Chemical design of nanoprobes for T_1-weighted magnetic resonance imaging

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Magnetic resonance imaging (MRI), which offers a number of advantages such as unlimited tissue penetration, zero ionizing radiation, and a noninvasive nature, has received considerable attention over the past two decades as a technique for clinical diagnosis. To improve imaging sensitivity, contrast agents have been employed to accelerate the relaxation rate of water molecules and thus to increase the contrast between specific tissues or organs of interest. However, conventional contrast agents such as Gd^{3+}-based T_1 complexes and iron oxide nanoparticle-based T_2 contrast agents have been proven to have adverse effects. The former may cause fatal nephrogenic systemic fibrosis (NSF) and difficulty in metabolism, while the latter is less sensitive due to the background interference. Also, their development has been well documented. Therefore, the orientation of this review will be geared toward the newly developed nanoparticulate agents that serve as better alternatives. In this regard, the recent advances on various nanostructured Mn/Fe-based T_1 contrast agents seem to fit these categories. As they reveal longer circulation half-life and better biocompatibility, they have demonstrated themselves as a promising T_1 candidate for MRI. The focus of this review will be on the preparation and fabrication of T_1 contrast agents that contain mainly paramagnetic manganese and iron ions, with special attention being paid to the growth mechanism. Additional emphasis is also put on their progressive development in an aim to overcome the drawbacks of classical iron oxide nanoparticle-based T_2 and Gd^{3+}-based T_1 contrast agents. Representative applications *in vitro* and *in vivo* will be presented for this new generation of contrast agents. The pros and cons of each case are also briefly summarized.

Introduction

Nanostructured materials exhibit different and unique properties (e.g., electronic, optical and magnetic) from their bulk counterparts and have been widely employed in various areas. In particular, imaging technologies (optical imaging [1], ultrasound [2], positron emission tomography (PET) [3], computed X-ray tomography (CT) [4], and magnetic resonance imaging (MRI) [5,6]) have been actively adopting these materials as imaging agents and tracking probes because of their diagnostic ability at the molecular level. Among those imaging technologies, MRI is currently one of the most powerful *in vivo* imaging technologies in both biological research and clinical diagnosis owing to a number of advantages, such as the ability to image soft tissue, unlimited tissue penetration, zero ionizing radiation, and its noninvasive nature. It visualizes contrasts in images by monitoring the response of aqueous protons to an external magnetic field [5].

In order to induce additional contrast in MR images, contrast agents have been introduced to accelerate the relaxation times of protons from bulk water. Contrast agents are generally classified into T_2-weighted and T_1-weighted agents according to the relaxation mechanism. T_2 contrast agents are superparamagnetic iron oxide nanoparticles (SPIONPs)-based. They generate an induced magnetic field under an external field, perturb the spin–spin

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relaxation (T_2) processes of protons in the vicinity, giving rise to negative (dark) MRI images. As this relaxation is induced through space by the inhomogeneity of the local magnetic field, it is easier to incorporate more versatile functions for advanced molecular imaging without severely diminishing T_2 MR ability [6]. In stark contrast, even though the spin-lattice relaxation (T_1) can give a positive (bright) MR image, it needs direct contact between the paramagnetic center and water molecule in the inner coordination sphere. The T_1 signal is thus very sensitive to any surface ligand/coating with different degrees of water penetration to the inner paramagnetic center and could disappear if the diffusion of water is blocked. Therefore, during the past two decades, the multi-funtionalized contrast agent has been intensively pursued in T_2-probes rather than T_1-probes [7–19]. Furthermore, dextran-coated SPIONPs and related commercial products (e.g., Feridex, Resovist, and Combidex) have been served as the only nanoparticulate contrast-enhancing T_2-probes in clinical application. However, these SPIONPs-based T_2 contrast agents have several intrinsic disadvantages that limit their extensive application in the clinic. The resulting dark signal produces images of lower contrast, so the location of the contrast agent can be easily confused with other hypointense areas, such as bleeding, calcification, and metal deposits, which can affect the clinical diagnosis. Moreover, the intrinsically high susceptibility of the superparamagnetic agents induces a long-range magnetic field that not only perturbs neighboring normal tissues but also distorts the background image (the blooming effect). This could become the main obstacle to diagnosing the exact locations of lesions. To address these problems, T_1 contrast agents containing paramagnetic species such as Gd^{3+}, Mn^{2+} and Fe^{3+} have been regarded as an advantageous alternative, for they can provide positive (bright) MRI images [20–35,38–50,52–82]. The bright signal can be clearly detected and distinguished from other pathogenic or biological conditions. Since T_1 contrast agents are mostly made up of paramagnetic ions, they do not disrupt the magnetic homogeneity and thus do not disturb other anatomic backgrounds.

