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Nanoparticles as magnetic resonance imaging contrast agents for vascular and cardiac diseases

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Abstract

Advances in nanoparticle contrast agents for molecular imaging have made magnetic resonance imaging a promising modality for noninvasive visualization and assessment of vascular and cardiac disease processes. This review provides a description of the various nanoparticles exploited for imaging cardiovascular targets. Nanoparticle probes detecting inflammation, apoptosis, extracellular matrix, and angiogenesis may provide tools for assessing the risk of progressive vascular dysfunction and heart failure. The utility of nanoparticles as multimodal probes and/or theranostic agents has also been investigated. Although clinical application of these nanoparticles is largely unexplored, the potential for enhancing disease diagnosis and treatment is considerable.

INTRODUCTION

Atherosclerosis is a chronic inflammatory response of arterial blood vessels where deposits of fatty substances, cholesterol, cellular waste products, calcium, and other substances build up in the inner lining of an artery, forming an atherosclerotic plaque. The plaque may further progress and ultimately rupture, leading to thrombus formation, which can occlude arteries and cause serious clinical events such as myocardial infarction and stroke when the coronary circulation supplying the heart and the cerebral circulation supplying the brain, respectively, are blocked.¹

In order to assess atherosclerotic plaques and myocardial infarctions, several invasive (e.g., X-ray angiography, intravascular ultrasound, and angiography) and noninvasive (surface B-mode ultrasound and ultrafast computed tomography) imaging techniques have been developed.² Among the many imaging techniques, high-resolution magnetic resonance (MR) has emerged as a valuable imaging modality for characterization of atherosclerotic arteries and infarct regions in a noninvasive and nondestructive way with high soft tissue contrast. The soft tissue contrast in MR imaging is generated by biophysical and

biochemical parameters such as chemical composition and concentration, water content, physical state, molecular motion, and diffusion.³ Although MR imaging can noninvasively produce high spatial resolution images rich in anatomical information, it has significantly lower sensitivity to contrast agents than positron emission tomography (PET), single photon emission computed tomography (SPECT), and fluorescence techniques. On the other hand, SPECT, PET, and fluorescence do not provide anatomical information.⁴ Therefore, a multimodal imaging approach is often necessary where the strengths of several modalities are exploited to fully validate the results of molecular imaging.

During the development of atherosclerotic plaques, many potential biomarkers, such as adhesion molecules [vascular cell adhesion molecules (VCAMs), intercellular adhesion molecules (ICAMs), selectins], macrophages and their scavenger receptors, matrix metalloproteinases (MMPs), oxidized low-density lipoprotein (oxLDL), $\alpha_v\beta_3$ integrin, extracellular matrix, and fibrin (summarized in a review by Lipinski et al.⁵), are upregulated. In the infarcted myocardium, inflammation, angiogenesis, MMPs, thrombin-activated factor XIII (FXIII), apoptosis/necrosis, and extracellular matrix are important factors in the processes of remodeling after injury.⁶⁻¹¹ It is important to point out that these molecules are often not unique to cardiovascular diseases, but they are present at increased levels under these disease conditions as compared to disease-free conditions. Moreover, these molecular targets are often present at relatively low levels (10^{-9} to 10^{-13} M/g tissue). To overcome sensitivity issues, high payload contrast agent vehicles are desired for molecular MR imaging in order to generate sufficient signal change. Nanoparticle-facilitated imaging is the most promising approach for molecular MR imaging purposes, since nanoparticles exhibit the possibility to include a high contrast agent payload, may be of relatively small size to facilitate penetration into tissue, and have a tunable circulation half-life, a large surface area available for conjugation of functional groups, and the potential to function as both imaging and therapeutic (i.e. 'theranostic') agents.

Two categories of contrast agents are frequently used for molecular MR imaging of atherosclerotic plaques and myocardial infarctions: (1) superparamagnetic iron oxide (SPIO) nanoparticles and (2) nanoparticles that incorporate gadolinium (Gd) chelates. The contrast agents change the longitudinal and transverse relaxation times (T_1 and T_2 , respectively), and thus affect relaxation rates ($R_1 = 1/T_1$; $R_2 = 1/T_2$). The efficiency of contrast agents is described by relaxivity (r_1 for longitudinal relaxivity, r_2 for transverse relaxivity), which is

defined as , where $i = 1$ or 2 and $[CA]$ is the concentration of contrast agents. SPIO nanoparticles have r_2/r_1 ratios much higher than 1 (usually higher than 10) and therefore are more suitable for T_2 - and T_2^* -weighted MR imaging, while Gd chelates have r_2/r_1 ratios close to 1 (typically between 1.1 and 2) and are exploited to create MR signal enhancement in T_1 -weighted imaging. Several nanoparticle platforms have been developed for target-specific imaging of cardiac disease and include dextran-coated iron oxides,¹² micelles,¹³ liposomes,^{14,15} oil-in-water emulsions,^{16,17} and lipoproteins¹⁸⁻²¹ as illustrated in Figure 1. In this review, we will focus on recent work that used nanoparticles for molecular MR imaging of vascular and cardiac diseases.

