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Targeted Probes for Cardiovascular MR Imaging

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Abstract

Background—Molecular magnetic resonance (MR) imaging plays an important role in studying molecular and cellular processes associated with heart disease. Targeted probes that recognize important biomarkers of atherosclerosis, apoptosis, necrosis, angiogenesis, thrombosis and inflammation have been developed.

Discussion—This review discusses properties of chemically different types of contrast agents including iron oxide nanoparticles, gadolinium based nanoparticles or micelles, discrete peptide conjugates and activatable probes. Numerous examples of contrast agents based on these approaches have been used in preclinical MR imaging of cardiovascular diseases. Clinical applications are still under investigation for some selected agents with highly promising initial results.

Conclusion—Molecular MR imaging shows great potential for the detection, characterization of a wide range of cardiovascular diseases and for monitoring response to therapy.

Keywords

Cardiovascular; molecular imaging; MRI; atherosclerosis; iron oxide nanoparticles; targeted peptides; smart probes; micelles; gadolinium

Background

Heart disease is the leading cause of mortality worldwide. MR imaging is widely used clinically to determine the extent of myocardial infarction or peripheral vascular disease and is playing an increasing role in characterizing atherosclerosis, cardiac hypertrophies, cardiac thrombosis, and congestive heart failure. MR imaging provides anatomical (e.g. quantifying the degree and extent of arterial narrowing), physiological (e.g. myocardial perfusion) and increasingly, molecular (e.g. protein overexpression) information.

Cardiovascular MRI is performed both with and without administration of a contrast agent. MRI without a contrast agent is the “gold standard” to determine myocardial thickening (or thinning) and to quantify myocardial wall motion abnormalities and ejection fraction. Contrast agents are paramagnetic complexes (typically Gd^{3+} -based) or superparamagnetic particles (typically iron oxides) that change the relaxation properties of water molecules that they encounter. Clinically, contrast agent usage is overwhelmingly dominated by small gadolinium (III)-based complexes that have a strong T1-shortening effect and serve to increase MR signal intensity on T1-weighted images [1]. Commercial extracellular gadolinium(III)-based contrast agents, such as $[Gd(DTPA)]^{2-}$, are used to measure blood flow to the myocardium by rapidly imaging the heart after contrast agent injection. After heart attack, regions of infarcted tissue show delayed enhancement after extracellular contrast agent injection [2]. This delayed or late

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gadolinium enhancement (LGE) enables MRI to differentiate the etiology of heart failure and to assess prognosis based on the location and extent of the LGE; this is currently one of the most clinically important uses of cardiac MRI. Recently, a compound termed gadofosveset (MS-325, Fig. 1) has been approved for blood vessel imaging [3]. This compound binds to serum albumin in blood, serving to localize the contrast agent to the blood vessels and while increasing its relaxivity [4,5]. Recent preclinical work with novel contrast agents has moved beyond anatomy and function to provide additional imaging information on the presence of specific proteins, cells, or enzymes. In the radiology field, this is termed “molecular imaging” [6].

In this brief review, we highlight recent advances in molecular imaging of cardiovascular disease. The review is structured around 4 different categories of molecular MR agents based on their chemical structure: superparamagnetic iron-oxide particles, discrete targeted peptides conjugated with gadolinium chelate(s), self-assembled gadolinium nanoparticles, and activatable “smart” probes. Each class of compounds is discussed along with benefits and drawbacks of each approach and examples are drawn from the cardiovascular imaging literature. This review is not meant to be exhaustive in scope. The interested reader is referred to several detailed articles for a more comprehensive review of cardiovascular imaging [7-10].

Iron oxide nanoparticles

Iron oxide nanoparticles are typically classified according to their size which impacts their magnetic and biological properties [11]. Ultrasmall particles of iron oxide (USPIO) sometimes termed monocrystalline iron oxide nanoparticles (MION) consist of a 2-3 nm single crystal iron oxide core surrounded by a coating to make them biocompatible. The coating is often dextran, citrate, or a polymer such as polyethylene glycol or polyvinyl alcohol [12,13]. The final construct has a size of 20-50 nm. Particles in the 50–200 nm range are termed small particles of iron oxide (SPIO) and contain multiple iron oxide crystals. Large particles of iron oxide (LPIO) range from 300–4000 nm. USPIO and SPIO are superparamagnetic, that is they become ferromagnetic in the presence of a strong magnetic field (e.g. an MR imager) but lose their magnetization in the absence of the field. All iron oxides are T2-contrast agents. They produce a large local magnetic susceptibility that dephases the spins of nearby water hydrogen nuclei resulting in signal loss on T2 or T2* weighted images. This relaxation mechanism does not depend on physical access to water molecules; it is a through space effect that decreases as (distance)⁻³. As a result these particles exhibit a “blooming” effect, creating contrast beyond the regions where they are localized. LPIO are typically too large to be administered intravenously and would cause embolic effects. These particles have a high magnetic susceptibility and have been used to label stem cells for cell tracking studies, including myocardial studies [14,15]. SPIO can be administered intravenously but are cleared rapidly via the reticuloendothelial system (RES) into the spleen and liver and there are approved SPIO for liver and spleen imaging. USPIO are small enough to evade the RES and typically have a long blood residency time (10's of hours) and are eventually cleared to the liver. This long blood half-life has caused most molecular imaging applications to focus on USPIO. USPIO also differ from larger particles in that they are capable of producing significant T1 relaxation at low fields (1.5T and lower) [11].

A common method for (U)SPIO synthesis is coprecipitation of ferrous and ferric salts in an alkaline medium. If this coprecipitation is done in the presence of a surface complexing agent like dextran, then the dextran will adsorb to the surface of the particle and stabilize the colloid. This results in a fairly polydisperse mixture, which can then be fractionated using sizing columns and/or magnetic separation where filtration is performed in the presence of a magnetic field. Other synthetic methods have been reviewed [11,16]. To functionalize the particles for

targeting, it is necessary to append reactive functional groups to the surface. The dextran coating can be modified by amination. A scheme representing the amination of the dextran coating and the conjugation with targeting ligands (TL) and fluorescent dyes is given in Fig. 2. Amination renders the nanoparticles easily modifiable by activated esters or isothiocyanates of ligands such as antibodies, peptides etc. that can readily be attached to the nanoparticles for targeting purposes. The dextran coating can also be modified with carboxylate groups but these need to be activated before further modification with targeting ligands.

Iron oxides exhibit high T2 relaxivity ($r_2 = \Delta(1/T_2)/[Fe]$) and this permits detection of sparsely expressed targets present at nanomolar concentration. In preclinical in vivo cardiovascular applications, USPIO have been used to image macrophages associated with atherosclerotic plaque [8,17], surface receptors such as vascular cell adhesion molecule-1 (VCAM-1) [8] and E-selectin [18], thrombosis components such as fibrin [19] and activated platelets [20], and apoptosis [21]. Micron-sized nanoparticles have also been synthesized and used to target surface receptors such as VCAM-1 and P-selectin [22] as well as activated platelets via a conjugated antibody for the glycoprotein II_b/III_a receptor [23].

