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Strategies for Target-Specific Contrast Agents for Magnetic Resonance Imaging

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Abstract

This review describes recent research efforts focused on increasing the specificity of contrast agents for proton magnetic resonance imaging (MRI). Contrast agents play an indispensable role in MRI by enhancing the inherent contrast of images; however, the non-specific nature of current clinical contrast agents limits their usefulness. This limitation can be addressed by conjugating contrast agents or contrast-agent-loaded carriers—including polymers, nanoparticles, dendrimers, and liposomes—to molecules that bind to biological sites of interest. An alternative approach to conjugation is synthetically mimicking biological structures with metal complexes that are also contrast agents. In this review, we describe the advantages and limitations of these two targeting strategies with respect to translation from *in vitro* to *in vivo* imaging while focusing on advances from the last ten years.

Keywords

Contrast agents; gadolinium; magnetic resonance imaging; relaxivity; superparamagnetic iron oxide particles; target specificity

1. INTRODUCTION

Imaging techniques play a vital role in diagnostic medicine and medical research by aiding with the detection of diseases as well as the monitoring of disease progression and treatment. These imaging modalities include single photon emission computed tomography, positron emission tomography, ultrasound, magnetic resonance imaging (MRI), photoacoustic tomography, and fluorescence imaging, and reviews describing the advantages and limitations of each modality have been published elsewhere [1–6]. This review focuses on MRI, which is a powerful non-invasive imaging technique that does not use ionizing radiation and has the capability to generate three-dimensional images of deep tissue with good spatial resolution (25–100 μm) [6].

Conventional MRI images are produced by mapping either the relaxation rates or densities of the nuclear spins of water protons in a magnetic field. Differences in relaxation rates are due in part to the chemical composition of the surrounding environment; however, the inherent levels of signal intensity that result from the differences in relaxation rates of water protons are often not sufficient to produce satisfactory contrast. Therefore, chemicals known as contrast agents are used in MRI to catalytically accelerate the relaxation rate of water

CONFLICTS OF INTEREST

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protons to obtain contrast-enhanced images. Paramagnets including Gd^{III} , Mn^{II} , and Mn^{III} are referred to as “ T_1 agents” because of their influence on longitudinal (T_1) relaxation rates, and superparamagnetic iron oxide (SPIO) particles are referred to as “ T_2 agents” because of their influence on transverse (T_2) relaxation rates [7]. The ability of a contrast agent to influence contrast is measured in terms of relaxivity, and relaxivity changes in response to changes in physical parameters including magnetic field strength, temperature, and solvent composition. This dependence of relaxivity on measurement conditions often prevents a meaningful direct comparison of agents in different studies. Hence, in this review, we list the physical parameters reported for each example to avoid misinterpretation that could arise due to direct comparison of agents measured under different conditions (Table 1).

In current clinical MRI (1.5–3 T), Gd^{III} -based complexes are the most commonly used contrast agents, and these agents are non-specific; they do not accumulate in particular tissues or organs of interest. Non-specific contrast agents are useful in the imaging of pathologies like tumors, lesions, and inflammation because these agents aid in the differentiation of healthy and diseased tissues based on vascular volume, vascular perfusion, and vascular permeability [8]. While these non-specific contrast agents are used in ~35% of clinical scans [9], their utility in the early detection of disease and imaging of specific biological regions is limited. This limitation arises because diseases often do not display changes in vasculature, and if observable changes in vasculature occur, they are likely to occur in later stages of diseases. One method to address the limitation of non-specificity is the use of targeted contrast agents. Since the first descriptions of targeted contrast agents [10, 11], this topic has become a focus in the field of MRI and contrast agent research because of the potential to enable molecular imaging in addition to anatomical imaging. The importance of targeted contrast agents has been described in several recent reviews [8, 3 12–18]. These reviews discuss the development and properties of T_1 and T_2 agents and the use of different biomarkers as targets. In this review, we describe targeted contrast agents for proton MRI that were developed during the last ten years using two different synthetic strategies with a focus on agents that have been tested both *in vitro* and *in vivo* to emphasize limitations that must be overcome to enable the clinical use of targeted contrast agents.

The design of target-specific contrast agents involves two types of strategies: the most common strategy is to conjugate a contrast agent with an antibody, peptide, or a small organic molecule that enables interactions with a target. A second strategy is to transform a contrast agent directly into the targeting moiety by mimicking the structure of a molecule that naturally interacts with a desired target. The development of targeted contrast agents with these two strategies has led to successful targeting *in vitro*, and some agents have been successfully translated to enable *in vivo* visualization of targets. The remainder of this review is organized into these two strategies: conjugation and mimicking.

2. THE CONJUGATION STRATEGY

Direct conjugation of derivatives of clinically approved contrast agents to peptides, antibodies, or small organic molecules is a popular and relatively simple method of achieving target specificity. All directly conjugated contrast agents contain three parts: a targeting moiety, a contrast-enhancing unit, and a linker that connects the other two parts (Fig. 1). When linking the contrast-enhancing unit and targeting moiety, either metalate-then-conjugate or conjugate-then-metalate methods can be used [19], and conjugation generally results in the formation of biologically stable amides, ethers, thioesters, or triazoles. Low molecular weight (< 30 kDa) targeted contrast agents are desirable because their small size enables diffusion to small areas and facile clearance, but this type of targeted contrast agent faces the challenge of low sensitivity: with small Gd^{III} -based contrast agents, micromolar or greater concentrations are needed to achieve satisfactory contrast *in vivo* [20,

21]. Therefore, contrast agents that possess both high relaxivity and long circulation times are desirable to lower the dose of contrast agent. To increase sensitivity, targeting moieties and Gd^{III}-containing complexes are conjugated to macromolecules including dendrimers, polymers, proteins, liposomes, and nanoparticles. These agents obtain high relaxivity by slowing the molecular tumbling rate, by combining several contrast-enhancing units per targeting moiety, or both. In the following section, we describe the use of the conjugation strategy to synthesize targeted imaging agents using both a single contrast-enhancing unit (monomeric) and multiple contrast-enhancing units (multimeric), and we describe the selectivity and sensitivity of the resulting agents *in vitro* and *in vivo*.