SUPERPARAMAGNETIC IRON OXIDE NANOPARTICLES

SPIO nanoparticles are composed of iron oxide nanocrystals and a coating material such as dextran that prevents aggregation of the iron oxide cores and improves biocompatibility. There are four different categories of SPIO nanoparticles based on their size: large (>200 nm), standard (60–150 nm), ultrasmall (USPIO, 10–40 nm), and monocrySTALLINE (MION, 10–30 nm) agents.²² The association of iron oxide particles with cells can occur via passive or active targeting. Dextran-coated USPIO nanoparticles are inherently sequestered by monocytes/macrophages because of phagocytosis, which is therefore referred as passive targeting to monocytes/macrophages.^{23,24} This is valuable as high macrophage content is considered a hallmark of plaque vulnerable to rupture.¹ Ruehm et al. were the first to show that once these particles are internalized within the intraplaque macrophages, the high magnetization associated with the iron oxide crystal core induces significant T_2^* -weighted MR signal loss, allowing the detection of plaques that are macrophage rich.²⁵ Several reports have shown that active intraplaque macrophages can readily phagocytose iron oxide particles that diffuse or migrate into the atherosclerotic plaque in preclinical studies,^{25–34} while dextran-coated USPIOs have also been used clinically to target intraplaque macrophages.^{29,35–39} In Europe, Combidex (Sinerem/AMI-227, Guerbet) is typically infused (over 30 min) into patients at a dose of 2.6 mg Fe/kg of bodyweight. Iron oxide MR imaging may be used as a surrogate end point in clinical trials designed to test the efficacy of novel cardiovascular therapeutics.⁴⁰ In these studies signal loss in T_2/T_2^* -weighted MR images is usually observed at late time points (>24 h) after injection of iron oxide. To optimize the detection of low-concentration molecular targets, the relaxivity of iron oxide particles can be further increased by manganese doping.⁴¹

The size of iron oxide nanoparticles affects passive targeting to intraplaque macrophages. Feridex has a mean size of ~97 nm and, in a 2008 study, was not observed to accumulate in intraplaque macrophages.⁴² However, Feridex can be fractionated to isolate ultrasmall particles of 12 nm. After injection of a 4.8 mg Fe/kg of bodyweight dose, significant uptake of this fractionated Feridex was observed in intraplaque macrophages in a rabbit model (Figure 2).⁴² This size effect can be attributed to easier diffusion of these small nanoparticles into atherosclerotic plaques and their long blood half-life. Small nanoparticles (10–100 nm) are more likely to extravasate from the vasculature and accumulate into diseased tissue via the enhanced permeability and retention (EPR) effect^{43–47} than large particles (100–500 nm). This is an important aspect to consider because the nonspecific accumulation of nanoparticles contributes to signal attenuation and may result in an overestimation of the actual receptor expression. On the contrary, molecular imaging of an extravascular target requires nanoparticles to escape the blood vessels, which favors the use of small nanoparticles.

In addition to passively targeting monocytes/macrophages, iron oxide particles have also been modified to actively target molecular markers. Nahrendorf et al. functionalized monocrySTALLINE iron oxide nanoparticles with vascular cell adhesion molecule-1 (VCAM-1) targeting peptides.⁴⁸ *In vivo* MR imaging revealed that the aortic root of apoE^{−/−} mice became hypointense (dark) after injection of these nanoparticles, which was confirmed through fluorescence imaging to be due to nanoparticle accumulation in cells that

overexpressed VCAM-1 (Figure 3). Kang et al. have functionalized cross-linked iron oxide (CLIO) nanoparticles with anti-human E-selectin antibody fragments to detect E-selectin in endothelial cells.⁴⁹ A three times greater CLIO-induced MR signal decrease on T₂*-weighted images was observed in human umbilical vein endothelial cells implanted into mice in response to interleukin-1 β (a cytokine that induces E-selectin expression) treatment compared to untreated controls.⁴⁹ In addition, a dual targeted strategy has been used to image endothelial adhesion molecules in apoE^{-/-} mice,⁵⁰ where microparticles of iron oxide were conjugated with monoclonal antibodies against VCAM-1 (VCAM-MPIO) and/or P-selectin (P-selectin-MPIO). Using *ex vivo* MR imaging, dual targeted particles showed higher affinity to the endothelium under flow conditions in comparison to single targeted ones. Smith et al. have decorated SPIO with the protein Annexin V (Anx-SPIO, ~98 nm) to target apoptosis in atherosclerotic plaques.⁵¹ *In vivo* MR imaging revealed that Anx-SPIO induced signal hypointensity in atheromatous lesions, but not in healthy arteries, in a rabbit model. Annexin V decoration was required to produce hypointensity at a low dose (0.05 mg Fe/rabbit, or about 0.012 mg Fe/kg of bodyweight), while a 2000-fold higher dose of untargeted nanoparticles was needed to produce the same negative contrast in this animal model.⁵¹ Iron oxide nanocrystals can also be incorporated into nanocarriers, such as micelles,⁵² liposomes,¹⁴ nanoemulsions,⁵³ and high-density lipoproteins (HDLs)⁵⁴ for active targeting, which will be discussed in the following sections.