Imaging macrophages associated with atherosclerotic plaque

Atherosclerotic plaque rupture and subsequent thrombosis can result in unstable angina, myocardial infarction, or embolic stroke. Atherosclerosis is a progressive disease characterized by the thickening of the vessel wall. The disease is asymptomatic in its early stages; however, symptoms from stenosis (narrowing) of the lumen occur in the late stages due to the gradual growth of the plaque or the rapid rupture of a plaque resulting in thrombosis. Classically, narrowing of the coronary artery was considered the cause of most acute coronary syndromes such as unstable angina and myocardial infarction. However, it is now well established that the majority of vulnerable plaques (i.e. those at risk for rupture) do not restrict flow but pose a greater risk of rupture and subsequent thrombosis [24-26]. Plaques differ in their composition depending on the degree of disease progression. It is generally believed that collagen-rich plaques with low lipid content are less likely to rupture than plaques that have a large lipid component, a thin fibrous (collagen) cap, and significant macrophage activity. Currently there are no clinically established methods to non-invasively differentiate between stable and vulnerable plaques and to identify plaques prone to rupture. As a result, there has been much activity in this field.

Macrophages are an essential component of the inflammatory response governing atherosclerosis and are involved in lesion formation, disease progression and plaque disruption [27]. Given their broad role in atherosclerosis, macrophages, particularly activated macrophages are being recognized as a target for therapeutic intervention [28]. USPIO are known to be passively taken up by macrophages as part of the obligate response of phagocytes encountering foreign nanoparticles. Several investigators have successfully used iron-oxide nanoparticles to image plaque macrophages in animal models of atherosclerosis such as the hyperlipidemic rabbits [17]. Initial studies suggested that carotid plaque inflammation could be imaged using iron oxide nanoparticles in humans [29,30]. Recently, the feasibility of imaging macrophage content on the basis of iron oxide accumulation has been confirmed. These studies have also given insights into the mechanism of nanoparticle uptake and the optimal strategy to image them [17,31].

The extent of passive targeting can be altered by modifying the surface of the nanoparticles [32], and nanoparticle constructs with a range of binding affinities for activated macrophages have been synthesized. USPIO also accumulate in inflamed endothelial and smooth muscle cells in the plaque but to a significantly lesser degree. Thus the uptake of these nanoparticles is predominantly a marker of plaque macrophages and more accurately a marker of composite plaque inflammation.

The detection of iron oxide nanoparticles using MRI is traditionally performed with T2*-weighted gradient echo sequences. These sequences generate negative contrast in the vicinity of the nanoparticles, which creates detectable signal in the parenchymal organs such as the myocardium, pancreas and liver [21]. However, imaging of blood vessel walls is much more complex. The structures surrounding the vessel wall in the thorax and neck generally contain air and result in a background that is unsuitable for negative contrast. However recently pulse sequences to generate positive contrast from iron oxide particles have been developed and used successfully to image stem cells [33], macrophage infiltration in the infarcted myocardium [34] and atherosclerotic plaques.

Imaging of cell surface receptors in atherosclerosis

The expression of endothelial adhesion molecules such as VCAM-1 happens early on in the atherosclerotic cascade and thus, their detection is significant for early diagnosis of the disease. Several generations of VCAM-1 targeted magnetofluorescent contrast agents, based on CLIO-Cy5.5 nanoparticles, have been characterized and used for in vivo imaging. These are USPIO that have their surface cross-linked and aminated (termed cross linked iron oxide = CLIO) and then derivatized with the near IR carbocyanine dye Cy5.5. These CLIO-Cy5.5 nanoparticles provide both an MRI and fluorescent readout. The fluorescence is especially helpful for validation studies to identify particle distribution within the plaque ex vivo using fluorescence microscopy and for correlation with other plaque components. The first generation of these contrast agents had a VCAM-1 targeted antibody as appendage [35]. The second generation used a cyclic peptide to bind VCAM-1 [36] but the most recent version employs a phage-derived linear peptide with superior target affinity [8]. A scheme of the synthesis of this VCAM-1 targeted iron oxide nanoparticles is given in Fig. 3. The aminated iron oxide nanoparticles were conjugated with a Cy5.5 molecule by reacting the particles with an activated NHS ester of Cy5.5. Subsequently, some of the amine groups were reacted with activated iodoacetic acid (succinimidyl iodoacetate) and pendant iodides were introduced on the surface. These iodide groups readily reacted with the cysteine group on the C-terminus of the VCAM-1 targeted peptide. The surface of the product nanoparticle is adorned with an average of 20 peptide molecules, thereby increasing the affinity to VCAM-1 substantially. Fluorescence microscopy after probe injection showed that the accumulation of the agent in plaques is due to specific binding to VCAM-1 and not due to macrophage uptake of the nanoparticles. This study also indicated that a blood half-life of 1 to 3 h is optimal for a contrast agent to target an endothelial surface receptor because it favors ligand-mediated binding to the target with decreased nonspecific uptake of the probe.

In vivo imaging of VCAM-1 expression was studied in ApoE^{-/-} mice fed a high-cholesterol diet. Apolipoprotein E (ApoE) is a class of lipoproteins essential for the normal catabolism of triglyceride-rich lipoprotein constituents. Lipoproteins package cholesterol and other fats and carry them to the bloodstream. These lipoproteins also remove excess cholesterol from the bloodstream and transport it to the liver for processing. It is essential to maintain normal levels of cholesterol to prevent cardiovascular diseases. Since the ApoE^{-/-} mice have high levels of cholesterol they are more prone to cardiovascular diseases such as atherosclerosis. These mice develop atheromatous plaques along their aortas. Significant negative contrast was observed in the aortic wall consistent with VCAM-1 expression and accumulation of VCAM-1-targeted probe. Ex vivo fluorescence imaging and histology confirmed uptake of the probe in regions of the aortic root with plaques and high VCAM-1 expression. In addition, reduced uptake was observed in mice treated with statins to decrease VCAM-1 expression [8]. Thus, a VCAM-1-sensing probe provides adequate sensitivity and dynamic range to detect treatment effects on an important biomarker of plaque vulnerability.