2.1. Monomeric Target-specific Contrast Agents

Monomeric targeted contrast agents contain one contrast-enhancing unit per targeting moiety; therefore, to achieve satisfactory contrast enhancement with these agents, targets with high expression levels need to be selected. Examples of such highly expressed targets include biomarkers associated with tumors; consequently, many monomeric tumor-targeted contrast agents are reported. Conjugation of steroids with contrast agents enables targeting of receptors on tumor cells because hormone receptors are over-expressed by various tumors. 21-Hydroxyprogesterone and 17 β -estradiol are steroids that interact with progesterone receptors and estrogen receptors that are over-expressed by uterine, breast, ovarian, and prostate carcinomas. These two steroids were conjugated with Gd^{III}-containing complexes to produce monomeric contrast agents **1** and **2**, respectively (Fig. 2) [22, 23]. The relaxivity of **1** is 5.35 mM⁻¹s⁻¹ (1.41 T, 37 °C, and 1% dimethylsulfoxide in water), and the relaxivity of **2** is 6.8 mM⁻¹s⁻¹ (9.4 T and phosphate buffered saline (PBS)). Because these relaxivity values are similar to those of clinically used non-specific agents [20, 24], doses similar to those used for non-specific agents are needed to enable good contrast (0.15 mmol/kg for **1** and 0.075 mmol/kg for **2**). *In vivo* imaging of xenograft mice using intraperitoneal or subcutaneous injections of **1** at 7.05 T resulted in contrast enhancement of progesterone-receptor-positive tumors with respect to progesterone-receptor-negative tumors (Fig. 3). Furthermore, agent **1** labels tissues that express progesterone receptors including the uterus and ovaries [22]. Imaging of xenograft mice using **2** (tail-vein injection at 9.4 T) demonstrated the targeting efficiency of this agent toward estrogen-receptor-positive tumors by enhancing contrast relative to estrogen-receptor-negative tumors [23]. The success of these two agents in enhancing contrast in tumors relies on the over expression of receptors in tumors relative to normal tissues.

Integrins are other receptors targeted for imaging. Integrin-targeted contrast agents have the potential to enable the detection of angiogenesis and thrombosis. The cyclic peptide cRGD is a widely used targeting moiety for integrins [25–27]. An $\alpha_{IIb}\beta_3$ integrin-receptor-targeted agent reported by Fayad and co-workers to detect thrombosis demonstrates the importance of high receptor concentration to obtain good contrast enhancement with monomeric agents [25]. Their contrast agent, **3**, consists of cRGD conjugated to Gd^{III} 1,4,7,10-tetraazacyclododecane-*N,N',N'',N'''*-tetraacetic acid (DOTA). This agent has a relaxivity of 9 mM⁻¹s⁻¹ (1.41 T and 40 °C) and binds with the $\alpha_{IIb}\beta_3$ receptor as well as the $\alpha_v\beta_3$ receptor. *In vivo* studies with mouse models of thrombosis demonstrated the ability of **3** to increase contrast of activated platelets with a 0.1 mmol/kg dose injected in the tail-vein. The reason for the success of **3** as a thrombosis-targeted contrast agent is the high expression of the $\alpha_{IIb}\beta_3$ receptor (40,000 per platelet) in platelets relative to $\alpha_v\beta_3$ (~1,500 per platelet) [28]. When the expression level of a target is low, larger amounts of monomeric agents are needed to achieve contrast enhancement. For example, cRGD-conjugated contrast agent **4** (Fig. 2) was studied as a means to selectively image the $\alpha_v\beta_3$ integrin receptor that is over-expressed in hepatocellular carcinomas [27]. The relaxivity of contrast agent **4** is 7.4 mM⁻¹s⁻¹ (1.5 T and 25 °C), and *in vivo* imaging of H-ras12V transgenic mice bearing

hepatocellular carcinomas at 1.5 T (tail-vein injection of 1.43 mmol/kg of **4**) enhanced contrast of the tumor region. Tumor cells used in this study have a low concentration of $\alpha_v\beta_3$ receptors, and the contrast agent has a moderate specificity for these receptors; thus, the high dose used in this study was needed to saturate the receptors.

The integrin-receptor-targeted examples **3** and **4** demonstrate the importance of biomarker expression level for targeted imaging. However, *in vitro* success is often difficult to translate *in vivo* even with biomarkers expressed at high levels and high-affinity targeting moieties. For example, the estrogen-receptor-targeted contrast agent **5** (Fig. 2) reported by Degani and co-workers has a relaxivity of $4.7 \text{ mM}^{-1}\text{s}^{-1}$ (9.4 T and PBS). However, agent **5** (using same dose as **2**) was not able to produce significant enhancement *in vivo* despite a higher *in vitro* binding affinity with a binding inhibition constant, K_i of $0.13 \text{ }\mu\text{mol/L}$ for estrogen receptors than **2** ($K_i = 0.97 \text{ }\mu\text{mol/L}$) [23]. This discrepancy likely was due to the accumulation of **5** in muscle tissues causing a reduction in the effective dose for estrogen-receptor-positive tumors.

Further highlights of the inconsistencies that are often observed between *in vitro* and *in vivo* systems are exemplified by two studies that target the folate receptor, which is found in high concentrations in tumors. Folate-conjugated monomeric contrast agent **6** (Fig. 2) has a low relaxivity of $1.28 \text{ mM}^{-1}\text{s}^{-1}$ (4.7 T and aqueous solution) [29]. *In vitro* studies of **6** with human folate-receptor-positive ovarian carcinoma (IGROV-1) cell lines did not produce signal enhancement; however, inductively coupled plasma-mass spectrometry results with competitive binding experiments confirmed the uptake of **6** by the cells. Despite the negative imaging results of the *in vitro* studies, *in vivo* studies at the same field strength demonstrated increased contrast of tumors in xenograft mouse models using a micromolar dose of **6** delivered intravenously. Conversely, the folate-related agent **7** reported by Wang and co-workers containing a dimeric Gd^{III} chelate conjugated to folate has a high relaxivity of $21 \text{ mM}^{-1}\text{s}^{-1}$ per Gd^{III} ion (1.41 T, 37°C , and water) [30]. *In vitro* studies with IGROV-1 cells confirmed the uptake of **7** through folate receptors. While contrast agent **7** was unable to enhance contrast in tumors implanted in rat models *in vivo* at 2 T (0.03 mmol/kg dose), the relaxation rate in the tumor increased and ΔR_1 (the difference between the T_1 relaxation rates before and 1 h after contrast enhancement) was 0.214 s^{-1} for **7** relative to non-targeted agents (0.112 s^{-1}), suggesting that the agent was retained in the tumor. Hence, the poor enhancement obtained in this study likely is not related to the selectivity of the contrast agent.

Conjugating a high relaxivity agent to a targeting moiety often is advantageous because it leads to an increase in contrast with lower doses of agent. Recently, Yang and coworkers reported contrast agent **8** targeted to human epidermal growth factor receptor type 2 (HER2) (Fig. 2) using a designed protein chelator for Gd^{III} , and agent **8** has a high relaxivity ($21 \text{ mM}^{-1}\text{s}^{-1}$, 1.41 T, 37°C , and tris(hydroxymethyl)aminomethane (Tris) buffer at pH 7) [31]. The contrast agent is conjugated to a HER2 affibody as the targeting moiety, a fluorescent dye to enable tracking by fluorescence microscopy, and polyethylene glycol moieties to achieve biocompatibility. Agent **8** demonstrated increased *in vivo* enhancement of tumors in xenograft mice using a tail vein injection of 100 fold lower dose ($0.24 \text{ }\mu\text{mol}$ per mouse) than is used commonly with clinical contrast agents for routine MR imaging experiments.