Identifying hypointense regions in MR images of atherosclerotic plaques caused by iron oxide nanoparticles is challenging, since partial volume effects and artifacts may lead to overestimation of the target-rich areas. As an alternative to identifying hypointense regions in T₂(*)-weighted imaging of iron oxide contrast agents, positive contrast techniques have been developed by either designing new imaging sequences or tailoring iron oxide particles. The currently available positive contrast imaging sequences are summarized in recent reviews.^{12,55} T₁-weighted MR imaging with iron oxide nanoparticles is usually not performed because of their strong impact on tissue R₂ values, even though these nanoparticles often have very high r₁ values, comparable with those of nanoparticles that contain Gd chelates. However, some groups have tailored the magnetic properties of iron oxide particles to decrease the r₂/r₁ ratio, which thus enables T₁-weighted MR imaging.⁵⁶⁻⁵⁸ For example, iron oxide nanoparticles have been doped with manganese to form solid-solution nanocrystals (Mn_xFe_{1-x}O). These nanocrystals showed simultaneous T₁ and T₂ enhancements in MR imaging of rat liver *in vivo*.⁵⁶ Alternatively, magnetite nanoparticles whose surface was coated with Gd chelates were demonstrated to be contrast agents for both T₁- and T₂-weighted *in vivo* MR imaging of subcutaneously implanted hydrogels in nude mice.⁵⁸ Colloidal iron oxide nanoparticles (CION),⁵³ another example of a T₁ iron oxide contrast agent, are formed by entrapping magnetite particles within phospholipid nanoemulsion, which will be discussed in detail in the *Oil-in-Water Emulsion* section below.

Iron oxide nanoparticles have also been developed to target biomarkers of interest for cardiovascular diseases other than atherosclerosis, such as myocardial infarction.^{27,59-64} Dextran-coated iron oxide nanoparticles (~50 nm) induced significant negative contrast in infarcted, but not normal, myocardium on T₂-weighted images from 5 to 48 h postinfarction.⁶³ In another example, an annexin-based iron oxide nanoparticle AnxCLIO-Cy5.5 was used to target apoptotic cardiomyocytes in a mouse model of acute myocardial

ischemia.⁶¹ MR imaging revealed that AnxCLIO-Cy5.5 was most prominently localized in the mid-myocardium, in which apoptosis was observed most frequently using terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) *ex vivo*.⁶¹ Weissleder et al. used MION (~3 nm) attached to antimyosin antibody fragments (Fab) for MR imaging of cardiac infarcts by targeting myosin. A significant decrease in the signal intensity of infarcted myocardium was observed by MR imaging at 1 h postinjection, indicating successful uptake of this contrast agent in the target tissue.⁶⁴

MICELLES

As mentioned above, the trapping of iron oxide nanocrystals into micelles can serve as one of several methods that facilitate active targeting of biomarkers. A typical micelle in aqueous solution is an aggregate of amphiphilic surfactants (formed when above the critical micelle concentration), which has a structure where the hydrophilic 'head' regions of the amphiphile molecules are in contact with the surrounding water molecules, and the hydrophobic tail regions are sequestered in the core (Figure 1). As a result, micelle cores can incorporate hydrophobic materials such as oleic-acid-coated iron oxide nanocrystals. These micellular iron oxide nanoparticles sometimes possess polyethylene glycol (PEG) incorporated at the lipid headgroups to increase biocompatibility and to facilitate the introduction of targeting groups. For example, van Tilborg et al. conjugated human recombinant Annexin A5 to pegylated micellular iron oxide nanoparticles, which were able to target apoptotic cells.⁵²