Imaging cardiomyocyte apoptosis and necrosis

Cardiomyocyte apoptosis is implicated in numerous heart diseases including ischemia/reperfusion injury, heart failure, atherosclerosis, myocarditis and transplant rejection [37]. Apoptotic cells in all organs express the membrane phospholipid phosphatidylserine, on the outer surface of the cell membrane early on in the apoptotic process. This phospholipid binds to several proteins including Annexin V. A probe consisting of Annexin V conjugated to CLIO-Cy5.5 was synthesized (Fig. 4). CLIO-Cy5.5 was prepared in the same manner as in Fig. 3. The nanoparticles were reacted with N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP) and a pendant disulfide linkage was introduced. This disulfide linkage can further be exchanged with another molecule containing a thiol group. Annexin V with a terminal thiol group was reacted with the pendant disulfide linkage on the surface of the nanoparticle to produce a phosphatidylserine-targeted nanoparticle. Cardiomyocyte apoptosis was studied *in vivo* with Annexin V- CLIO-Cy5.5 in an acute reperfusion injury mouse model using MRI [21]. Loss of MR signal intensity due to probe accumulation was observed in the injured part of the myocardium, whereas no significant changes were observed in the signal intensity with the unlabeled probe. *Ex vivo* fluorescence imaging also confirmed the *in vivo* findings. Thus, annexin V conjugated nanoparticles could perform as a sensitive indicator of apoptosis in heart disease.

Cardiomyocyte necrosis has also been imaged *ex vivo* in the rat heart using MRI [38]. The probe used was MION conjugated to an antimyosin antibody. The simultaneous use of the apoptosis-sensing probe and necrosis-detecting probe could provide insight into the pathogenesis of cell death during myocardial injury, transplant rejection and myocarditis.

Discrete targeted peptide Gd-chelate conjugates

Gadolinium (Gd) based probes offer the benefit of positive (T1) contrast as compared to the negative contrast observed with iron oxide particles. Discrete small or medium-sized molecules (<10 kDa) comprising a targeting vector (e.g. peptide) and one or more Gd chelates (Fig. 5a) is one such approach. Gadolinium chelates used are often derivatives of the acyclic multidentate ligand GdDTPA or macrocyclic GdDOTA (Fig. 1). Recently a rare, but potentially lethal, gadolinium associated toxicity termed nephrogenic systemic fibrosis has demonstrated the need for thermodynamically stable, kinetically inert gadolinium complexes [39-41]. Here, the macrocyclic complexes based on GdDOTA are preferred.

A benefit to using small molecules is their pharmacokinetics. Unlike USPIO, these molecules are small enough to cross the endothelial barrier into the interstitial space of tissue opening up new areas to target. They generally have fast uptake at the target, fast blood clearance and fast renal excretion resulting in high target:background signal. Since these are small discrete molecules, synthesis is reproducible, unlike nanoparticles, which exist as distributions. On the other hand, the lower gadolinium payload means that they are less sensitive and are not adequate for imaging molecular targets that are present in low concentrations. The sensitivity issue can be appreciated by inspection of equation 1 which relates the effect of contrast agent on the relaxation rate (1/T1) of tissue, for a homogeneous sample:

$$1/T1 = 1/T1_0 + r_1[Gd] \quad (1)$$

1/T1₀ is the inherent tissue relaxation rate, for heart muscle 1/T1₀ is ~0.8 s⁻¹ at 1.5T. In order to induce detectable signal change, the relaxivity concentration product (r₁[Gd]) should be at least 10% of this inherent rate or 0.08 s⁻¹. The relaxivity of gadolinium complexes at 1.5T is compound dependent but is in the 4–40 mM⁻¹s⁻¹ range, so [Gd] required for minimal detection

is in the 2–20 μM range ($1 \mu\text{M} \sim 1 \text{ nmol Gd per g tissue}$). For more robust image contrast, concentration should be higher. For targeted imaging, one requires a target protein, receptor, etc expressed at the nmol/g concentration range and saturable binding. Most proteins and receptors are present at concentrations that are orders of magnitude lower.

For discrete targeted Gd-based agents the strategy has been to identify high concentration targets and to incorporate multiple high relaxivity Gd-chelates. MS-325, described above, was an early example of this strategy where the abundant plasma protein serum albumin ($\sim 600 \mu\text{M}$) was targeted. Fibrin, a key component of blood clots, is another micromolar level protein, as is type I collagen which is upregulated in fibrosis. Surprisingly, gadolinium-peptide conjugates targeted to matrix metalloproteinases (MMPs) [42,43] have been used in molecular imaging. The positive enhancement observed in those studies suggests either extremely high MMP levels or a significant non-specific enhancement effect.

Collagen targeted peptide conjugates for imaging fibrosis

Collagen is a highly abundant protein that forms the connective tissue in the body. In wound healing collagen synthesis is upregulated. The development of excess fibrous tissue, termed fibrosis, is characteristic of many cardiovascular diseases. After myocardial infarction (heart attack), fibrotic scars are generated as a result of wound healing. The contrast agent EP-3533, which contains a collagen-specific peptide as the targeting vector and 3 Gd chelates for signal enhancement was synthesized and evaluated in a mouse myocardial infarction model [44,45].

One of the major challenges of Gd-based MR contrast agents is their sensitivity and target affinity. MR signal can be increased by conjugating multiple chelates to the peptide but this may hamper the affinity of the peptide for the target. For fibrosis imaging, it is critical that the contrast agent has both high relaxivity to produce a detectable MR signal and high binding affinity and specificity for collagen [46]. The lead peptide in the collagen-targeted probe was identified from a phage display study. It was a disulfide bridged 17 amino acid cyclic peptide: GQWHCTTRFPHHGYCLYG. An alanine walk was performed to determine which amino acid side chains were critical for strong binding to collagen. Each amino acid, except for the cysteines, was systematically substituted with L-alanine and the binding affinity to type I collagen measured by an in vitro plate assay. The alanine walk demonstrated that the systematic replacement of tryptophan, proline, phenylalanine and the tyrosine closest to the C-terminus with alanine dramatically reduced the collagen binding affinity [46]. The amino acids whose side chains are critical for binding are represented in bold in Fig. 6. Replacement of the glutamine, histidines, arginine or leucine by alanine did not affect the affinity significantly. One strategy to introduce multiple Gd chelates in the contrast agent is to introduce lysines in the peptide sequence and conjugate the Gd chelate to the $\epsilon\text{-NH}_2$ of the lysine. For this particular sequence lysine can be introduced at the N-terminus and in the sequence where the alanine substitution was well tolerated. The amino acids Arg and Leu could each be replaced with Lys conjugated to GdDTPA via a thiourea linkage with no effect on collagen binding; these positions are shown italicized in Fig. 6. Several different peptides with 2 GdDTPA moieties at the N-terminus were screened for their affinity to collagen. It was observed that modification of Arg to Tyr, Leu to Val, and C-terminal Gly to aromatic substitutions improved the affinity of peptide-bis chelate conjugates. As a result of this structure activity data, EP-3533 was synthesized (Fig 6). This compound had a higher affinity to collagen than the initial lead peptide and contained three GdDTPA chelates (one conjugated to a lysine in the cyclic portion of the peptide and two GdDTPA chelates at the N-terminus) for increased relaxivity.