Although, a protein was used as the chelating moiety in the previous example, more often proteins are used as targets. There are several contrast agents that label human serum albumin, including Gd^{III} 3,6,10,16-tetraazabicyclo [10.3.1]hexadecane-3,6,10-tris(methanephosphonates) **9**, MS-325 **10**, and Gd^{III} DOTA-deoxycholic acid **11** (Fig. 2) [26, 32, 33]. These agents bind to albumin leading to increased circulation times; consequently, they are used as blood pool imaging agents. Fibrin and collagen are other well explored

proteins for targeted imaging because they are found in high concentrations in angiogenesis, thrombosis, atherosclerosis, and other wound-healing processes. The cyclic decapeptide cCGLIIQKNEC (CLT1) conjugated to Gd^{III} diethylenetriaminepentaacetic acid (DTPA), **12**, (Fig. 2) is a fibrin–fibronectin-labeling agent with a relaxivity of 4.22 mM⁻¹s⁻¹ (3 T and room temperature) [34]. *In vivo* tumor imaging of human colon cancer xenografts in mice showed enhancement of tumors using a 0.1 mmol/kg dose because of the high concentration of fibrin–fibronectin associated with the neovascularization of tumors.

Imaging diseases in brain is more challenging than other parts of the body because of the tight regulation in transportation of compounds across the blood–brain barrier (BBB). Some diseases like brain tumors disrupt the BBB allowing contrast agents to accumulate in the interstitial spaces that outline the tumor region. However, many other abundant neuropathologies including Alzheimer's disease and multiple sclerosis do not disrupt the BBB during early stages of the diseases. Hence, the synthesis of targeting biomarkers to image neuropathology with MRI is an active area of research because of the current lack of *in vivo* detection methods. β -Amyloid peptides and myelin are two components that have been investigated as targets [35–39].

β -Amyloid plaques are potential biomarkers for imaging Alzheimer's disease [35–37]. A derivative of amyloid peptide conjugated with Gd^{III}-containing complexes, **13**, was found to label amyloid plaques *in vivo* [35]. MRI imaging at 7 T of brains removed from transgenic mice (APP-PS1) that were injected intravenously with **13** demonstrated contrast enhancement of plaques. This result implies that **13** has the ability to cross the BBB. However, *in vivo* imaging was not reported; hence, the applicability of **13** for *in vivo* imaging cannot be predicted without further research.

Myelin is another biomarker for various neurodegenerative diseases including multiple sclerosis. Wang and coworkers reported myelin-targeted contrast agent **14** (Fig. 2) [38, 39]. Contrast agent **14** contains a coumarine derivative as the targeting unit conjugated to Gd^{III}DOTA, and this agent has a relaxivity of 5.2 mM⁻¹s⁻¹ (9.4 T, 21 °C, and water). *Ex vivo* T₁ mapping of mouse brains incubated with **14** demonstrated the specificity of **14** for myelin [38]. Subsequent *in vivo* studies were carried out with intracerebroventricular infusions (~1 mg (1.14 μ mol)/kg) because of the poor BBB penetration of **14**. These studies demonstrated the specificity for myelin and the ability of **14** to highlight demyelination with T₁ mapping at 9.4 T [39]. However, conventional MR imaging was not reported with this agent, which likely was due to low sensitivity that needs to be improved for clinical translations of **14**.

While monomeric agents are desirable because of their low molecular weight, in general, studies of monomeric targeted agents reveal the need for biomarkers with high local concentrations or for the use of large doses of contrast agents to achieve enhancement of targets due to the low sensitivity of these agents. Therefore, it is essential to increase the sensitivity of target-specific agents to image biomarkers with relatively low expression levels.

2.2. Multimeric Target-specific Contrast Agents

Compared to monomeric agents, multimeric contrast agents can be used to image biomarkers that have relatively low expression levels. Also, the blood circulation of multimeric contrast agents tends to be longer than that of monomeric agents because the large size of multimeric agents prevents rapid clearance via the kidneys; hence, multimeric agents often have a longer time to interact with targets. Furthermore, high molecular weights result in slow tumbling rates that increase relaxivity, and multimeric targeted contrast agents contain up to thousands of contrast-enhancing units and targeting moieties that also increase

relaxivity. The long circulation times and high relaxivities of multimeric agents enable the use of smaller doses than monomeric agents for *in vivo* imaging.

Fibrin-targeted agent **15** (Fig. 4) contains four Gd^{III}-DOTA complexes conjugated to an 11-amino-acid fibrin-targeting peptide. The relaxivity of this tetrameric contrast agent is 10.1 mM⁻¹s⁻¹ per Gd^{III} ion in the absence of fibrin and increases to 17.8 mM⁻¹s⁻¹ per Gd^{III} ion upon binding to fibrin (1.41 T, 37 °C, and Tris buffered saline (TBS)) [40]. The effectiveness of **15** as a targeted contrast agent *in vivo* was demonstrated by imaging pulmonary emboli and cerebral venous sinus thrombosis [41, 42]. Both studies were performed using swine models containing engineered human blood clots, and contrast enhancement was reported at 1.5 T after intravenous injection of 4 μmol/kg of **15**. These studies reveal the specificity of **15** for fibrin and the applicability of **15** to the detection of fibrin-associated diseases *in vivo* with low doses.

A structurally similar agent to **15** is collagen-targeted contrast agent **16** (Fig. 4). Agent **16** was synthesized by conjugating three Gd^{III}DTPA complexes to a 16-amino-acid peptide [43]. Contrast agent **16** has a relaxivity of 16.1 mM⁻¹s⁻¹ per Gd^{III} ion (1.41 T, 37 °C, and PBS) or 5.4 mM⁻¹s⁻¹ per Gd^{III} ion (4.7 T, 25 °C, and PBS). *In vivo* imaging with **16** (tail-vein injection of 0.025 mmol/kg) in mouse models enabled specific imaging of myocardial fibrosis at 4.7 T [43, 44]. The higher dose used for **16** relative to **15**, is likely necessary due to the large amounts of collagen present in many organs and low relaxivity reported for **16** at 4.7 T field strength. With agents **15** and **16**, three or four Gd^{III}-containing units were conjugated to the targeting peptide via functional groups present in the amino acid side chains resulting in increased efficiency.

To increase relaxivity by increasing Gd^{III}-loading, macromolecules including polymers, dendrimers, nanoparticles, and liposomes are used [45–60]. These macromolecules act as carriers to transport large numbers of contrast agents and targeting moieties. The expectation with this method is that contrast enhancement will be observed even if only a small number of target molecules are labeled. Conjugation of several Gd^{III}-containing complexes and targeting moieties to poly(L-glutamic acid)cystamine or *N*-(2-hydroxypropyl)methacrylamide produced contrast agents **17** and **18** (Fig. 4) for targeting integrins [45, 46]. Agent **17** has a relaxivity of 9.7 mM⁻¹s⁻¹ per Gd^{III} ion (3 T and water) and **18** has a relaxivity of 20.6 mM⁻¹s⁻¹ per Gd^{III} ion (1.5 T and water). Both agents effectively bind α_vβ₃ integrin *in vitro*, and quantitative T₁ mapping demonstrated that polymers **17** and **18** interact with integrins in the tumors of xenograft mice using tail-vein injections of 5 μmol Gd^{III}/kg and 0.03 mmol Gd^{III}/kg, respectively. However, a contrast enhancement was not observed with **17** or **18**, which likely was due to insufficient loading of Gd^{III}-containing complexes onto the polymers [45, 46].