Gadolinium (Gd) chelates are another category of MR contrast agents that are frequently incorporated into micelles. Unlike iron oxide, Gd-chelate-based MR contrast agents speed up longitudinal relaxation by shortening tissue T_1 values, resulting in positive enhancement in T_1 -weighted MR imaging. Micelles containing Gd chelates have been developed for targeting specific epitopes in atherosclerotic plaque, allowing detection of their expression by MR imaging.⁶⁵ For instance, immunomicelles, i.e. micelles conjugated with antibodies, have been used to assess macrophage burden in atherosclerotic plaques by *in vivo* MR imaging.⁶⁵⁻⁶⁸ To demonstrate this concept, the macrophage scavenger receptor (MSR) CD204 was chosen as a target for molecular MR imaging.⁶⁶ *In vivo* MR imaging revealed that at 24 h postinjection, immunomicelles with MSR antibody (anti-CD204) caused significant enhancement of atherosclerotic aortas in apoE^{-/-} mice compared to untargeted micelles (Figure 4). This enhancement was found to be related to the macrophage content of the atherosclerotic vessel areas imaged. Immunomicelles may thus aid in the detection of high macrophage content, which is associated with plaques vulnerable to rupture.⁶⁶

Oxidized low-density lipoprotein (oxLDL) plays a key role in the initiation, progression, and destabilization of atherosclerotic plaques and is present in macrophages and the lipid pool. Immunomicelles containing murine (MDA2 and E06) or human (IK17) antibodies that bind unique oxidation-specific epitopes (i.e. oxLDL) induced enhancement in T_1 -weighted MR images of the aorta wall of apoE^{-/-} mice.⁶⁷

Micelles have also been used to detect myocardial infarctions. Lukyanov et al. reported that long circulating micelles accumulated in the infarction zone in greater quantities as

compared to a non-damaged part of the heart muscle, as evidenced by *ex vivo* gamma camera imaging.⁶⁹ The micelle accumulation was primarily due to the EPR effect in the infarct areas. This study demonstrated that untargeted micelles have the potential for the delivery of therapeutic or diagnostic agents to an area of myocardial infarction.

LIPOSOMES

Liposomes are spherical, self-closed structures composed of natural and/or synthetic amphiphilic lipids with diameters in the range of 50–500 nm and can be functionalized with targeting ligands to allow molecular imaging (Figure 1). These biocompatible particles can be further coated with polymers (e.g. PEG) to increase stability and to prolong their blood circulation half-life. A wide range of amphiphilic and hydrophobic molecules can be incorporated in the bilayer of liposomes, such as phospholipids whose headgroup is modified with a Gd chelate or a fluorophore. The aqueous interior can be loaded with water-soluble drugs, proteins, or other therapeutics for treating diseases or with contrast-generating materials such as Gd-DTPA. Untargeted liposomal nanoparticles have been demonstrated to deliver Gd chelate contrast agents to detect lipid-rich atherosclerotic plaques by MR imaging in a study by Mulder et al.⁷⁰ Neointimal lesions were induced in apoE^{-/-} mice by placing a constrictive collar around the right carotid artery. After injection of paramagnetic liposomes (mean size = 90 nm) that include amphiphilic Gd chelates in the lipid bilayer, a pronounced MR signal enhancement of the lesions was observed, while injection of the control agent Gd-DTPA did not result in signal enhancement.⁷⁰ This result is likely due to accumulation of the liposomes in plaque via the EPR effect.

Apart from MR imaging exploiting nonspecific uptake of liposomes, targeted imaging of molecular markers, such as E-selectin, apoptosis, and collagens, has also been explored.^{52,71,72} Paramagnetic liposomes enriched with phosphatidylserine (PS) have been used to target macrophages and enable molecular MR imaging of atherosclerosis.⁷³ In this case, the targeting exploits the fact that PS residues on the plasma membrane are exteriorized in apoptotic cells, triggering rapid phagocytosis by macrophages, so that macrophages would also take up these PS-rich liposomes. A rapid and significant enhancement of the aortic wall was observed *in vivo* after injection of PS-enriched liposomes in apoE^{-/-} mice. The colocalization of the liposomes with macrophages in mouse atherosclerotic plaques was revealed by confocal microscopy.⁷³

Collagen is an important component of the extracellular matrix and plays important roles in atherosclerosis and myocardial infarction. The degradation of collagen in fibrous caps correlates with rupture of atherosclerotic plaques.¹ In myocardial infarctions, the deposition of collagen heals injury in the infarct area on one hand, and contributes to ventricular stiffness and dysfunction in the uninfarcted region on the other hand.⁷⁴ A collagen-specific, bimodal liposomal MR contrast agent (Figure 5) has been developed by functionalizing liposomes with CNA35, a collagen adhesion protein of the *Staphylococcus aureus* bacterium. This CNA35 functionalized liposomal contrast agent decreased the T₁ value of collagen coated on plate by 1000 ± 50 ms compared to controls, which indicates the potential of this technology for molecular imaging of collagen.⁷² In addition to CNA35, peptides⁷⁵⁻⁷⁹ have also been investigated as collagen-specific targeting ligands for MR

contrast agents. These peptides have been successfully used for molecular imaging of fibrosis and postinfarction myocardial scars.^{78,79}

Hiller et al. used liposomes that include Gd chelates and are targeted with Annexin V to assess apoptosis in myocardial infarctions.¹⁵ When these liposomes were used in perfused hearts, a significant increase in signal intensity was visible in MR images of the heart regions where apoptotic cardiomyocytes were present. Consequently, these Annexin-V-enriched liposomes have potential applications in MR imaging of apoptotic cells in the ischemic and reperfused myocardium, enabling visualization of apoptotic cell death noninvasively.

