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Emerging concepts in molecular MRI

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Molecular magnetic resonance imaging (MRI) offers the potential to image some events at the cellular and subcellular level and many significant advances have recently been witnessed in this field. The introduction of targeted MR contrast agents has enabled the imaging of sparsely expressed biological targets *in vivo*. Furthermore, high-throughput screens of nanoparticle libraries have identified nanoparticles that act as novel contrast agents and which can be targeted with enhanced diagnostic specificity and range. Another class of magnetic nanoparticles have also been designed to image dynamic events; these act as 'switches' and could be used *in vitro*, and potentially *in vivo*, as biosensors. Other specialized MR probes have been developed to image enzyme activity *in vivo*. Lastly, the use of chemical exchange and off-resonance techniques have been developed, adding another dimension to the broad capabilities of molecular MRI and offering the potential of multispectral imaging. These and other advances in molecular MRI offer great promise for the future and have significant potential for clinical translation.

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Introduction

The attributes of magnetic resonance imaging (MRI; see Box 1) allow high-resolution anatomical and functional images of numerous organ systems to be obtained and also make it highly suited to the molecular imaging mission, namely the imaging of events at the cellular and subcellular level [1••,2,3]. The detection of events at this level, however, often requires nanomolar sensitivity, thus precluding the use of conventional gadolinium chelates as molecular MR imaging agents, as they display only micromolar sensitivity. The principal response to the inadequate sensitivity of conventional gadolinium chelates has been to synthesize novel MR contrast agents with significantly higher relaxivities [3]. These include paramagnetic gadolinium-containing liposomes [4,5] or micelles [6,7], and superparamagnetic iron oxide nanoparticles [8,9]. The use of iron oxide nanoparticles for liver imaging [10], lymph node characterization [11], diagnosis of tissue inflammation [12,13•,14], and stem-cell labeling [15,16] is becoming fairly well established, and will thus not be further discussed in this review.

Several novel techniques and applications in molecular MRI have emerged in the past few years and will be highlighted here. These include the *in vivo* imaging of sparsely expressed biological targets using targeted MR contrast agents, the surface modification of nanoparticles to yield nanoparticle libraries with enhanced target specificity, the use of iron oxide containing magnetic relaxation switches as *in vitro* and *in vivo* biosensors, *in vivo* imaging of enzyme activity with activatable MR contrast agents, and the development of CEST and PARACEST techniques that allow multispectral proton MRI to be performed.

Cardiovascular disease remains the leading cause of mortality in industrialized societies and applications in this area will thus be highlighted, although the techniques listed above are applicable to a broad range of disease states. Particular, but not exclusive, attention will also be paid to applications involving iron oxide nanoparticles. The interested reader is referred to other articles in this volume for a more detailed discussion on other nanoparticles and nanostructures. Finally, given the scope of this review, the discussion will be limited to proton MRI.

Targeted MR contrast agents

Fibrin-targeted MR contrast agents were amongst the first molecular MR probes to be developed, and exploited the high levels of fibrin expressed within thrombi. These agents were successfully used to detect both arterial and venous thrombi in a variety of models [5,17,18]. Recently, however, more sparsely expressed targets such as surface integrins [19], surface phospholipids on apoptotic cells [20••], vascular adhesion molecules [21••], and macrophage receptors [7], amongst others, have been imaged non-invasively *in vivo*. The detection of a sparsely expressed surface target, particularly one within an organ parenchyma or an atherosclerotic plaque, ideally requires the MR contrast agent to have a long half-life in the blood, the ability to penetrate the tissue of interest, and the ability to detect and amplify the biological signal of interest. The superparamagnetic iron oxides, in particular, fulfill many of these criteria [22].

Cross-linked iron oxide (CLIO) is a highly stabilized form of monodisperse iron oxide or MION [9,22]. The

Box 1 MRI technology and its attributes.

Magnetic resonance imaging (MRI) is a non-invasive diagnostic technique that has the potential to image some events at the cellular or subcellular level. This imaging technology is based on the interaction of protons with each other and with surrounding molecules in a tissue of interest. When placed in a strong magnetic field protons precess or rotate at a given frequency and are able to accept energy from a radiofrequency wave applied at this rotational or resonance frequency. The behavior of the energy inserted in to the system is described by two relaxation constants: the T2 or transverse relaxation time and the T1 or longitudinal relaxation time. Different tissues have different relaxation times, and this can be used to produce endogenous contrast between different tissues. Exogenous contrast agents can further enhance tissue contrast by selectively shortening either the T1 or T2 in a tissue of interest. The MR image can be weighted to detect differences in either T1 or T2 by adjusting parameters in the acquisition.