Another type of macromolecular targeting agent uses proteins as scaffolds for targeting moieties and Gd^{III}-containing chelates. Maleylated bovine serum albumin conjugated to Gd^{III}DOTA, **19**, was studied as a macrophage-scavenger-receptor-targeted contrast agent *in vitro* [47]. The maleyl groups acted as the targeting moieties and up to 22 Gd^{III}-chelates were linked to the protein. The relaxivity of **19** is 29.1 mM⁻¹s⁻¹ per Gd^{III} ion (0.61 T, 25 °C, and water at pH 7), and *in vitro* cell imaging demonstrated increased contrast for macrophages after incubation with **19**.

Dendrimers are also used as carriers for multimeric agents. Polyamidoamine (PAMAM) dendrimers are widely used as a macromolecular delivery system because they can be functionalized easily and are biocompatible [48–50]. *In vivo* imaging using agents **20** and **21** composed of folic acid and Gd^{III}-containing chelates conjugated to PAMAM dendrimers enabled contrast enhancement of human epidermoid carcinomas in xenograft mice [49, 50].

The relaxivity of these contrast agents is higher than monomeric agents, for example 64.88 $\text{mM}^{-1}\text{s}^{-1}$ per Gd^{III} ion (3 T and water) for **20** and 26.0 $\text{mM}^{-1}\text{s}^{-1}$ per Gd^{III} ion (2 T and 20 °C) for **21**. These high relaxivities enable low-dose imaging (tail-vein injections of 0.05 mmol Gd^{III} /kg and 0.029 mmol Gd^{III} /kg for **20** and **21**, respectively).

Nanoparticles and liposomes that contain Gd^{III} chelates are other classes of multimeric targeted contrast agents. These systems enable the incorporation of different types of imaging, therapeutic, and targeting agents to the same carrier without great synthetic burden. Tens to thousands of Gd^{III} or Mn^{II} ions have been loaded into nanoparticles for use as targeted contrast agents for *in vitro* and *in vivo* studies [54, 56, 57]. Lanza and co-workers reported integrin-targeted contrast agents for tumor imaging using Gd^{III} -containing nanoparticles [54, 55]. These nanoparticles, **22**, containing an $\alpha_v\beta_3$ integrin antagonist and Gd^{III} DTPA, were used to visualize early tumor angiogenesis in xenograft mice using intravenous injections of 0.5 mL/kg (~ 0.03 nmol particles/kg or 2.7 μmol Gd^{III} /kg based on data in [54]). Each nanoparticle contained $\sim 90,000$ Gd^{III} ions with a relaxivity of 1,800,000 $\text{mM}^{-1}\text{s}^{-1}$ per particle (~ 20 $\text{mM}^{-1}\text{s}^{-1}$ per Gd^{III} ion, 0.47 T, and 40 °C). Nanoparticle **22** produced a 173% contrast enhancement in melanoma xenografts at 1.5 T [54]. Lu and co-workers recently reported a nanoglobular system **23** (Fig. 4) containing the decapeptide CLT1 and Gd^{III} DOTA to target fibrin–fibronectin [56]. The generation 3 nanoglobular system with a molecular relaxivity of 353 $\text{mM}^{-1}\text{s}^{-1}$ (8.2 $\text{mM}^{-1}\text{s}^{-1}$ per Gd^{III} ion) (3 T, 25 °C, and water) was used for *in vivo* imaging of tumor-bearing mice. A tail-vein injection of 0.03 mmol Gd^{III} /kg of **23** produced image enhancement in the area of the tumor (Fig. 5). The nanoparticles were excreted via the renal pathway, which is important for the translation of these contrast agents into clinical applications.

Although, nanoparticles are potential carriers to increase the local concentration of contrast agent at sites of interest, maintaining a size smaller than the renal excretion threshold (~ 8 nm) and a high relaxivity is needed to enable the clinical application of these agents. To achieve high relaxivity with nanoparticles, attachment of Gd^{III} -containing chelates to the surface of nanoparticles is more desirable than encapsulation [57] because encapsulation decreases relaxivity of nanoparticles. For example, Gd^{III} DOTA encapsulated in chitosan nanoparticles **24** (Fig. 4) and Gd^{III} DTPA encapsulated in liposomes **25** have lower relaxivities (1.04 $\text{mM}^{-1}\text{s}^{-1}$ per Gd^{III} ion, 0.47 T, and 40 °C for **25**) than Gd^{III} DOTA and Gd^{III} D-TPA, respectively [58, 59]. These low relaxivities resulted from the reduced accessibility of water molecules to the Gd^{III} ions due to the encapsulation inside of the hydrophobic nanosystems. Attaching Gd^{III} -containing chelates on particle surfaces enables facile access of water molecules to Gd^{III} ions; however, attaching a large number of Gd^{III} -containing chelates on the nanoparticle surface increases the risk of leaching Gd^{III} ions.

Because of the toxicity of unchelated Gd^{III} ions, several research groups have studied Mn^{II} -containing nanosystems for targeted imaging [60, 61]. Although, Mn^{II} (spin 5/2) has a lower relaxivity than Gd^{III} (spin 7/2), nanosystems with large Mn^{II} loadings can produce contrast enhancement of targets. Lu and co-workers reported a nanoglobular system **26** (Fig. 4) that contained the fibrin-targeted decapeptide CLT1 and Mn^{II} DOTA monoamide [60]. This Mn^{II} nanosystem has a relaxivity of 3.13 $\text{mM}^{-1}\text{s}^{-1}$ per Mn^{II} ion and 131 $\text{mM}^{-1}\text{s}^{-1}$ per nanoglobule (3 T and room temperature). *In vivo* imaging of breast carcinoma xenograft mice that were injected (tail-vein) with 0.03 mmol Mn^{II} /kg of **26** produced contrast enhancement of tumor with respect to non-targeted controls, and the contrast agents were excreted through the kidneys. Manganese oxide nanocolloidal systems also were reported to target fibrin [61]. Mn^{II} oxide nanocolloids **27** and Mn^{II} oleate nanocolloids **28** both labeled fibrin clots *in vitro*. The relaxivities of **27** and **28** were 4.1 and 14.6 $\text{mM}^{-1}\text{s}^{-1}$ per Mn^{II} ion (91,127 and 423,420 $\text{mM}^{-1}\text{s}^{-1}$ per particle at 3T, 25 °C), respectively. These high relaxivity

values and *in vitro* studies suggest that Mn^{II} oxide nanocolloids could be useful for *in vivo* fibrin detection.