OIL-IN-WATER EMULSIONS

Oil-in-water emulsions are composed of hydrophobic oil cores coated with amphiphilic molecules, and are dispersed in water (Figure 1). Emulsions are capable of carrying both a high payload of hydrophobic materials in their core and an amphiphilic payload in their surfactant corona. There have been many reports from the group of Lanza, and from others, of submicron size emulsions (around 250 nm in diameter) formulated using liquid perfluorocarbons as a core. Some of the formulations include Gd-chelating lipids in the surfactant corona.⁸⁰⁻⁸⁴ Morawski et al. have shown that sufficient MR imaging quality can be achieved at picomolar concentration with these high payload emulsion nanoparticles.⁸⁵ The lipid surface of these emulsions has been functionalized with several different ligands as targeting moieties. For example, anti-fibrin antibody fragments were conjugated to the lipids in the emulsion surface, allowing target-specific MR imaging of thrombi. This is of interest in cardiovascular disease not only because they are usually formed after atherosclerotic plaque rupture and can occlude the arteries⁸⁰ but also because they occur before occlusive rupture, serving as an early indicator for severe clinical events.

When small molecules mimicking the RGD-peptide were coupled to the lipid membrane, the resulting emulsions can target the $\alpha_v\beta_3$ integrin, which is overexpressed in angiogenic endothelial cells. An enhancement was therefore observed when these nanoparticles were used for *in vivo* MR imaging of the abdominal aortic wall of atherosclerotic rabbits, due to angiogenesis occurring in their plaques.⁸⁴ A theranostic agent has been developed by loading fumagillin, a water-insoluble angiostatic drug, in $\alpha_v\beta_3$ -integrin-targeted emulsions.⁸² When these theranostic nanoemulsions were applied in atherosclerotic rabbits, MR signal enhancement was observed at baseline. Seven days after the first injection, a lesser MR signal enhancement was observed in the aortic wall of the rabbits treated with $\alpha_v\beta_3$ -integrin-targeted fumagillin loaded emulsions, while the contrast enhancement for targeted emulsions without fumagillin was unchanged.⁸² This result indicated the anti-angiogenic effect of the fumagillin loaded emulsions. In a subsequent report on these $\alpha_v\beta_3$ -targeted fumagillin emulsions it was revealed that their anti-angiogenic effects are acute, but can be prolonged when combined with atorvastatin, resulting in a marked and sustainable anti-angiogenic effect.⁸³

Perfluorocarbon emulsions have also been used in ^{19}F MR imaging.⁸⁶ The MR-active isotope of fluorine, ^{19}F , is 100% naturally abundant. However, there is no ^{19}F background in

the body, as fluorine is not used in biological processes. Therefore, significant signals observed in ^{19}F MR can only originate from exogenous, injected ^{19}F -containing compounds. Flögel et al. used ^{19}F MR imaging to show that the accumulation of an injected untargeted perfluorocarbon nanoemulsion into the border of infarcted myocardium in a myocardial ischemia mouse model increased from 1 to 6 days postinjection, due to nonspecific accumulation (Figure 6).⁸⁶

Jarzyna et al. have recently developed stable nanoemulsions whose size can be controlled in the 30-to 100-nm size range.¹⁷ These nanoemulsions were loaded with oleic-acid-coated iron oxide nanocrystals in the core and Cy5.5 fluorophores were attached to the distal ends of PEG chains that were anchored in the phospholipid coating to create a multifunctional nanoplatform for MR/optical imaging.¹⁷ This contrast agent was employed for tumor imaging in a mouse model of cancer, with the nanoparticles accumulating in the tumors because of the EPR effect. As the EPR effect occurs in atherosclerotic plaque also, these nanoemulsions could also be used for plaque imaging or targeted to epitopes of interest in plaque. As mentioned before, Senpan et al. have used CIONs as T_1 -weighted MR contrast agents, which are nanoemulsions whose strength of magnetic flux experienced by surrounding protons is reduced due to cross-linking of the shell of the particles.⁵³ The shielding effect of nanoemulsions resulted in a lengthening of T_2 and also higher r_1 values, which permitted the T_1 contrast to be detected. CIONs with fibrin-specific functional groups caused signal enhancement in T_1 -weighted MR *ex vivo* imaging of fibrin in ruptured atherosclerotic plaques from human carotid endarterectomy specimens without blooming artifacts (Figure 7). Fumagillin was incorporated into these CIONs in an *in vitro* demonstration of their potential as theranostic agents.⁵³

