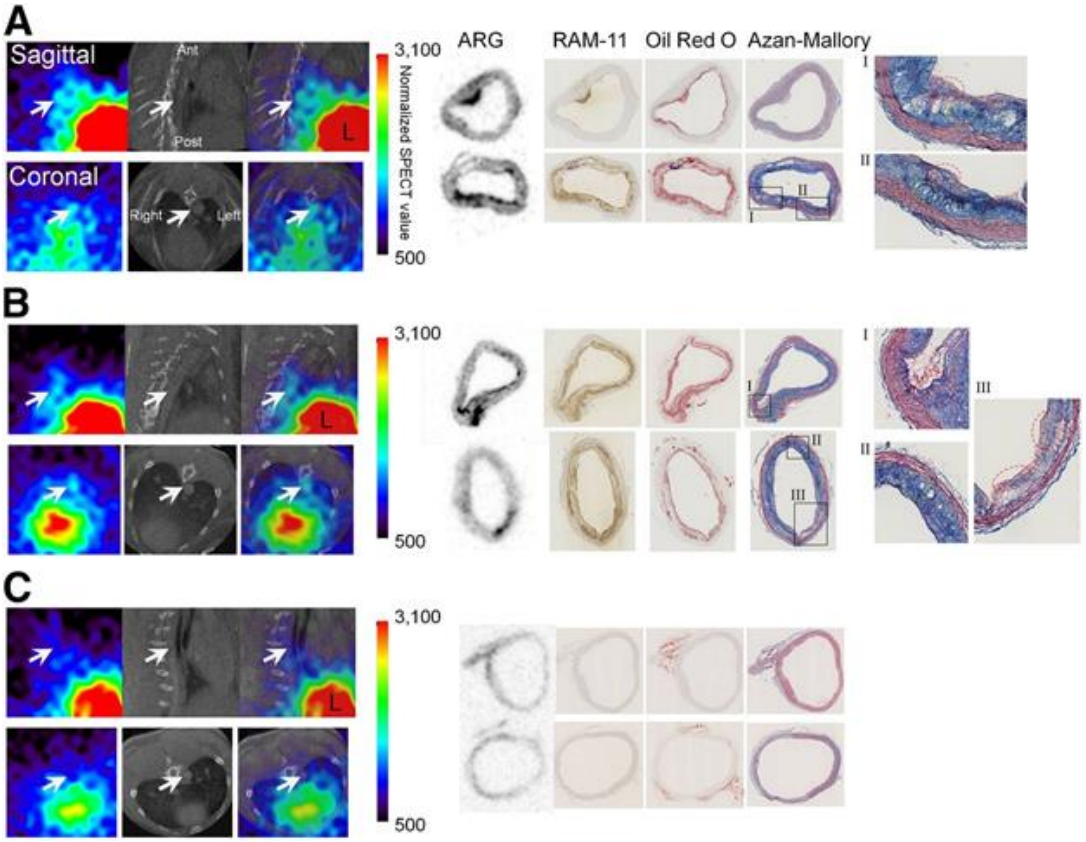


Figure 3. Title*.* Aortic wall enhancement by smaller liposomal contrast agent (d ~ 112 nm) in Watanabe heritable hyperlipidemic (WHHL) rabbits **(a,b)** and balloon-denuded cholesterol-fed New Zealand white (NZW) rabbits **(c,d)**. Animals were injected intravenously with control liposomes [phosphatidylcholine (PC)-liposomes] (a,c) or phosphatidylserine (PS)-liposomes (b,d). Imaging was performed with a GE eXplore flat-panel computer tomography (CT) scanner at baseline and 48 h after contrast injection. Section thickness was 0.2 mm and reconstructed images were displayed with a maximum intensity projection of 1.0 mm. Specific contrast enhancement in the aorta was determined by comparing images obtained at baseline and 48 h after injection. Focal enhancement was detected in the atherosclerotic segments in the aortas in both animal models injected with PS-liposomes (yellow arrows) but not with control liposomes. **(e)** CT enhancement of highlighted regions at baseline and 48 h after injection of various liposomal preparations in both animal models. Reproduced, with permission, from [123].