MRI offers several advantages over other imaging modalities. Firstly, it is non-ionizing as it detects the magnetic signals generated by protons and other molecules. The technique is also tomographic, enabling any tomographic plane through a three-dimensional volume to be imaged. High-resolution images with excellent soft tissue contrast between different tissues can be obtained. Lastly, multiple contrast mechanisms are possible using MRI and the technique can be used to provide anatomical as well as physiological readouts.

cross-links on CLIO are aminated, allowing a large variety of ligands to be conjugated to the nanoparticle with a high degree of stability and relative ease. Near-infrared fluorochromes can be attached to the amine groups to form a dual modality magnetofluorescent nanoparticle [23]. Thereafter, many copies of the targeting ligand can be attached to the nanoparticle to form a multivalent (i.e. more than one copy of the targeting ligand) magnetofluorescent nanoparticle. Recently reported examples of two such ligands include annexin for apoptosis imaging [24] and a phage-derived peptide specific for the adhesion molecule VCAM-1 [21••,25•].

The properties of the magnetofluorescent annexin probe, AnxCLIO-Cy5.5, have been previously described [24]. Cardiomyocyte apoptosis in a mouse model of ischemia-reperfusion was successfully imaged with this probe by MRI *in vivo* [20••]. Preliminary results with the probe also suggest that it can successfully detect even the low levels of cardiomyocyte apoptosis seen in heart failure. The adhesion molecule VCAM-1 is expressed by damaged endothelium early in the atherosclerotic process and was successfully imaged in the aortic roots of apoE$^{-/-}$ mice *in vivo* (Figure 1) [21••]. In addition, the beneficial effect of statin therapy on VCAM-1 expression could be documented with the probe *in vivo* by demonstrating decreased probe accumulation in the statin-treated mice [21••]. Thus, this VCAM-1-sensing iron oxide probe not only demonstrated adequate sensitivity to detect a sparsely expressed molecular marker, but also exhibited an adequate dynamic range to detect a treatment effect.

The therapeutic effect of the anti-angiogenic agent fumagillin has also been demonstrated in a rabbit model of vascular injury with a gadolinium-containing liposome targeted to the $\alpha_V B_3$ integrin. This integrin is a marker of angiogenesis, inflammation and instability within an atherosclerotic plaque [26••]. Although, larger than iron oxide probes, the hyperpermeability of vessels involved in plaque angiogenesis allows the gadolinium-containing liposome to penetrate the plaque to enable imaging of new vessel formation. Gadolinium-containing immunomicelles have also recently been developed and, when decorated with an antibody to the macrophage scavenger receptor, have been shown by *ex vivo* MRI to detect plaque macrophages [7].

In the near future it is likely that superparamagnetic iron oxides with even higher relaxivities will be generated [27]. Further advances are also likely to be seen in the synthesis of novel gadolinium chelates and constructs. The imaging of a wide variety of sparsely expressed molecular targets by MRI in small animal models will thus become an established technique in molecular biology and pharmaceutical development. Targeted molecular MRI in large animal models will require ligand synthesis to be scaled up to support this, and will thus require careful selection. Further translation into the clinical realm will require even more careful consideration, as each new construct will require a completely separate profile of pharmacokinetic and toxicity screening. Nevertheless, the translation of a few well-selected targeted iron oxide probes into the clinical realm remains highly likely.

Iron oxide nanoparticle libraries

Although the clinical translation of targeted MR contrast agents will require careful selection of the most appropriate agents, this restriction will not apply in the preclinical arena. In this realm, high-throughput screening and synthesis techniques are likely to be used to generate a diverse array of nanoparticles with small surface modifications. Minor modification of the surface of the iron oxide nanoparticle CLIO-Cy5.5 has already been shown to drastically alter its cellular uptake [28••]. In addition, these modifications can now be performed quickly and at room temperature using convenient 'click' chemistry techniques [29].

The generation of a library of nanoparticles for macrophage imaging has recently been described [28]. In its baseline state, CLIO-Cy5.5 has a high affinity for both resting and activated macrophages. Conjugation of the chemical moiety succinimidyl iodo acetate (SIA) to the aminated sidechains on CLIO-Cy5.5, however, almost completely abolished its uptake by any macrophages whatsoever. High-throughput screening also revealed CLIO conjugates with specific affinity for either resting or activated macrophages: the agent CLIO-Gly, for