EP-3533 binds reversibly to mouse type I collagen with a K_d of $1.8 \mu\text{M}$ and has a relaxivity of $56.2 \text{ mM}^{-1}\text{s}^{-1}$ at 0.47 T. In a mouse model of aged myocardial infarction, EP-3533 enhanced the infarct zone and provided persistent positive image contrast [44]. Histological studies indicated that areas stained for collagen correlated well with areas of prolonged enhancement

observed with EP-3533. Gd concentrations measured by inductively coupled plasma-mass spectrometry also demonstrated twofold higher concentration of Gd in the scarred myocardium as compared to the normal myocardium. To demonstrate specificity, an isomer with an L-cysteine replaced by a D-cysteine was prepared. This isomer had >100-fold lower affinity to collagen. In the same animal model it was shown that the non-binding isomer did not enhance the infarct zone [45]. Thus EP-3533 enabled in vivo imaging of fibrosis in a mouse infarct model.

Fibrin targeted peptide-conjugates for imaging thrombosis

Thrombosis plays a central role in cardiovascular diseases including myocardial infarction, pulmonary embolism and deep-vein thrombosis [47]. Rupture of an atheromatous plaque creates a thrombogenic surface and subsequent thrombosis of the vessel or embolism [24,25]. The presence of fibrin is indicative of stage VI plaque, the most advanced stage of the disease. Fibrin is a useful molecular imaging target for thrombosis since it is present in all thrombi (arterial and venous, chronic and acute) resulting in potentially high sensitivity of detection; but fibrin is not found in the vasculature outside of thrombi resulting in a potentially high specificity for disease.

Circulating fibrinogen is present at a concentration of 5-10 μM in the blood. When a blood vessel is damaged or an atherosclerotic plaque ruptures, cytokines are released that trigger the coagulation cascade. The enzyme thrombin, a serine protease, is activated and cleaves short peptides from the ends of fibrinogen, which results in an end-to-end polymerization of fibrin monomers. This fibrin polymer is further stabilized by the transglutaminase Factor XIII which crosslinks the fibrin polymers. The net effect is a fibrin mesh, which helps to stop blood flow and results in localized fibrin monomer concentration in the 10-100 μM range. This high concentration is adequate to produce sufficient enhancement with MRI using targeted small molecules. Botnar et al. reported a short fibrin specific peptide that contained four GdDTPA units (termed EP-1873) and was used to identify thrombi in a rabbit model of plaque rupture [48]. This probe was further optimized to give EP-2104R, with a similar peptide sequence but containing the more substitutionally inert GdDOTA chelates. A scheme of the synthesis of EP-2104R is given in Fig. 7. EP-2104R was synthesized using a mixed solid and solution phase synthesis. The peptide was built on 2-chlorotrityl resin. First a xylylenediamine was conjugated to the resin such that on cleavage of the peptide from the resin, the C-terminus also has a pendant amino group. The rest of the amino acids were coupled by standard Fmoc solid phase synthesis. At the N-terminus, a diaminobutyric acid (Dab) group was added on solid phase to provide a di-amino linkage. The peptide was cleaved from the resin with TFA keeping the amino acids still protected. In solution phase, a second Dab molecule was conjugated to the C-terminus. All the amino acids were deprotected and the peptide was cyclized. Activated DOTAGA molecules were reacted to the four amino groups, two at each terminus. Gd was then chelated to all the DOTAGA molecules to prepare EP-2104R. The four Gd chelates, two at each terminus of the peptide enhance the metabolic stability of the peptide. EP-2104R binds equally to two sites of human fibrin ($K_d = 1.7 \mu\text{M}$) [49]. The binding is 100 times more specific for fibrin as compared to fibrinogen and 1000 times more specific as compared to serum albumin. The relaxivity of EP-2104R at 1.4 T and 37 $^{\circ}\text{C}$ ($71.4 \text{ mM}^{-1}\text{s}^{-1}$ per molecule) was about 25 times higher as compared to Gd-DOTA under the same conditions [49].

After evaluation in a number of preclinical rabbit [50] and swine models [51-55], EP-2104R was used for imaging in humans. EP-2104R was evaluated for thrombus detection in six regions including the deep veins of the legs, pulmonary arteries, carotid arteries, aortic arch, left ventricle and the atria of the heart [56]. Fig. 8 shows thrombus enhancement post-injection of the probe in the thoracic aorta on an inversion recovery black-blood gradient echo image.

Contrast enhanced CT in this subject demonstrated a filling defect consistent with the thrombus in the aortic wall.

Nair et al. described a different class of cyclic peptide that also targeted fibrin [57]. They synthesized a compact fragment containing 4 GdDTPA moieties and two hydroxylamine groups for conjugation. This fragment could be coupled in water to the N-terminus of a peptide containing an N-terminal serine that had been oxidized to the aldehyde resulting in a stable oxime bond. This approach gave contrast agents with one or two peptides for targeting coupled to 4 Gd moieties for signal enhancement. The bis(peptide) construct had 5-fold higher affinity than the mono-peptide analog, and both displayed high relaxivity of $>20 \text{ mM}^{-1}\text{s}^{-1}$ per Gd (>80 per molecule at 37°C , 1.5T) when bound to fibrin compared to ca. $4 \text{ mM}^{-1}\text{s}^{-1}$ for [Gd(DTPA)]²⁻ itself.

Self-assembled Gd-containing nanoparticles

The discrete gadolinium-peptide conjugates require targets present at micromolar concentrations. To access targets present at lower concentration, signal amplification strategies are required. One approach is to use nanoparticles to deliver a high payload of paramagnetic ions as in the case of the iron oxides discussed above. Similarly Gd-based nanoparticles have been employed to provide positive T1-contrast. Most often, a self-assembly approach is used to create emulsions, micelles or liposomes containing 10's to 1000's of Gd chelates, thus amplifying the per particle relaxivity. These particles can be appended with target-specific antibodies or peptides to achieve target recognition. A schematic representation of micelles and liposomes is shown in Fig. 5b-c. Micelles are generally 5-30 nm in size but unilamellar liposomes are somewhat bigger, about 50 nm. Stable emulsions can be larger still. Micelles are often formed using lipids with large head groups such as PEG-lipid where poly (ethyleneglycol) (PEG) polymers are conjugated to a phosphate moiety of a lipid such as distearoyl-phosphoethanolamine (DSPE). Two lipid molecules that are often employed as building blocks for creating micelles are given in Fig. 5d-e. Liposomes are typically obtained from lipid mixtures containing the dual chain phospholipids which provide liposomes their stability, and less than 7% PEG-lipid. Perfluorocarbon emulsions are typically composed of 20% of perfluorocarbon such as perfluoro-15-crown-5 or perfluorooctylbromide and 2% of other lipids such as safflower oil, 2% of surfactant comixture containing 30 mol% of GdDTPA-bis-oleate, 60 mol% lecithin and 10 mol% cholesterol, 1.7% glycerin and water [58].