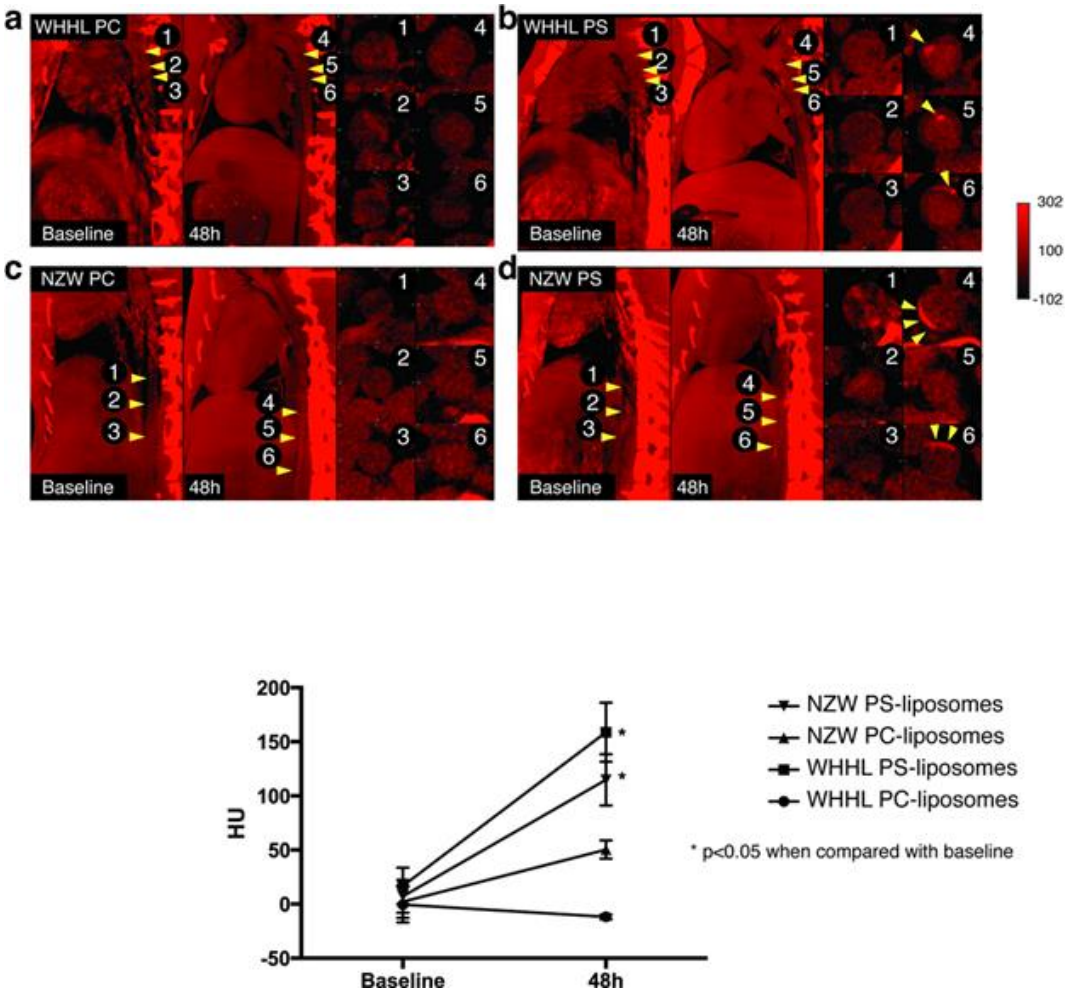


Figure 4. Images of the delivery and localization of glucocorticoid-loaded gadolinium (Gd)-containing liposomes by magnetic resonance imaging (MRI) and confocal laser scanning microscopy. **(a)** *In vivo* MRI of the abdominal aorta before (left) and 2 days after (right) the administration of liposomes. A marked signal intensity increase was observed throughout the atherosclerotic lesion. **(b)** Near-infrared fluorescence images of an atherosclerotic aorta excised from a rabbit injected with liposomes (left) and untreated aorta (right). The color bar represents photon count. **(c)** Confocal laser scanning microscopy of liposomes (red), cell nuclei (blue), and macrophages. **(d)** A high degree of colocalization of liposomes