2.3. Superparamagnetic Target-specific Contrast Agents Using the Conjugation Strategy

Superparamagnetic iron oxide (SPIO) nanoparticles coated with polymers are another class of contrast agents for MRI. These agents enhance the contrast of images by decreasing signal intensity in T_2 - and T_2^* -weighted imaging and, consequently, darkening the target area relative to surrounding tissues. Although, the darkening effect (negative contrast) is not ideal for anatomical imaging, many SPIO-based target-specific contrast agents are reported with T_2 -weighted imaging because they can be used for target detection in post-injection images when compared to pre-injection images. Additionally, SPIO-based agents can be converted to positive imaging agents using different pulse sequences and post-processing positive-contrast techniques [62]. Although, these new positive imaging techniques with SPIO nanoparticles have been reported with cell labeling and tracking studies, we have limited our discussion on SPIO-based target-specific contrast agents in this section to conventional T_2 - or T_2^* -weighted imaging.

SPIO nanoparticles are used in MRI because of the biocompatibility of iron oxide nanoparticles and high T_2 relaxivity for contrast enhancement. These biocompatible SPIO nanoparticles are taken up by the reticuloendothelial system (RES) in cells; hence, in early studies, SPIO nanoparticles were used to detect lesions in RES-rich organs including the liver, spleen, and lymph nodes without the use of a conjugated targeting moiety [17]. However, recent research has focused on conjugating targeting moieties to the nanoparticle surface to achieve selectivity to areas other than hepatic lesions. Conjugation of targeting moieties to the iron oxide core is possible because of the polymeric coatings used to stabilize the SPIO nanoparticles. Rajabi and Tsourkas and co-workers reported the use of targeted SPIO nanoparticles for selective detection of tumors *in vivo* [63, 64]. The contrast agents reported by these groups target the HER2 receptor in tumor tissues and consisted of magnetic nanoparticles modified with the monoclonal antibody Trastuzumab, **29**, or with a HER2-affibody, **30** (Fig. 4). To synthesize particle **29**, the targeting antibody was conjugated to the nanoparticle using an amine-containing linker incorporated into a dextran coating [63]. Whereas, in particle **30**, the azide-alkyne Huisgen cycloaddition (often referred to as “click” chemistry) was used to conjugate an alkynated linker containing the HER2-targeted affibody to a polymer coating functionalized with azides [64]. Agent **29** enhanced the tumor regions in mouse models relative to surrounding tissues with 30 $\mu\text{mol/kg}$ (tail-vein injections). For agent **30**, a 10 mg (0.18 mmol) Fe/kg dose was used (retro-orbital injection) to enhance contrast of tumors (Fig. 6). Iron oxide nanoparticles conjugated with urokinase-type plasminogen activator (uPA) peptide, **31**, used carboxylates on the polymer coating to conjugate with targeting moieties [65]. The uPA peptide was engineered to contain an amine that enables conjugation with nanoparticles via amide linkages. Contrast agent **31** has relaxivity (T_2) value of 124 $\text{mM}^{-1}\text{s}^{-1}$ (3 T) and targets uPA receptors that are over-expressed in tumors. *In vivo* imaging with intravenous injections of **31** at 3 T using mammary carcinoma bearing mouse models demonstrated enhanced contrast in the tumor region, but contrast enhancement within the tumor was heterogeneous. This heterogeneous distribution of **31** in tumor tissue and the resultant decrease in signal intensity enabled an understanding of receptor distribution in addition to tumor detection. Another cancer-cell-targeted SPIO-nanoparticle-based contrast agent, **32** (Fig. 4), acts as a drug delivery vehicle as well as an imaging probe [66]. In this study, SPIO nanoparticles were labeled with a monoclonal antibody, a fluorescent probe, and four anticancer drugs that release from the SPIO nanoparticle in response to changes in pH. Although, *in vivo* imaging was not performed, *in vitro* studies of this potential theranostic agent using human colon cancer cells

(LS174T) demonstrated a reduction of T_2 in cells incubated (0.03 mg/mL and 37 °C) with **32** (55.5 ms) with respect to untreated control cells (117.3 ms) at 7 T.

Other than tumor imaging, SPIO nanoparticles are reported to detect renal inflammations. Thurman and coworkers reported the use of SPIO nanoparticles, **33**, conjugated with a recombinant protein that targets the complement receptor type 2 in kidneys for *in vivo* studies of glomerulonephritis. A dose of 10–16 mg of particles/kg enhanced contrast of the kidneys of diseased mice and demonstrated the potential for selective visualization of renal inflammation without the need for invasive renal biopsies [67, 68].

The direct conjugation of targeting moieties with contrast agents or carrier molecules containing contrast agents is synthetically a simple approach to achieve selectivity. Therefore, both *in vitro* and *in vivo* imaging are reported for many targeted agents synthesized with this strategy. Monomeric targeted agents interact with or are internalized by targeted cells and can clear from the body easily because of their small size. But, monomeric agents often have low sensitivity. Multimeric agents that bear up to thousands of contrast-enhancing units and targeting moieties have high relaxivities because of their large size and the number of conjugated contrast agents. However, large size can result in difficulties in clearance. Therefore, while each class of targeted agents has potential niche uses, multimeric agents of intermediate size that have high relaxivities but remain within the renal-clearance threshold likely will be the agents with more potential for *in vivo* applications in the future.

3. TARGET-SPECIFIC CONTRAST AGENTS DESIGNED WITH THE STRUCTURE-MIMICKING STRATEGY

Transforming contrast agents into structural mimics of molecules that interact with specific tissues and receptor molecules is a targeting strategy that converts contrast agents directly into targeting moieties. However, the synthesis of contrast agents that mimic the structure of biologically active molecules can be synthetically challenging. Several attempts to synthesize targeted biomimetic contrast agents have been reported and include porphyrin-, high-density-lipoprotein-, and ferritin-based agents. [69–79].

Porphyrins selectively accumulate in necrotic tissues and interact with neurons [69, 80, 81]; porphyrins also form stable complexes with some metal ions and possess optical properties that are advantageous for imaging studies. Consequently, several porphyrin-based contrast agents are reported for tumor targeting. Mn^{III}-containing porphyrin–dextran system **34** (Fig. 7) was used to visualize tumors *in vivo*. Porphyrin agent **34** has a relaxivity of 8.90 mM⁻¹s⁻¹ (4.7 T and aqueous solution) and enhances tumor contrast in mice bearing hepatoma tumors using a 0.05 mmol Mn^{III}/kg dose (intravenous injection) [70]. Another Mn^{III}-based porphyrin, **35** (Fig. 7), was reported as a brain-tissue-specific contrast agent that is cell permeable and labels neuronal cell bodies in the hippocampus [71]. However, the introduction of contrast agent **35** to rat brains (0.017–0.02 μmol/kg dose) using direct injection and the long retention time reported in brain tissues ($t_{1/2} \approx 10$ days) suggest areas for future research with this contrast agent.