LIPOPROTEINS

Lipoproteins are nanoparticulate aggregates of lipids and proteins that are responsible for the transport of water insoluble nutrients through the vascular and extravascular spaces to cells. Usually, lipoproteins have a spherical shape and consist of a lipid core [triglycerides (TGs) and cholesteryl esters (CEs)] surrounded by a monolayer of phospholipids and cholesterol in which a family of proteins called apolipoproteins are embedded (Figure 1). Traditionally, lipoproteins are classified into five major classes on the basis of ascending density: chylomicrons, very-low-density lipoproteins (VLDLs), intermediate-density lipoproteins (IDLs), low-density lipoproteins (LDLs), and high-density lipoproteins (HDLs).

LDL nanoparticles bind to low-density lipoprotein receptors (LDLRs) for cholesterol endocytosis with high selectivity, which makes LDL an interesting carrier for targeted delivery of therapeutic drugs and diagnostic contrast agents. A large numbers of reports can be found in literature that deal with imaging of atherosclerosis using radiolabeled LDL or radiolabeled modified LDL.⁸⁷ However, reports on LDL or modified LDL as contrast agents for MR imaging of atherosclerosis remains limited, although the *in vivo* MR efficacy of paramagnetic LDL particles for LDLR targeting has been explored in tumor models.⁸⁸⁻⁹⁰ In the context of atherosclerosis, LDL that is enriched with a hydrophobic contrast agent, manganese-mesoporphyrin, caused reduction in T_1 for foam cell pellets that were incubated

with this agent.¹⁹ Despite the potential of these nanoparticles, *in vivo* MR imaging of atherosclerotic plaque by LDL nanoparticles has not yet been reported.

In comparison with LDL, HDL nanoparticles have some advantages for atherosclerotic plaque imaging. First, they are the key players in reverse cholesterol transport, which is of potential benefit for regression of atherosclerotic plaque. During reverse cholesterol transport, HDL binds to scavenger receptor B type I (SR-BI) and ATP-binding cassette transporters,⁹¹ and thus targets to macrophages expressing these receptors. High HDL cholesterol levels are associated with reduced carotid atherosclerotic plaque burden and lipid content whereas LDL promotes atherosclerosis.^{1,92} Second, they have a small size (diameter of 7–12 nm), which enables them to penetrate the vascular endothelium more easily than LDL, although the larger size of LDL permits larger payloads to be carried. Third, HDL-like particles can easily be reconstituted, whereas LDL is difficult to reconstitute. HDL-based MR contrast agents were first reported in 2004. They were synthesized via reconstituting HDL nanoparticles with Gd-chelating phospholipids included in the lipid layer (rHDL).^{20,21} These rHDL nanoparticles are approximately 9 nm in diameter and have longitudinal relaxivity values of about 10 mM⁻¹ s⁻¹. In hyperlipidemic apoE^{-/-} mice, MR imaging revealed significant enhancement of atherosclerotic plaque at 24 h postinjection of paramagnetic rHDL nanoparticles as compared with preinjection images. Confocal microscopy revealed the association of macrophages with these rHDL nanoparticles. The targeting of rHDL to macrophages can be further enhanced by incorporating P2A2, an apolipoprotein-E-derived lipopeptide, into the lipid layers.⁹³ P2A2-modified HDL resulted in an average normalized enhancement ratio (NER) of 93% for MR images of aortic vessel wall at 24 h postinjection, which was significantly higher than HDL (NER of 53%). In addition, HDL nanoparticles can be converted to a versatile platform by rerouting them to other biomarkers than their natural receptors.⁹⁴⁻⁹⁶ For example, conjugation of cyclic RGD peptides to the apoAI component of rHDL redirected these nanoparticles so that they bound to angiogenic endothelial cells, as demonstrated by MR/optical imaging *in vitro* and in a tumor model.⁹⁴

This rHDL nanoplatform can also be modified to incorporate hydrophobically coated nanocrystals such as iron oxide (FeO-HDL), quantum dots (QD-HDL), or gold nanoparticles (Au-HDL) in the core. Fluorescent and/or paramagnetic lipids were included in the lipid coating to form multimodal contrast agents.⁵⁴ A clear increase in MR signal was observed in the aortic wall of apoE^{-/-} mice *in vivo* 24 hours after injection of the paramagnetic Au-HDL and QD-HDL, while a clear decrease of MR intensity in the aortic wall was observed for the FeO-HDL (Figure 8). *Ex vivo* computed tomography (CT) and fluorescence imaging confirmed the results found with MR imaging: nanocrystal-core HDL is taken up into the aorta wall to a much greater extent than aspecific control nanoparticles. Immunofluorescence staining revealed these nanoparticles to accumulate in macrophage cells in the plaque.