with macrophages was observed. **(e)** Although liposomes were found throughout the entire lesion areas, a vessel wall reconstruction of multiple confocal laser scanning microscopy images revealed heterogeneous accumulation of liposomes. (f) The corresponding MRI slice of the histological section depicted in (e) revealed a similar heterogeneous distribution. Reproduced, with permission, from [89].

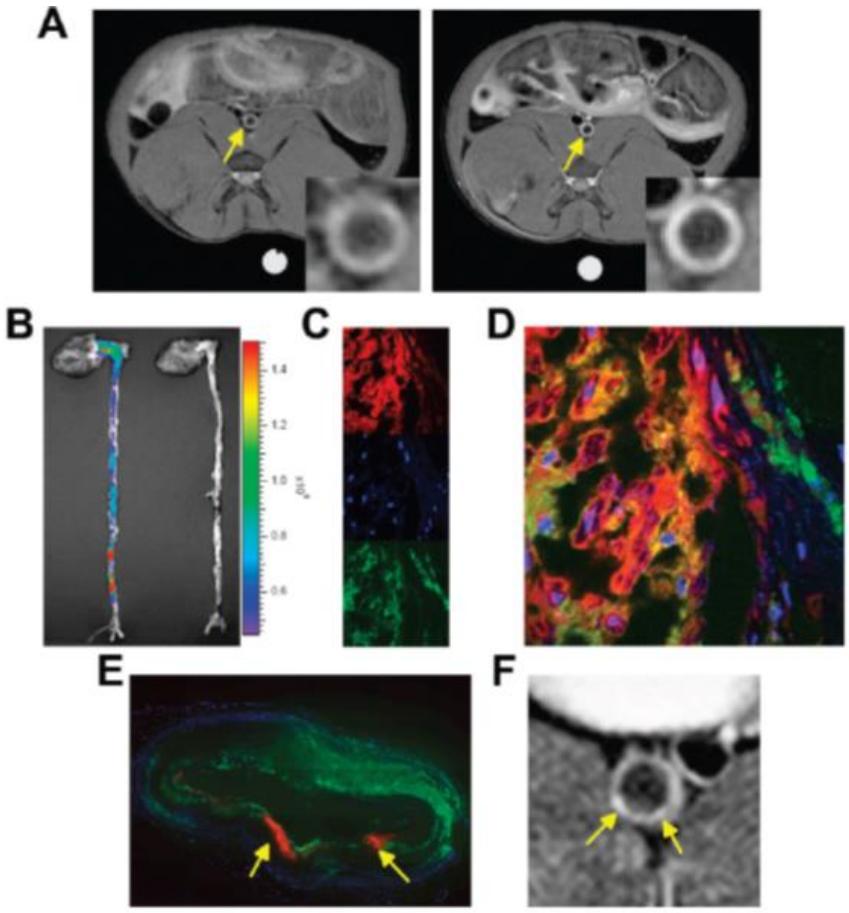


Figure 5. Transvascular ultrasound images of an atherosclerotic left carotid artery of a Yucatan mini swine. **(a)** After injection of saline. **(b)** After injection of antifibrinogen-labeled liposomes (arrows indicate liposomal attachment to fibrinogen in the atherosclerotic plaque). Reproduced, with permission, from [58].