Although, Mn^{III}-containing porphyrins have higher relaxivities than clinical contrast agents, they have lower relaxivities than Gd^{III}-containing porphyrins [70, 72]. Two Gd^{III}-containing porphyrins **36** and **37** (Fig. 7) were reported for *in vivo* melanoma imaging (relaxivities of 16.3 and 31.7 mM⁻¹s⁻¹ at 7 T, 23 °C, and aqueous solutions for **36** and **37** respectively) [72, 73]. *In vivo* imaging of xenograft nude mice using 0.1 mmol/kg of **36** and **37** (intravenous bolus injections) showed contrast enhancement in tumors due to specific accumulation [72]. However, Gd^{III}-containing porphyrins are kinetically unstable in solution because of the size

disparity between the metal ion and porphyrin cavity [74, 82]. Hence, expanded porphyrin systems, such as texaphyrins, are used to form stable complexes with Gd^{III} . Like porphyrins, Gd^{III} -containing texaphyrins accumulate in tumors and enhance contrast [83]. The relaxivity of Gd^{III} -containing texaphyrin **38** (Fig. 7) is $18 \text{ mM}^{-1}\text{s}^{-1}$ (1.2 T, 25 °C, and aqueous saline) [84]. Because of the specific accumulation of texaphyrins in tumors and the optical properties of these molecules, texaphyrins are used as radiation sensitizers for tumor therapy and as tumor-specific imaging probes [85].

Another porphyrin structural mimic used as a targeted contrast agent is the copper phthalocyanine dye Luxol fast blue MBS (LFB MBS) **39** (Fig. 7). Complex **39** is a histology stain for myelinated neurons that was studied as a contrast agent for MRI in *ex vivo* brain tissues [81]. The relaxivity of this agent is low ($0.15 \text{ mM}^{-1}\text{s}^{-1}$, 4.7 T, and ethanol) partially because the Cu^{II} ion has a spin of 3/2. Heavily myelinated regions appeared brighter in images when stained with LFB MBS with respect to control experiments, but to attain good contrast enhancement, similar molecules containing metals with higher spin values need to be synthesized.

Synthetic high-density lipoprotein (HDL) nanoparticles are another class of targeted contrast agents that mimic natural molecular entities to achieve selectivity. HDL particles are made into contrast agents by incorporating chelated Gd^{III} into the outer phospholipid coating or by loading the core of the HDL particle with iron oxide [75, 76]. Synthetic HDL nanoparticles **40** and **41** (Fig. 7) target atherosclerosis. *In vivo* imaging of genetically engineered hyperlipidemic (ApoE KO) mice with tail-vein injections of 3.25 and 4.36 $\mu\text{mol/kg}$ doses of **40** (relaxivity (T_1) of $10.4 \text{ mM}^{-1}\text{s}^{-1}$ per particle, 1.5 T, 25 °C, and water at pH 7.4) demonstrated contrast enhancement of plaques (Fig. 8) [75]. *In vivo* imaging of ApoE KO mice with **41** (relaxivity (T_2) of $94.2 \text{ mM}^{-1}\text{s}^{-1}$, 1.41 T, and 40 °C) also demonstrated contrast enhancement of plaques using 30 mg (0.54 mmol) Fe/kg [76].

Another iron-oxide-containing biomimetic used as a targeted agent is ferritin. Ferritin is a protein that stores iron and regulates levels of iron in the body. Consequently, endogenous ferritin can be used to recognize some ferritin-related diseases using MRI [77]. Thus, ferritin provides a template to generate SPIO-based contrast agent mimics. Recombinant human H chain ferritin, **42**, was used *in vitro* as a macrophage-targeted contrast agent, and these studies showed selective uptake of **42** by macrophages (incubated with 165 μg (3 μmol) Fe/mL of **42**) resulting in contrast enhancement at 1.5 T [78]. In another study, cationic ferritin **43** was used as a basement-membrane-selective contrast agent to detect glomerulosclerosis [79]. Both *ex vivo* and *in vivo* imaging using **43** in rat models at 11.7 T detected disruptions of basement membranes; however, five intravenous injections of a relatively high dose (~5 mg (0.089 mmol) Fe/kg) was required to detect cationic ferritin accumulation in glomeruli *in vivo*; this high dose is a challenge for translation into clinical applications.

Recently, Yang and co-workers reported engineered proteins that chelate Gd^{III} ions to produce stable high relaxivity contrast agents for MRI [86]. An example is a contrast agent containing an engineered cell adhesion protein **44** (Fig. 7) with a domain to chelate Gd^{III} ions. Agent **44** has a relaxivity of $117 \text{ mM}^{-1}\text{s}^{-1}$ (1.5 T) enabling doses as low as 2.4 μmol Gd^{III} /kg for contrast-enhanced imaging. Using this approach, endogenous proteins can be mimicked to synthesize targeted contrast agents with higher efficiency.

While the structure-mimicking strategy enables the use of endogenous interactions to deliver agents, synthesizing targeted agents with the structure-mimicking strategy is challenging and not generalizable like the conjugation strategy. Therefore, the scope of targets and number of examples are limited relative to agents synthesized with the conjugation strategy. Some of these agents have high relaxivities and enhance contrast *in vivo*; however, more research is

needed in this area to improve current mimetic agents and to find new biomimetics that form stable paramagnetic complexes and are specific for various tissues and diseases.

4. CONCLUSION

Targeted contrast agents enable the visualization of structural changes in organs or the expression of biological molecules, and these agents have the potential to aid in the diagnosis of diseases at early stages. In general, targeting ability is achieved by conjugating a targeting moiety to a contrast agent or by mimicking the structural features of targeting moieties with contrast agents. A large number of targeted agents have been synthesized with the conjugation strategy, and while conjugation of a single contrast-enhancing unit per targeting moiety enables selective imaging in the presence of high local concentrations of targets, many such contrast agents suffer from low sensitivity and require large doses to achieve contrast enhancement. The use of multiple contrast-enhancing units in multimeric agents overcomes the limitation of sensitivity, but the large size of multimeric agents can cause difficulties with excretion. Synthesis of macromolecules smaller than the renal excretion threshold is a strategy to avoid this problem, but this strategy also influences relaxivity. Also, polydispersity of these systems can lead to difficulties with reproducibility. Relatively few examples of the mimicking strategy are reported for the design of targeted contrast agents because of the limited availability of biomimetics that form stable complexes with paramagnetic cations; however, recent advances in nanochemistry and bio-technology will likely lead to more opportunities for mimicking biologically important structures with contrast agents.

Except for one or two target-specific contrast agents that are clinically approved (MS-325) or in clinical-trials (texaphyrins), the majority of target-specific agents are in pre-clinical levels of research; however, successful *in vivo* imaging was reported with many pre-clinical studies, and these results shine positive light onto the future of the field. An area of where extra attention to detail will likely aid the field is the inclusion of as many experimental details as possible: the direct comparison of different studies is difficult because the conditions used for relaxivity measurements and imaging experiments are different (Table 1), and improper comparisons could lead to misinterpretations of the efficiency of one agent compared to another. Finally, improvements to the selectivity, sensitivity, and biocompatibility of target-specific agents and the selection of new molecular targets using both the conjugation and mimicking strategies will likely enable translation into clinical applications for MRI.