Peptides that mimic apoAI can be used instead of the natural HDL apolipoprotein components to form rHDL contrast agents that target macrophages for MR imaging of atherosclerosis. In 2009 it was reported that two peptides have been explored for this purpose, 18A (an amphiphatic, α -helical peptide with 18 amino acid residues, whose

hydrophobic face binds to the acyl chains of the phospholipids) and 37pA (a similar 37 amino acid residue peptide).^{97,98} *In vitro* experiments with J774A.1 macrophages indicated that peptide-based rHDL nanoparticles could produce cholesterol efflux from these cells comparable to native HDL and were taken up in a saturable, specific fashion by these cells. Both 18A- and 37pA-based rHDL contrast agents produced enhancements of about 90% in MR imaging of atherosclerotic plaques of apoE^{-/-} mice by specifically targeting macrophages.

QUANTUM DOTS

Quantum dots (QDs) are colloidal nanocrystals with attractive fluorescence features, such as excellent photostability, high molar extinction coefficient, and narrow and tunable emission spectrum.⁹⁹ As a consequence, QDs have been used in a variety of applications such as fluorescence imaging, immunofluorescence, and fluorescence microscopy.¹⁰⁰ QDs have been adapted for use as MR/optical contrast agents by incorporating them into the core of paramagnetic micelles,¹⁰¹ which were composed of CdSe/ZnS core/shell QDs coated with a mixture of paramagnetic Gd-chelate lipids and PEG phospholipids. Via functionalization with cyclic RGD peptides, these paramagnetic micellular QDs have potential for imaging angiogenesis in atherosclerotic plaques by targeting $\alpha_v\beta_3$ integrin.¹⁰¹ These micellular QDs have also been conjugated with MSR CD204-specific antibodies for molecular imaging of macrophages in atherosclerosis.⁶⁸ These CD204-specific micellular QDs caused pronounced signal enhancement for MR imaging of aortic vessel wall in apoE^{-/-} mice. The QDs in the micelles allowed fluorescence microscopy and optical imaging of the excised aorta, which identified the regions with high macrophage content under ultraviolet (UV) illumination.⁶⁸

Paramagnetic QDs with Gd chelates have also been utilized as multimodal contrast agents for noninvasive *in vivo* MR imaging of angiogenesis in a mouse model of myocardial infarction. The QDs were functionalized with cyclic NGR peptides (cNGR-pQDs), which are specific for CD13, an aminopeptidase that is strongly upregulated during myocardial angiogenesis. Injection of cNGR-pQDs resulted in strong negative contrast of MR images mainly in the infarcted myocardium, although paramagnetic agents usually result in positive contrast (Figure 9).¹⁰²

DISCUSSION

In this review, we have highlighted several nanoparticle platforms for molecular imaging of cardiovascular diseases. Each system has its own advantages and disadvantages in terms of ease of synthesis, toxicity, payload, and biodistribution.

Among the nanoparticle platforms discussed, lipid-based systems, such as micelles, liposomes, and HDL, are relatively easy to synthesize. Iron oxide nanoparticles are of interest since they can be degraded by the liver and therefore have low toxicity and exhibit good biocompatibility.¹⁰³ Gd chelates are, however, linked with nephrogenic systemic fibrosis (NSF) in patients with renal disease.¹⁰⁴ Many QD formulations are toxic because of the leakage of the heavy metal element Cd, which can partially be reduced by appropriate surface coatings.¹⁰⁵ Large particles in general have a higher payload than small particles,

which improves their detectability. Small particles, however, can penetrate deeper into tissue, which offers advantages for targeting extravascular biomarkers in cardiovascular diseases.⁴⁵

Molecular imaging of cardiovascular disease faces several challenges as compared to other pathologies, such as cancer. First, as atherosclerosis is a systemic disease it requires investigators to focus on different structures, which may include the carotids, the aortic root and arch, the abdominal aorta, the renal arteries, the aortic bifurcation and femoral arteries. Moreover, movement, flow effects, the beating of the heart, as well as the small size of vessels hamper molecular imaging sensitivity. Therefore, nanoparticles used for cardiovascular imaging usually need to be designed to allow their detection with superb sensitivity and should strongly and very specifically bind to the targeted biomarker to enable sufficient accumulation of the contrast-generating material.

CONCLUSION

With the advances in nanoparticle contrast agents for cellular and molecular imaging, the roles for different factors in cardiovascular diseases can be investigated *in vivo* and be better understood. The multimodal capacity of nanoparticle contrast agents can enable visualization of both the anatomical morphology and pathological characteristics of cardiovascular disease at high resolution by combinations of MR with complimentary imaging techniques. The theranostic potential of nanoparticles may aid in the early detection of high-risk patients with cardiovascular diseases as well as in the advancement of therapy, and thus, it is hoped, will contribute to reductions in patient morbidity and mortality rates.