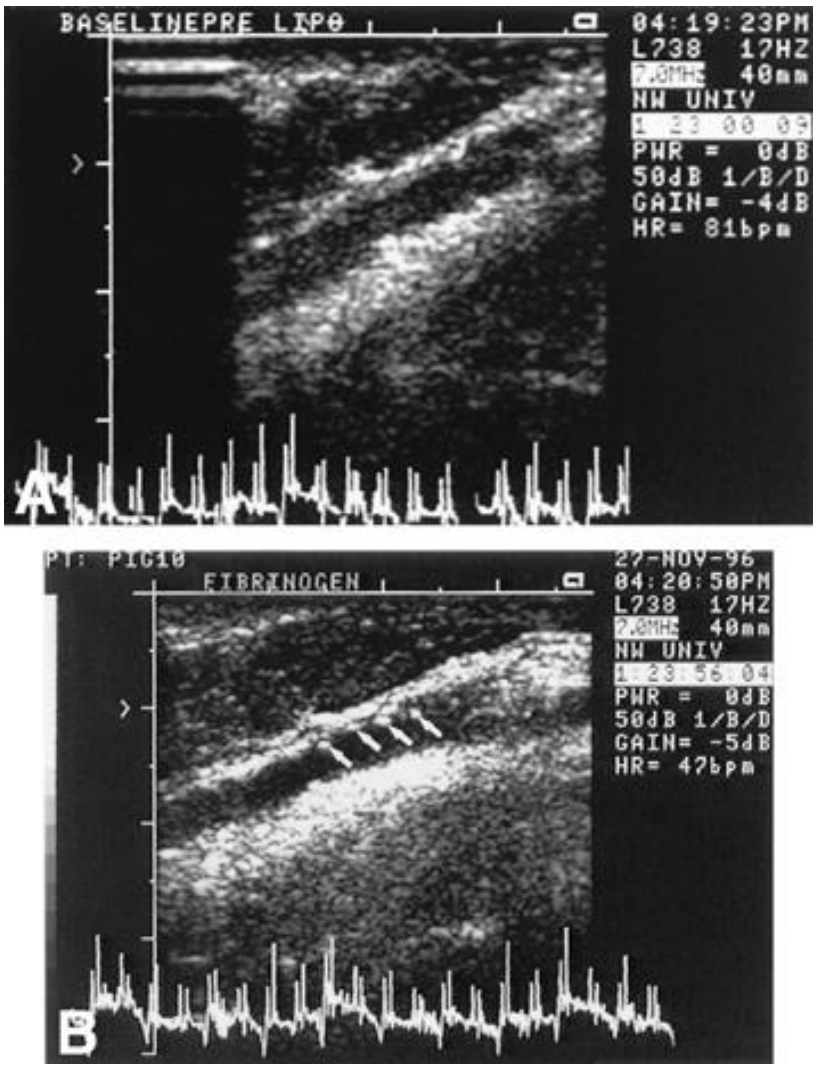


Figure 6. The progression of atherosclerosis by different imaging modalities. This figure depicts how atherosclerosis begins with endothelial dysfunction, followed by expression of proadhesive molecules that recruit monocytes in response to stimuli such as hypertensive pressure or lipid build-up. These monocytes then take up lipids to become foam cells. Additionally, smooth muscle cells migrate from the tunica media to the tunica intima, where they produce elastin and collagen, which create a cap that covers the plaque. Plaque growth can compromise blood flow to distal regions, resulting in stable angina or peripheral arterial disease. Alternatively, erosion of the fibrous cap can expose prothrombotic mediators, resulting in clot formation and infarction, either in the myocardium or the brain. Imaging modalities for these stages should be selected based on the physiological changes expected at each stage. Abbreviations: CAC, coronary artery calcium; CT, computer tomography; FDG-PET, 18-fluorodeoxyglucose positron emission tomography; FMD, flow-mediated dilatation; IVUS, intravascular ultrasound; NaF, sodium fluoride; PAT, peripheral arterial tonometry, small arteries; PET, positron emission tomography; PWV, pulse wave velocity, large arteries. Reproduced, with permission, from [109].