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SYMBOLS

ΔR_1	Difference between the T_1 relaxation rates before and 1 h after contrast enhancement
K_i	Binding inhibition constant
R_2	Transverse relaxation rate
$t_{1/2}$	Half-life
T_1	Longitudinal relaxation time
T_2	Transverse relaxation time

ABBREVIATIONS

BBB	Blood-brain-barrier
CLT1	Cyclic decapeptide CysGly-LeuIleIleGlnLysAsnGluCys
cRGD	Cyclic ArgGlyAsp
DMSO	Dimethylsulfoxide
DOTA	1,4,7,10-tetraazacyclododecane- <i>N,N',N'',N'''</i> -tetraacetic acid
DTPA	Diethylenetriaminepentaacetic acid
HDL	High-density lipoprotein
HER2	Human epidermal growth factor receptor type 2
LFB MBS	Luxol fast blue MBS
MRI	Magnetic resonance imaging
PAMAM	Polyamidoamine
PBS	Phosphate buffered saline
PR	Progesterone receptor
RES	Reticuloendothelial system
SPIO	Superparamagnetic iron oxide
TBS	Tris(hydroxymethyl)aminomethane buffered saline
Tris	Tris(hydroxymethyl)aminomethane
uPA	Urokinase-type plasminogen activator

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Fig. 1.

Schematic representation of the basic units of targeted contrast agents synthesized using the conjugation strategy.

Fig. 2. Monomeric targeted contrast agents synthesized using the conjugation strategy. Contrast agent **8** is adapted with permission from Qiao J, Li S, Wei L, *et al.* HER2 Targeted molecular MR imaging using a *de novo* designed protein contrast agent. PLoS ONE 2011; 6(3): e18103. Copyright 2011 Qiao *et al.*

Fig. 3. Representative *in vivo* images of targeted contrast agents: contrast enhancement of progesterone-receptor (PR)-positive and PR-negative tumors using monomeric conjugated contrast agent **1**. Scale bars represent 5 mm, and arrows point to tumors. Adapted with permission from Sukerkar PA, MacRenaris KW, Meade TJ, Burdette JE. A steroid-conjugated magnetic resonance probe enhances contrast in progesterone receptor expressing organs and tumors *in vivo*. *Mol Pharmaceutics* 2011; 8(4): 1390–400. Copyright 2011 American Chemical Society.

Fig. 4.

Multimeric targeted contrast agents synthesized using the conjugation strategy. Contrast agents **23**, **24**, **26**, **30**, and **32** were adapted with permission from Tan M, Wu X, Jeong E-K, Chen Q, Lu Z-R. Peptide-targeted nanoglobular Gd-DOTA monoamide conjugates for magnetic resonance cancer molecular imaging. *Biomacromolecules* 2010; 11(3): 754–61. Copyright 2010 American Chemical Society; Nam T, Park S, Lee S-Y, *et al.* Tumor targeting chitosan nanoparticles for dual-modality optical/MRI cancer imaging. *Bioconjugate Chem* 2010; 21(4): 578–82. Copyright 2010 American Chemical Society; Tan M, Wu X, Jeong E-K, Chen Q, Parker DL, Lu Z-R. An effective targeted nanoglobular manganese(II) chelate conjugate for magnetic resonance molecular imaging of tumor extracellular matrix. *Mol Pharmaceutics* 2010; 7(4): 936–43. Copyright 2010 American Chemical Society; Elias DR, Cheng Z, Tsourkas A. An intein-mediated site-specific click conjugation strategy for improved tumor targeting of nanoparticle systems. *Small* 2010; 6(21): 2460–8. Copyright 2010 Wiley-VCH verlag GmbH & Co. KGaA, Weinheim; and Zou P, Yu Y, Wang A, *et al.* Superparamagnetic iron oxide nanotheranostics for targeted cancer cell imaging and pH-dependant intracellular drug release. *Mol Pharmaceutics* 2010; 7(6): 1974–84. Copyright 2010 American Chemical Society, respectively.

Fig. 5.

Representative *in vivo* images of targeted contrast agents: contrast enhancement of fibronectin–fibrin complex in tumor tissues using multimeric conjugated contrast agent **23**. Arrows point to tumors. Reprinted with permission from Tan M, Wu X, Jeong E-K, Chen Q, Lu Z-R. Peptide-targeted nanoglobular Gd-DOTA monoamide conjugates for magnetic resonance cancer molecular imaging. *Biomacromolecules* 2010; 11(3): 754–61. Copyright 2010 American Chemical Society.

Fig. 6.

Representative *in vivo* images of targeted contrast agents: contrast enhancement of HER2-positive tumors using conjugated SPIO nanoparticle **30**. Arrows point to tumors. Adapted with permission from Elias DR, Cheng Z, Tsourkas A. An intein-mediated site-specific click conjugation strategy for improved tumor targeting of nanoparticle systems. *Small* 2010; 6(21): 2460–8. Copyright 2010 Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim.

Fig. 7.

Targeted contrast agents synthesized using the structure mimicking strategy. Contrast agents **40**, **41**, and **44** were adapted with permission from Frias JC, Williams KJ, Fisher EA, Fayad ZA. Recombinant HDL-like nanoparticles: A specific contrast agent for MRI of atherosclerotic plaques. *J Am Chem Soc* 2004; 126(50): 16316–17. Copyright 2004 American Chemical Society; Cormode DP, Skajaa T, van Schooneveld MM, *et al.* Nanocrystal core high-density lipoproteins: A multimodality contrast agent platform. *Nano Lett* 2008; 8(11): 3715–23. Copyright 2008 American Chemical Society; and Yang JJ, Yang J, Wei L, *et al.* Rational design of protein-based MRI contrast agents. *J Am Chem Soc* 2008; 130(29): 9260–7. Copyright 2008 American Chemical Society; respectively.

Fig. 8. Representative *in vivo* images of targeted contrast agents: contrast enhancement of atherosclerotic plaques using high-density lipoprotein mimic **40**. Arrows point to abdominal aorta. Adapted with permission from Frias JC, Williams KJ, Fisher EA, Fayad ZA. Recombinant HDL-like nanoparticles: A specific contrast agent for MRI of atherosclerotic plaques. *J Am Chem Soc* 2004; 126(50): 16316–7. Copyright 2004 American Chemical Society.