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FIGURE 1.

Schematic structures for several nanoparticles: iron oxide with surface coating, micelle, micelle with core of nanoparticles, liposome, discoidal high-density lipoprotein (HDL), spherical HDL, and oil-in-water emulsion. (Targeting molecules: ligands, peptides, and antibodies; apoA1: apolipoprotein A I; CE: cholesteryl ester; TG: triglyceride).

FIGURE 2.

Typical gradient echo (GRE) and GRE acquisition for superparamagnetic particles (GRASP) images of the rabbit aorta (red arrow) pre- and 24 h postinjection of fractionated Feridex or Feridex at 4.8 mg Fe/kg of bodyweight. (Reprinted with permission from Ref 42. Copyright 2008 John Wiley and Sons, Ltd).

FIGURE 3.

Representative *in vivo* magnetic resonance (MR) images of apoE^{-/-} heart (a) pre- and (b) 48 h postinjection of VCAM-1 targeting iron oxide nanoparticles (VINP-28). Dotted line shows the location of short-axis view for the insets (lower panel with color-coded signal intensity). (c) The location of VINP-28 under fluorescent microscopy was associated with (d) VCAM-1 expression in immunohistochemistry. (Reprinted with permission from Ref 48. Copyright 2006 American Heart Association, Inc.).

FIGURE 4.

Typical *in vivo* magnetic resonance (MR) images pre- and postinjection of macrophage-targeted immunomicelles (a and b), untargeted micelles (c), and Gd-DTPA (d) in apoE^{-/-} mice (insets are enlargements of the aortas). The right side of (a)–(d) shows H&E staining of the aorta at the identical anatomic level as the MR images from the same animal. (Reprinted with permission from Ref 9. Copyright 2007 National Academy of Sciences, USA).

FIGURE 5.

(a) Cryo-TEM image of bare liposomes. (b) 3D reconstruction of CNA35-functionalized liposomes from a series of TEM images. Red: lipid bilayer; Blue: individual CNA35 proteins. (c) T_1 -map of bovine collagen type I matrices treated with (1) buffer, (2) bare liposomes and (3) CNA35-functionalized liposomes. (d) Surface relaxation rate of bovine collagen type I showed a linear correlation with the molar fraction of Gd-DTPA-BSA in the CNA35-functionalized liposomes. (Reprinted with permission from Ref 72. Copyright 2009 John Wiley and Sons, Ltd).

FIGURE 6.

Typical *in vivo* ^{19}F magnetic resonance (MR) images of myocardial infarction after injection of perfluorocarbon emulsions. (a) ^1H and ^{19}F MR images at the same position of a mouse thorax recorded 4 days after ligation of the left anterior descending artery. ^{19}F signal is near the infarcted region (I) and at the location of surgery (T). (b) Sections of ^1H images superimposed with ^{19}F images (red) at same position acquired 1, 3, and 6 days after surgery. (Reprinted with permission from Ref 86. Copyright 2008 American Heart Association, Inc.).

FIGURE 7. Schematic illustration (left) and TEM image (middle) of colloidal iron oxide nanoparticles (CIONs), and a T₁-weighted magnetic resonance (MR) image of a human carotid endarterectomy specimen (right). (Reprinted with permission from Ref 53. Copyright 2009 American Chemical Society).

FIGURE 8.

Typical T₁-weighted magnetic resonance (MR) images of the apoE^{-/-} mouse aorta (a, b) pre- and (d, e) 24 h postinjection with either Au-HDL or QD-HDL. Arrows point to areas enhanced in the post images. T₂*-weighted images of an apoE^{-/-} mouse aorta (c) pre- and (f) 24 h postinjection with FeO-HDL. (g, h, i) Confocal microscopy images of the apoE^{-/-} mouse aortic sections. Red: nanocrystal HDL; Green: macrophages; Blue: nuclei. The arrowheads indicate colocalization of nanocrystal HDL with macrophages. (Reprinted with permission from Ref 54. Copyright 2008 American Chemical Society).

FIGURE 9.

Typical short-axis magnetic resonance (MR) images with an echo time (TE) of (a) 2.9 ms and (b) 6.0 ms for a myocardial infarction (MI) mouse injected with cNGR-pQDs (left), an MI mouse injected with unlabeled QDs (middle), and a sham-operated mouse injected with cNGR-pQDs (right). Arrowheads indicate the hypointense area for MI mouse injected with cNGR-pQDs. (c) Two-photon laser-scanning microscopy revealed that cNGR-pQDs were mainly in the (2) border zone and (3) infarct areas, but not in (1) remote myocardium.

Arrows indicate the colocalization of nanoparticles with vasculature. Red: quantum dots; Green: α -CD31-FITC. (Reprinted with permission from Ref 102. Copyright 2010 American Heart Association, Inc.).