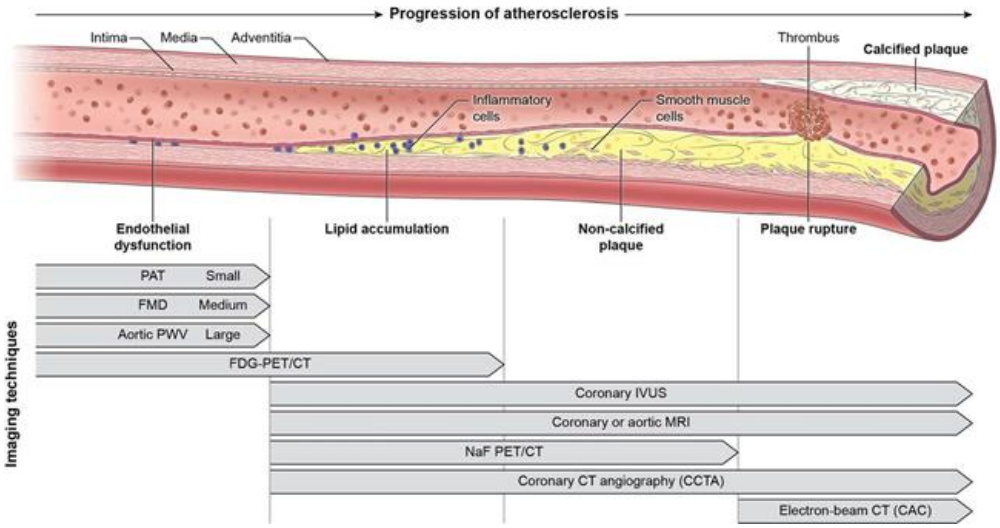


Figure 7. Scanning electron microscopy of bifunctional echogenic immunoliposome (BF-ELIP)-targeted CD34+ cells adhering to the luminal surface of porcine aorta following ultrasound treatment. **(a)** Scattered cell attachment to normal, nonatherosclerotic aortic surface, and **(b)** increased cell attachment and penetration in region with fatty streaks. Arrows indicate BF-ELIP-stem cell complexes adhering to the aorta. Reproduced, with permission, from [109].