Several different types of Gd(III) containing amphiphiles have been incorporated in these nanoparticles to impart paramagnetic character to them. Typically there are two types of Gd amphiphiles, those where the hydrophilic part solely consists of the Gd chelate conjugated to fatty acyl chains and those where the Gd chelate is conjugated to the hydrophilic part of an existing amphiphile such as phosphoethanolamine (PE). An example of the first type is GdDTPA-bistrearylamine (GdDTPA-BSA) and for the second type is GdDOTA-DSPE (Fig. 9). Gd chelates that have been included in paramagnetic nanoparticles include GdDTPA, and mono- or bis(amide) derivatives of GdDTPA, e.g. GdDTPA-BSA; recently macrocyclic chelates based on monoamides of GdDOTA have been used (Fig. 1). Due to the prolonged blood circulation times for these nanoparticles, it is critical that the Gd complexes that are incorporated in them be sufficiently stable so that Gd is not released by either transmetallation or decomposition of the complex. Macrocyclic chelators, which are more inert to Gd dissociation and transmetallation are preferable as compared to the acyclic chelators.

There is a rich liposome literature from the drug delivery field that can be consulted to influence particle stability and distribution. For instance, incorporation of PEG groups often results in "stealth" particles that are able to evade the RES and have extended blood half-lives of hours to days. In general, most of the reported constructs show slow blood clearance and optimal

imaging times may be 24 or 48 hrs post injection to minimize vascular background signal. Micelles are smaller than liposomes and have some potential to extravasate beyond the endothelial wall and access targets outside the vasculature that would not be possible for liposomes. Self-assembled Gd nanoparticles have been used to target atherosclerotic plaque components such as type I collagen [59], macrophages [60] and integrins involved in plaque angiogenesis [61,62].

Targeting macrophages and oxidized LDL using Gd nanoparticles

Fayad and coworkers have used nanoparticles 15-20 nm in diameter and built from mixed micelles prepared from phospholipids, palmitoylcholine phosphatidylcholine (POPC), 1,2-dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-7-nitro-2-1,3-benzoxadiazol-4-yl (DPPE-NBD), 1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine-N-biotinyl (DPPE-Biotin). The structure of POPC and DPPE is shown in Fig. 5d-e. Initially, using untargeted micelles, they observed aortic vessel wall enhancement in ApoE^{-/-} mice but not in wild-type mice [63]. Using immunomicelles, which incorporate an antibody for the macrophage scavenger receptor, they achieved an even bigger (79%) enhancement of the aortic wall in ApoE^{-/-} mice [60]. Ex vivo fluorescence microscopy showed colocalization of the fluorescent immunomicelles with the macrophages in the plaques. This study suggests that immunomicelles may aid in the detection of high macrophage content, which is associated with vulnerable plaques.

Fayad and coworkers have also targeted oxidized low-density lipoprotein (LDL) by using micelles containing Gd, rhodamine and antibodies that bind to unique oxidation-specific epitopes of LDL [64]. These micelles were built using PEG-DSPE, PEG-maleimide-DSPE and GdDTPA-BSA. The controls in this study were micelles without antibodies or with nonspecific immunoglobulin G (IgG). In ApoE^{-/-} mice, MR signal enhancement observed at the arterial wall was 125% to 231% as compared to the adjacent muscle at 72 and 96 hours post injection. IgG micelles and untargeted micelles showed only minimal signal enhancement (15% to 20%). Confocal microscopy revealed that oxidized LDL-targeted micelles accumulated within the macrophages in the atherosclerotic lesions.

Gadofluorines

Gadofluorine (or Gadofluorine-M) is a Gd-bearing amphiphile, which makes micelles 5-6 nm in diameter [65]. The molecule consists of a C8 perfluoroalkyl tail as the hydrophobic part and a macrocyclic Gd-chelate as the hydrophilic part as shown in Fig. 1. These agents have been used successfully to image atherosclerotic plaques [65-69]. Enhancement was observed in the aortic wall of Watanabe heritable hyperlipidemic rabbits post injection of Gadofluorine and not with [Gd(DTPA)]²⁻ [69]. No enhancement was observed on the vessel wall of the control group (New Zealand white rabbits). Ex vivo analysis showed that the area of enhancement on the vessel walls observed by MRI correlated with the area of plaques stained with Sudan red.

The underlying mechanism behind Gadofluorine-enhanced MRI of plaques has also been studied [65]. Derivatives of Gadofluorine, fluorescently labeled and radioactively labeled, were prepared and allowed to bind blood serum and plaque components in vitro. It was observed that Gadofluorine binds to albumin in vitro and it does not form micelles in presence of albumin. The binding affinity of Gadofluorine to albumin is similar to the binding affinity to extracellular matrix (ECM) components of the plaque such as collagen, proteoglycans and tenascin, all of which showed K_d ~2 μ M for Gadofluorine binding. The self-assembly of Gadofluorine in water and its indiscriminate protein binding suggests its hydrophobic character. On the other hand, the K_d for binding to LDL was only 2 mM, suggesting the lipophobic properties of this compound. Based on these properties of Gadofluorine M, a mechanism of plaque uptake was suggested. It was proposed that Gadofluorine binds to albumin and leaks into the plaque with albumin. The exchange of albumin between plasma and plaque is high in the outer layers of

the plaque. Once the complex is in the plaque, the hydrophobic components of the ECM compete with albumin for Gadofluorine binding. In the deeper areas of the plaque, where albumin exchange is low but the ECM components are high, there is accumulation of Gadofluorine. In summary, Gadofluorine detects the increased leakage of albumin and the accumulation of hydrophobic ECM proteins, which are both associated with fibroatheromas. In combination with high-resolution MRI, Gadofluorine may be able to identify plaques susceptible to rupture.

Activatable “Smart” Probes

Thus far, to address the challenge of molecular MR imaging we have discussed nanoparticles which provide a large boost in signal or small molecules that bind highly concentrated target proteins. Another approach is to exploit some type of *in situ* amplification. Activatable probes are chemically engineered substrates that undergo a physicochemical change after interacting with their intended target. This physicochemical change usually results in a product, which has high target:background ratios and is readily detectable either using MRI or fluorescence. A general scheme for this process is given in Fig. 10a. The physicochemical change in the probe can result from enzymatic cleavage, pH change, cell internalization or interaction with ions, and is either an increase in the number of inner sphere water molecules, a polymerization of the substrate, or initiation of protein binding, all resulting in higher relaxivities. Activatable probes that are based on enzyme activity of myeloperoxidase [70,71], tyrosinase [72], blood coagulation factor XIII [73], thrombin-activatable fibrinolysis inhibitor (TAFI) [74] and galactosidase [75] have been used in molecular MR imaging. A specific example of a contrast agent activated by TAFI is shown in Fig. 10b. A Gd^{3+} prodrug complex with poor affinity for human serum albumin (HSA) and concomitant low relaxivity is transformed by the TAFI enzyme to a species with stronger affinity to HSA and higher relaxivity (Fig. 10b). The relaxivity in HSA of the prodrug increased from 9.8 to 26.5 $mM^{-1}s^{-1}$ upon activation by TAFI.