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Table 1

Conditions Used for Relaxivity Measurements and MR Imaging

Contrast agent	Field strength (T)		Temperature for relaxivity (°C)	Medium for relaxivity measurements	Relaxivity (T_1) (mM ⁻¹ s ⁻¹) ^a	Relaxivity (T_2) (mM ⁻¹ s ⁻¹) ^a	<i>In vivo</i> imaging dose	<i>In vitro</i> imaging concentrations	Reference
	Relaxivity measurements	<i>In vivo/In vitro</i> studies							
1	4.7	7.05	21	NR	4.73	NR	0.15 mmol/kg	NR	22
1	1.41	7.05	37	1% DMSO in water	5.35	6.14	0.15 mmol/kg	NR	22
2	9.4	9.4	NR	PBS	6.8	NR	0.075 mmol/kg	NR	23
3	1.41	9.4	40	NR	9	NR	0.1 mmol/kg	NR	25
4	1.5	1.5	25	NR	7.4	4	1.43 mmol/kg	NR	27
5	9.4	9.4	NR	PBS	4.7	NR	0.075 mmol/kg	NR	23
6	4.7	4.7	NR	aqueous	1.28	NR	0.2 μmol/mouse	0.5 mM	29
7	1.41	2	37	water	21	30	0.03 mmol/kg	NR	30
8	1.41	4.7	37	Tris buffer, pH 7	21	30	0.24 μmol/mouse	NR	31
9	0.47	NR	25	pH 7.4	7.7	NR	NR	NR	33
10	4.7	NR	37	water	5.5	6.9	NR	NR	24
10	3	NR	37	water	5.3	6.1	NR	NR	24
10	1.5	NR	37	water	5.2	5.9	NR	NR	24
11	0.47	NR	37	water	5.6	NR	NR	NR	26
12	3	3	RT	NR	4.22	4.45	0.1 mmol/kg	NR	34
13	NR	7	NR	NR	NR	NR	0.4–2 mg/mouse ^b	NR	35
14	9.4	9.4	21	water	5.2	NR	1.14 μmol/kg	0.1 mM	38, 39
14	1.41	9.4	40	water	5.1	NR	1.14 μmol/kg	0.1 mM	38, 39
14	0.47	9.4	40	water	5.8	NR	1.14 μmol/kg	0.1 mM	38, 39
15	1.41	1.5	37	TBS, pH 7.4	10.1, 40.3 ^c	12.8, 51.1 ^c	4 μmol/kg	NR	40, 41
15	0.47	1.5	37	TBS, pH 7.4	11.1, 44.4 ^c	NR	4 μmol/kg	NR	40, 41
16	4.7	4.7	25	PBS, pH 7.4	5.4, 16.2 ^c	NR	0.025 mmol/kg	NR	43, 44
16	1.41	4.7	37	PBS, pH 7.4	16.1, 48.4 ^c	NR	0.025 mmol/kg	NR	43, 44
16	0.47	4.7	37	PBS, pH 7.4	18.7, 56.2 ^c	NR	0.025 mmol/kg	NR	43, 44
17	3	3	NR	water	9.7	NR	5 μmol Gd/kg	NR	45

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Contrast agent	Field strength (T)		Temperature for relaxivity (°C)	Medium for relaxivity measurements	Relaxivity (T_1) (mM ⁻¹ s ⁻¹) ^a	Relaxivity (T_2) (mM ⁻¹ s ⁻¹) ^a	<i>In vivo</i> imaging dose	<i>In vitro</i> imaging concentrations	Reference
	Relaxivity measurements	<i>In vivo/In vitro</i> studies							
18	1.5	9.4	NR	water	20.6	NR	0.03 mmol Gd/kg	NR	46
19	0.6	7	25	water, pH 7	29.1	NR	NR	50 μM	47
20	3	3	NR	water	64.88	NR	0.05 mmol Gd/kg	NR	49
21	2	2	20	NR	26, 598 ^c	NR	0.029 mmol Gd/kg	0.01 mM	50
22	0.47	1.5	40	NR	~20, 1,800,000 ^c	NR	2.7 μmol Gd/kg	NR	54
23	3	3	25	water	8.2, 353 ^c	16.1, 692 ^c	0.03 mmol Gd/kg	NR	56
24	4.7	1.5	NR	NR	NR	NR	3.3 μmol/kg	NR	58
25	0.47	4.7	40	NR	1.04	NR	0.2 mmol Gd/kg	NR	59
26	3	3	RT	water	3.13, 131 ^c	8.74, 367 ^c	0.03mmol Mn/kg	NR	60
27	3	3	25	NR	4.1, ~91,127 ^c	18.9, ~441,120 ^c	NR	NR	61
28	3	3	25	NR	14.6, ~423,420 ^c	70.7, ~2,135,482 ^c	NR	NR	61
29	NR	1.5	NR	NR	NR	NR	30 μmol particles/kg	0.54 mM	63
30	1.41	9.4	NR	NR	NR	89	0.18 mmol Fe/kg	2.68 mM	64
31	3	3	NR	NR	3.6	124	NR	NR	65
32	NR	7	NR	NR	NR	NR	NR	0.03 mg/mL	66
33	NR	4.7	NR	NR	NR	NR	10–16 mg/kg	NR	67, 68
34	4.7	4.7	25	aqueous	8.9	NR	0.05 mmol Mn/kg	NR	70
35	NR	9.4	NR	NR	~3.1 ^d	NR	0.017–0.02 μmol/kg	NR	71
36	7	7	23	aqueous	16.3	20	0.1 mmol/kg	NR	72
37	7	7	23	aqueous	31.7	38.2	0.1 mmol/kg	NR	72
38	1.2	1.5	25	aqueous saline	18	NR	0.017 mmol/kg	NR	82, 84
39	4.7	4.7, 14	NR	ethanol	0.15	NR	NR	1 % w/v	81
39	14	4.7, 14	NR	ethanol	0.12	NR	NR	1 % w/v	81
39	4.7, 14	4.7, 14	NR	aqueous	0.09	NR	NR	1 % w/v	81
40	1.5	9.4	25	water, pH 7.4	10.4 ^c	NR	3.25–4.36 μmol/kg	NR	75
41	1.41	9.4	40	NR	8.9 ^c	94.2 ^c	0.54 mmol Fe/kg	0.072 mM Fe	76
42	1.5	1.5	NR	Dulbecco's PBS	8.4 ^c (for 5000Fe)	93 ^c (for 5000Fe)	NR	3 mM Fe	78

Contrast agent	Field strength (T)		Temperature for relaxivity (°C)	Medium for relaxivity measurements	Relaxivity (T_1) (mM ⁻¹ s ⁻¹) ^a	Relaxivity (T_2) (mM ⁻¹ s ⁻¹) ^a	<i>In vivo</i> imaging dose	<i>In vitro</i> imaging concentrations	Reference
	Relaxivity measurements	<i>In vivo/In vitro</i> studies							
43	NR	11.7	NR	NR	NR	1–10	0.089 mmol Fe/kg ^e	NR	79
44	9.4	3, 9.4	NR	NR	6	50	~2.4 µmol Gd/kg	NR	86
44	3	3, 9.4	NR	NR	48	88	~2.4 µmol Gd/kg	NR	86
44	1.5	3, 9.4	NR	NR	117	129	~2.4 µmol Gd/kg	NR	86

NR = not reported RT = room temperature

^arelaxivity per ion is reported except for those that denoted

^b*ex vivo* imaging

^crelaxivity per molecule or particle

^destimated *in vivo*

^efive doses used