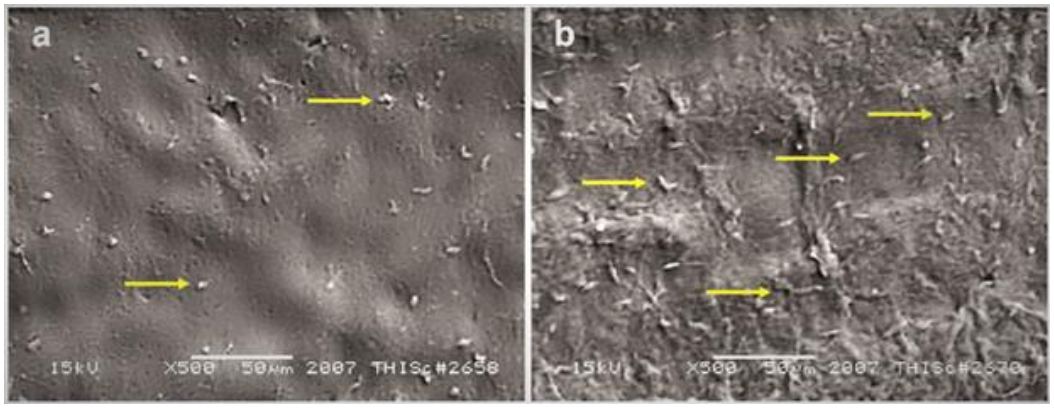
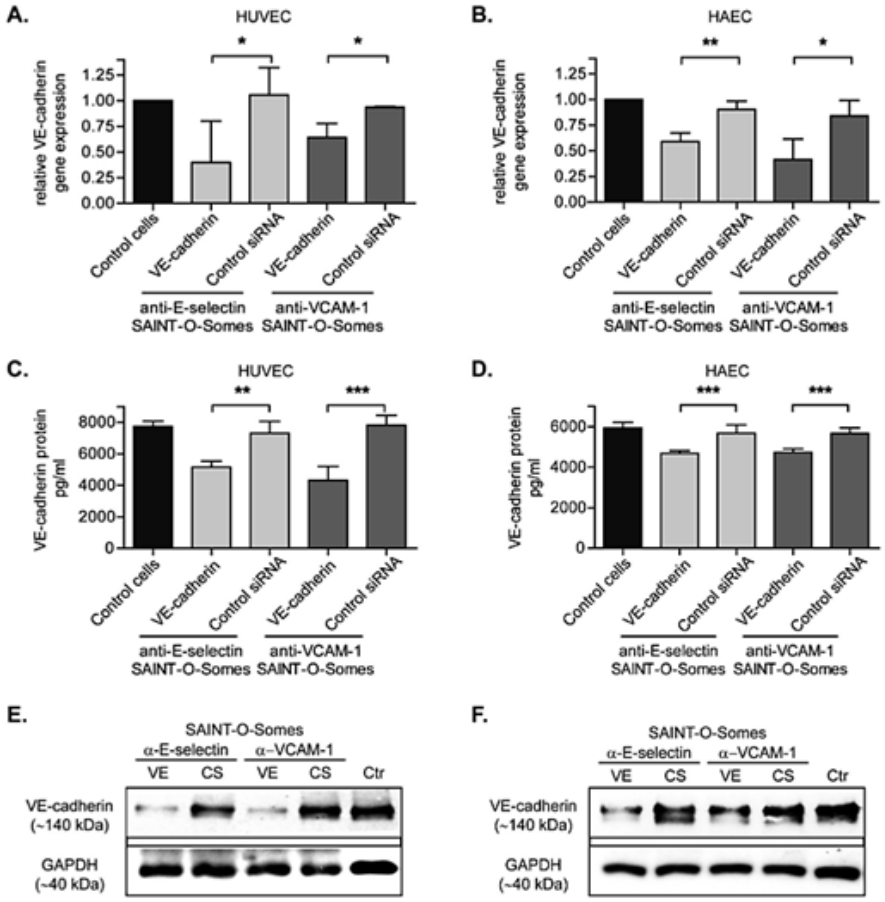


Figure 8. Effective downregulation of VE-cadherin in primary endothelial cells via targeted SAINT-O-Somes (cationic liposomes fabricated from amphiphilic SAINT-C18) small interfering (si)RNA delivery. Tumor necrosis factor (TNF)-αactivated human umbilical vein endothelial cells (HUVEC) and human aortic endothelial cells (HAEC) were incubated with targeted SAINT-O-Somes containing VE-cadherin (VE) or control siRNA (CS) at a 1 μM concentration for 48 h. After incubation, RNA or cell lysates were used for RT-qPCR **(a,b),** ELISA **(c,d)**, and western blot **(e,f)**. (a,b) Data are presented as relative expression ± SD, compared with cells treated only with TNF-α(Ctr), of a minimum of three independent experiments. **P* <0.05. (c,d). Data are presented as VE-cadherin protein expression ± SD of three independent experiments. (e,f) Images show a representative western blot. Reproduced, with permission, from [113].


A.
HUVEC
relative VE-cadherin gene expression
1.25
1.00
0.75
0.50
0.25
0.00
*
*
Control cells
VE-cadherin
Control siRNA
VE-cadherin
Control siRNA
anti-E-selectin SAINT-O-Somes
anti-VCAM-1 SAINT-O-Somes
B.
HAEC
relative VE-cadherin gene expression
**
*
C.
HUVEC
VE-cadherin protein pg/ml
8000
6000
4000
2000
0
**
***
D.
HAEC
VE-cadherin protein pg/ml
6000
4000
2000
0
***
***
E.
SAINT-O-Somes
α-E-selectin
α-VCAM-1
VE
CS
VE
CS
Ctr
VE-cadherin (~140 kDa)
GAPDH (~40 kDa)
F.
SAINT-O-Somes
α-E-selectin
α-VCAM-1
VE
CS
VE
CS
Ctr
VE-cadherin (~140 kDa)
GAPDH (~40 kDa)