Myeloperoxidase targeting

Advanced human atherosclerotic plaques contain neutrophils and phagocytes that actively express and secrete the heme-containing enzyme myeloperoxidase (MPO) [76,77]. A study in more than 600 patients established that a single measurement of MPO in the plasma could predict the risk of adverse cardiac events for the subsequent 6 months [78]. MPO consumes hydrogen peroxide and produces hypochlorite that contributes in the erosion and rupture of plaques. MPO also generates other highly reactive molecular species such as tyrosyl radicals and aldehydes that participate in the covalent modification of LDL to an atherogenic form. These products may also inactivate high-density lipoproteins and activate MMPs, consequently causing endothelial cell apoptosis and tissue factor release [79,80].

Chen et al. chemically engineered a paramagnetic electron donor compound that rapidly oxidizes and polymerizes in presence of MPO [71]. They covalently conjugated GdDOTA via a monoamide linkage to serotonin (3-(2-aminoethyl)-5-hydroxyindole, 5-HT), and this compound efficiently polymerizes in presence of human neutrophil MPO with a 70-100% increase in proton relaxivity. Using this probe, they were able to detect MPO activity in enzyme solutions and in a model tissue system. These studies suggested that activatable MR probes could be used to detect MPO activity. A second generation of these MPO-targeted activatable agents was developed where GdDTPA was conjugated to two 5-HT groups via amide linkages [81-83]. The structure of the compound is shown in Fig. 1 and it is termed GdDTPA-bis-5-HT. This probe was used to image atherosclerotic plaques in the thoracic aorta [70]. Focal areas of increased contrast were observed in the diseased wall but not in the normal wall after probe injection. These areas colocalized and correlated with MPO-rich areas infiltrated by macrophages on histopathological evaluations. This study shows that inflammation in plaques can be detected by examining macrophage function and activity of an effector enzyme.

In another study, transgenic mice were used to investigate the specificity of GdDTPA-bis-5-HT for MPO activity [84]. They used homozygous MPO^{-/-} mice, heterozygous MPO^{+/-} mice and wildtype MPO^{+/+} mice in the study and observed decrease in signal enhancement in both MPO^{-/-} mice and MPO^{+/-} mice as compared to the wildtype mice. Moreover, the enhancement in case of MPO^{-/-} mice was even lower than the MPO^{+/-} mice. The areas of signal enhancement on MRI correlated well with MPO rich areas by immunoreactive staining and histology, thus reemphasizing the specificity of the probe. The washout kinetics of GdDTPA-bis-5-HT from acutely infarcted myocardium was also investigated and compared to that of GdDTPA [84]. It was observed that the contrast to noise ratios (CNR) for GdDTPA peaked at 10 min and for GdDTPA-bis-5-HT at 60 min. Furthermore, the CNR for GdDTPA-bis-5-HT were significantly higher than GdDTPA. This group also studied the effect of the anti-inflammatory drug atorvastatin in an ischemia-reperfusion injury model using in vivo MR imaging with this probe. The signal enhancement 24 hours after the onset of reperfusion was significantly attenuated when atorvastatin was administered. This study suggests that GdDTPA-bis-5-HT may be able to assess MPO activity in vivo and may also help in non-invasively evaluating inflammation in ischemia.

Comparison of different strategies

There is no one ideal strategy for molecular MR contrast agents. Each has its pros and cons and the best approach to take will likely depend on the scientific or clinical question to be answered. Superparamagnetic iron oxide particles are primarily T2 contrast agents resulting in negative image contrast. These are generally potent relaxation agents and can cause detectable in vivo signal change at nanomolar particle concentrations. The susceptibility-based relaxation mechanism does not require direct (contact) access of the particle to water molecules and is thus effective even if the particle is compartmentalized in tissue. The blood half-life of the ultrasmall (<50 nm) nanoparticles can be quite long (hours to days). This can be useful in rodent studies where animals can be injected and imaged the next day when target:background is high, but it is less attractive for clinical translation because patients would need to be seen twice, once for injection and once for imaging. The negative contrast is effective if the surrounding tissue is bright on T2-weighted images as is the case in the myocardium; on the other hand these agents are less effective in the lungs and vessels in the thorax and neck where surrounding tissue is already dark. Because of their large size, target choice is mainly limited to the vasculature although these particles can be taken up by cells and there are applications in imaging macrophage and tumors. Size can also be an advantage in that it is possible to conjugate multiple targeting vectors to the surface to increase avidity for the target. Likewise it is possible to introduce additional functionality to alter pharmacokinetics (e.g. adding PEG groups) or an additional imaging reporter (e.g. fluorophore). Iron oxides are eventually trafficked to Kupffer cells in the liver where the particle is eventually broken down and the iron stored as ferritin. As with all nanoparticle approaches, the targeted SPIO exist as distributions and reproducible synthesis and characterization is a challenge for clinical development. As particles become more complex, there are more variables to control: size distribution, number of targeting groups per particle, number of unreacted functional groups on surface, etc.

Self-assembled gadolinium-based nanoparticles share many of the same positive and negative properties as iron oxide particles. A key difference is contrast mechanism where the Gd based agents primarily affect T1 and show positive contrast, although for this to occur the contrast agent must come into direct contact with water molecules. Typically it is much easier to identify areas of signal increase than areas of decreased signal. Size limits where these particles can go and it also impacts blood clearance and excretion. Unlike the SPIO where iron eventually becomes part of ferritin, there is no endogenous pool of gadolinium and this toxic ion must be

eliminated from the body. For clinical translation this must be addressed, as must questions of particle formulation stability and reproducibility.

The synthesis of the discrete Gd based peptide-conjugates is very reproducible and scalable. These conjugates are small enough to rapidly reach targets in the blood and interstitial space. They are also small enough to be renally eliminated thereby removing the gadolinium after the imaging study. However, they are limited to applications where the target is present at high concentrations. Moreover development of these compounds is often time consuming as there is a balance between increasing target affinity while increasing relaxivity and gadolinium content, all the while maintaining metabolic stability of the probe.

Activatable probes offer the potential of relatively simple small molecules, which are cost effective and reproducibly synthesized and small enough to be completely excreted after the imaging study. A major challenge is demonstrable efficacy. The MR signal will depend both on the probe concentration and its relaxivity. Diseased tissue may be more or less permeable than healthy tissue and non-specific concentration differences may result in signal changes that are independent of activation. For instance, commercial $[\text{Gd}(\text{DTPA})]^{2-}$ can be used to enhance atherosclerotic plaques in the coronary arteries via a completely passive mechanism [85]. Development of activatable probes will require an imaging paradigm that can distinguish activation from passive accumulation.

Conclusions

Molecular MRI has shown tremendous strides over the last decade. The large prevalence of cardiovascular disease in society and the morbidity and mortality associated with cardiovascular diseases continues to drive new diagnostics and tools for monitoring therapy. The direct accessibility of the cardiovascular system after intravenous administration overcomes one barrier, probe delivery, that is present in other diseases, e.g. the blood brain barrier in neurological disorders. One of the key challenges in this field has been in detection and characterization of vulnerable atherosclerotic plaques. Perhaps it is not surprising that the first molecular MR probe to advance to clinical trials is used for a cardiovascular indication [56].

The small dimension of the arterial wall, signal from the adjacent vessel, breathing artifacts and artifacts from the systolic expansion of the arterial wall continue to make molecular MRI a challenge. MRI is one of the most favorable techniques for imaging atherosclerosis due to its lack of ionizing radiation and high spatial resolution. The low sensitivity limitation of molecular MRI is being overcome by new contrast agents, which possess high relaxivities or have larger payloads of the contrast creating moieties. For imaging the much larger myocardium, there are fewer physical challenges and benefits may be expected in imaging infarction, hypertrophy, and cardiac thrombi.

Iron oxide nanoparticles have already proven their utility in detection of hepatic and lymph node metastases in humans [86]. They are safe for administration in humans and several preparations are clinically approved. Targeted iron oxide nanoparticles offer great potential, but to date none have advanced to human trials. Gd-based probes have also shown promise for imaging in humans. Initial studies with a fibrin-detecting peptide, EP-2104R, were successful in the detection of thrombi, including intracardiac, arterial and venous thrombi, in patients at 1.5 T and this compound displayed an excellent safety profile [56]. The use of larger constructs carrying Gd, such as Gd micelles and liposomes, is still under investigation. The concerns related to the long-term accumulation of Gd, especially using the Gd based agents such as micelles and Gd-based nanoparticles that deliver huge payloads, need to be addressed.

Future Perspectives

Molecular MRI has shown great promise in imaging of cardiovascular diseases. But new avenues are now being explored. MR probes are being investigated as vehicles for delivering therapeutics, multi-modal probes that use multiple imaging reporters are being utilized for detection of disease and integrated imaging systems that simultaneously image using two different modalities are being constructed and employed in imaging. Large constructs such as liposomes that carry 10-1000s of Gd ions have the potential to carry therapeutic loads as well. For instance, gadolinium(III) loaded liposomes targeted to $\alpha_v\beta_3$ integrin (involved in plaque angiogenesis) were used in detection of neovasculature in plaques [62]. On incorporation of the anti-angiogenic agent fumagilin into the probe, reduction in angiogenesis was observed which in turn decreased the uptake of the probe [87]. These constructs act both in diagnosis and therapy of the disease and are sometimes called theranostics. These theranostics may help in detecting the disease and administering and monitoring therapy.

Although molecular MRI is well suited to image many important processes, multimodal-imaging strategies are being actively pursued concurrently. Dual MR-fluorescence probes have been developed and allow direct detection of plaque components using fluorescence imaging techniques. The high spatial resolution of fluorescence microscopy enables the fluorescence reporters in the probe to confirm probe localization *ex vivo*, providing an important validation strategy. Recently a trimodal probe using PET, fluorescence and magnetic-detectable moieties was synthesized by conjugating ^{64}Cu to a magneto-fluorescent probe [13]. *In vivo* imaging of the plaque macrophages in the aortic roots of ApoE^{-/-} mice was successfully performed using MR and PET after injection of the trimodal probe. Similar type of multi-modal probes will probably be very significant in the future of molecular imaging of cardiovascular diseases.

Integrated MR-PET systems have been constructed for human imaging and will allow MR and PET imaging to be performed simultaneously, rather than sequentially as done with PET-CT [88-90]. These systems will likely play an important role in the clinical translation of molecular imaging.

Within MRI, newer strategies of molecular MR imaging are being developed using either heteronuclei such as ^{19}F [91] and hyperpolarized ^{13}C [92,93] or exchange of magnetization from one pool of protons to the other as is done in chemical exchange saturation transfer (CEST) [94].

^{19}F -MRI uses fluorinated compounds such as perfluorocarbon emulsions to produce contrast. ^{19}F is a suitable nucleus for MR due to its high magnetogyric ratio and high natural abundance. In addition, ^{19}F is not present in the body; hence the background in ^{19}F -MRI is very low. However this technique also has its limitations. ^{19}F -MRI has low sensitivity with a detection limit in the millimolar range, which is not suitable for detection of most *in vivo* targets (recall that conventional ^1H MRI detects water present at about 90 molar). So molecular imaging probes that can deliver very large numbers of ^{19}F per molecule will be required.

Hyperpolarized ^{13}C MRI uses a water-soluble ^{13}C -enriched compound with a long relaxation time for imaging. Typically the ^{13}C concentration of the injected compound is 0.3-0.5 M and it is hyperpolarized to 20-40%. A major benefit of hyperpolarized contrast media is the excellent sensitivity with no background (high signal-to-noise ratio). Challenges include the distribution and availability of the hyperpolarization equipment and imaging hardware compatibility for imaging nonhydrogen nuclei, which is not available on all clinical scanners. However, commercial polarizers are now available. Hyperpolarized ^{13}C contrast agents were used for magnetic resonance angiography in rats and swine [93]. Hyperpolarized ^{13}C MRI also offers the potential for investigating metabolic pathways. For instance, Merritt and colleagues

studied the metabolism of [1-¹³C]-pyruvate in a perfused rat heart. This field is likely to expand in the coming years.

CEST agents are based on the transfer of magnetization from a pool of exchangeable protons on the probe (e.g. amide N-H) to the free water protons [95]. CEST agents contain exchangeable protons with a resonance frequency well separated from that of the free water peak. Upon selective saturation of the exchangeable proton peak, a drop in the free water peak is observed due to transfer of saturated magnetization. Polypeptides and sugars are used as diamagnetic CEST agents. Paramagnetic lanthanide (Eu, Dy, Ho, Er, Tm, Yb) chelates are employed as paramagnetic CEST agents (PARACEST) because of their large chemical shifts and fast exchange rates [94]. CEST agents are attractive from the perspective of being “turned on” by the appropriate pulse sequence and also because of the potential for detecting multiple probes at once (provided the different probes have exchangeable hydrogens with different resonance frequencies) [94]. A major challenge for this class of compounds remains sensitivity; thus far it appears that detection limits are an order of magnitude or more higher than for gadolinium or iron oxide based probes.

Molecular MRI is already playing an important role in basic science investigation and development of novel pharmaceuticals and has the potential of becoming an important tool in the detection of cardiovascular diseases. Serial, noninvasive imaging is also contributing to preclinical development where repeated measurements can be made to assess the extent of disease and response to treatment. Molecular MRI provides complementary information to that provided by genomic and proteomic readouts. It will help to detect and follow the changes in atherosclerotic plaque composition in vivo and identify the most relevant markers of plaque instability, which is the major cause of heart diseases.

Executive Summary

- There are 4 different categories of molecular MR agents; superparamagnetic iron-oxide particles, discrete targeted peptides conjugated with gadolinium chelate(s), self-assembled gadolinium nanoparticles, and activatable “smart” probes.
- The superparamagnetic iron oxide nanoparticles are T2-contrast agents and produce negative contrast. They are prepared in variable sizes from 2-4000 nm and differ in their clearance properties. USPIO have been used to image macrophages associated with atherosclerotic plaque, surface receptors such as vascular cell adhesion molecule-1 (VCAM-1) and E-selectin, thrombosis components such as fibrin and activated platelets, and apoptosis.
- The discrete targeted peptides conjugated to Gd chelates are T1-contrast agents and produce positive contrast. These compounds are smaller than 10 kDa. They show fast uptake at the target and fast blood and renal clearance resulting in high target:background signal. Peptides-Gd conjugates have been used to target fibrin, an important marker of thrombosis and collagen, a protein which is upregulated in fibrosis.
- Self-assembled Gd constructs such as micelles or liposomes can carry large Gd payloads, 10s to 1000s of Gd³⁺ ions. They show slow blood clearance, and optimal imaging times may be 24 or 48 hrs post injection to minimize vascular background signal. Self-assembled Gd nanoparticles have been used to target atherosclerotic plaque components such as ECM proteins, macrophages and integrins involved in plaque angiogenesis.
- Activatable “smart” agents are chemically engineered substrates that undergo a physiochemical change as a result of enzymatic cleavage, pH change, cell

internalization or interaction with ions. The effect of this activation is either an increase in the number of inner sphere water molecules, a polymerization of the substrate, or initiation of protein binding, all resulting in higher relaxivities and high target:background signal. Activatable probes that are based on enzyme activity of myeloperoxidase, tyrosinase, blood coagulation factor XII, thrombin-activatable fibrinolysis inhibitor (TAFI) and galactosidase have been used in molecular MR imaging.

- There is no one ideal strategy for molecular imaging. Each has its benefits and limitations and the best approach to take will likely depend on the scientific or clinical question to be answered.
- The challenges in the field of molecular imaging include optimization of the relaxivity of the probe without compromising the binding affinity for a particular target, the toxicity concerns associated with the probes and the economic and regulatory hurdles for clinical translation.
- The future of molecular MR imaging will likely include the use of multi-modal probes, exploitation of integrated systems for imaging and combined diagnostic-therapeutic applications for the nanoparticle-based contrast agents. The contribution of other MRI strategies including MRI using nuclei such as ^{19}F and hyperpolarized ^{13}C , and chemical exchange saturation transfer (CEST) agents will likely grow in the coming years.

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Gd chelates

Figure 1.

Chemical structures of some Gd(III) containing contrast agents discussed in this review. These include acyclic chelates such as Gd-DTPA and GdDTPA-bis(methylamide) (Gd-DTPA-BMA); macrocyclic chelates such as Gd-DOTA and Gd-HPDO3A; the albumin binding agent MS-325; the micelle forming amphiphile Gadofluorine M; and activatable agents such as GdDTPA-bis-5HT.

Iron oxide nanoparticles

Figure 2. Schematic representation of the synthesis of targeted and fluorescently labeled superparamagnetic iron oxide nanoparticles. Coprecipitation of ferrous and ferric salts in an alkaline medium in the presence of a biocompatible surface complexing agent such as dextran gives a polydisperse mixture of nanoparticles that is fractionated using sizing columns and/or by magnetic separation. The dextran coating is then crosslinked and functionalized. These functionalized nanoparticles can be conjugated to targeting ligands and fluorescent reporters.

VCAM-1 targeting nanoparticles

Figure 3.

Synthesis of VCAM-1 targeting nanoparticles. Crosslinked iron oxide nanoparticles (CLIO) are first labeled with Cy5.5. The other amino groups on the surface of the nanoparticles are reacted with activated iodoacetic acid to create pendant iodides, which can then react with the thiol groups on the targeting peptide.

Synthesis of CLIO-Cy5.5-Annexin V

Figure 4.

Synthesis of CLIO-Cy5.5-Annexin V. CLIO-Cy5.5 was reacted with the SPDP linker which contains a disulfide linkage. The pyridine thiol in this disulfide bond can be exchanged with the cysteine thiol in Annexin V.

Gd based agents

Figure 5.

(a – c) Schematic representation of Gd based agents. (a) Targeting peptide/vector conjugated to Gd chelates; (b) Gd loaded micelles conjugated to targeting ligand and fluorescent label; (c) Gd loaded liposomes conjugated to targeting ligand and fluorescent label; (d, e) commonly used micelle/liposome constituents: (d) palmitoyl-oleoyl phosphatidylcholine (POPC); (e) 1,2-Dipalmitoyl-sn-glycero-3-phosphoethanolamine (DPPE).

EP-3533

Figure 6.

Structure of EP-3533. Amino acids are represented by their one-letter abbreviations. The targeting peptide is highlighted in gray. The amino acids whose side chains are critical for binding are shown in bold. The amino acid positions that can be replaced with lysine for conjugation of Gd chelates with no effect on binding are shown in italics.

Figure 7.
Mixed solid phase, solution phase synthesis of the fibrin targeted peptide-gadolinium chelate conjugate EP-2104R.

EP-2104R enhancement of thoracic aorta

Figure 8.

MRI of thrombus in the thoracic aorta post injection of EP-2104R. The thrombus appears bright on an inversion recovery black blood gradient echo sequence after EP-2104R injection. Arrow shows the localization of the clot. A corresponding multiplanar reconstruction from a contrast-enhanced CT indicates a filling defect consistent with the thrombus in the MR image. At the cranial end of the plaque a small calcification is also visible (arrowhead). (From Spuentrup et al. [56] with kind permission of Springer Science+Business Media).

Amphiphiles

Figure 9.

Examples of types of Gd micelle forming amphiphiles. (a) Amphiphiles where the hydrophilic part solely consists of the Gd chelate conjugated to fatty acyl chains. Structure shown is GdDTPA-bistrearylamine (GdDTPA-BSA). (b) Amphiphiles where the Gd chelate is conjugated to the hydrophilic part of an existing amphiphile such as phosphoethanolamine (PE). Structure shown is GdDOTA-distearoyl-phosphoethanolamine (GdDOTA-DSPE).

Figure 10.

Activatable probes. (a) A general schematic of the process of activation for “smart” probes. (b) A specific example of a “smart” probe activated by the thrombin-activatable fibrinolysis inhibitor (TAFI). A Gd^{3+} prodrug complex with poor affinity for human serum albumin (HSA) and concomitant low relaxivity is transformed by the TAFI enzyme to a species with stronger affinity to HSA and higher relaxivity. The relaxivity in HSA solution increased from 9.8 to 26.5 $\text{mM}^{-1}\text{s}^{-1}$ upon activation by TAFI.