Prior to the design of a MRI contrast agent, parameters that control the r_1 and r_2 relaxivities should be well understood. A T_1 contrast agent should have a high r_1 relaxivity with the r_2/r_1 ratio close to one. A T_2 contrast agent, however, should possess a high r_2 relaxivity and a large r_2/r_1 ratio. The r_2/r_1 ratio is theoretically always greater than one. As longitudinal relaxation is dependent on a dipolar mechanism of the ion-nuclear distance to the inverse 6th power, metal ions with a large spin number, S, are highly desired (i.e., a large number of unpaired S-state (not L-state) electrons). This is because a slow electron spin relaxation of S-state electrons closely matches with a water proton spin relaxation and can efficiently induce the longitudinal water proton relaxation [43]. However, the L-state electrons possess a fast electron spin relaxation which hardly matches with the water proton spin relaxation. Thus, they cannot efficiently induce the longitudinal water proton relaxation. As the r_1 relaxivity is proportional to $S(S + 1)$, taking Mn^{2+} as an example, the manganese ion satisfies this criterion because it has $S = 5/2$ and $L = 0$. Moreover, a T_1 contrast agent with paramagnetic spin structure for minimizing the transverse water proton relaxation (suppress r_2) is also the key to ensure low r_2/r_1 ratio. As the transverse water proton relaxation is accelerated by a fluctuating local magnetic field produced by the

magnetization (M) of a nanoparticle, a paramagnetic nanoparticle (e.g., Gd_2O_3) having zero M can thus ensure low r_2/r_1 ratio. In contrast, the T_2 contrast agent should possess a large M to achieve both a high r_2 relaxivity and a high r_2/r_1 ratio, since the r_2 relaxivity is proportional to M^2. The spin structure of T_2 contrast agent is therefore either ferromagnetic or ferromagnetic [43].

The classical theory for predicting the efficiency of T_1 contrast agents is based on the work of Solomon, Bloembergen and Morgan (SBM) [20], which is particularly applicable in the medium-to-high field regime (>0.1 T). According to the SBM theory, the paramagnetic relaxation enhancement of Gd-complexes originates from both the inner-sphere (IS) and the outer-sphere (OS) mechanisms. The overall longitudinal relaxivity (r_1) is the sum of the inner-sphere relaxivity, r_1^{IS}, and the outer-sphere relaxivity, r_1^{OS}. For r_1^{IS}, the most influential parameters are (i) the number, q, of fast-exchanging water molecules within the inner sphere, (ii) the characteristic tumbling time, τ_R, of the agent, together with its inner-sphere water molecules, and (iii) the characteristic water proton residence lifetime, τ_M, of the inner-sphere water molecules. For r_1^{OS}, which arises from the translational diffusion of water molecules near the Gd^{3+} ions, the most influential parameter is the diffusion correlation time τ_D. Recently, Ananta et al. [21] extended this theory from molecular Gd-complexes to explain the enhanced r_1 relaxivity in its aggregated form inside the nanoporous structure. In that report, under sufficiently large fields ($B > 0.25$ T) and a slow tumbling construct (aggregated Gd-complexes), the contribution to longitudinal relaxivity (r_1) from the outer-sphere (r_1^{OS}) can be neglected, compared with that from the inner sphere (r_1^{IS}) (the r_1^{OS}/r_1^{IS} ratio <0.1). In other words, the inner-sphere relaxivity (r_1^{IS}) contributes most of the overall longitudinal relaxivity (r_1). They showed that geometrical confinement can alter the original parameters q, τ_R, τ_M, and τ_D by reducing the ability of the Gd-complexes to tumble via either decreasing the mobility of the water molecules or favorable cluster formation and interactions between the loaded Gd-complexes. Standing on this basis, Shi's group has provided deeper insight into the origin of contrast enhancements of water soluble Gd^{3+}-doped upconversion nanoparticles and accordingly proposed useful strategies for achieving both high MR relaxivities and tunable r_2/r_1 ratios of Gd^{3+}-ion-containing nanoparticle probes such as Gd_2O_3, $GdPO_4$, and GdF_3 [22].

Since, as discussed above, r_1^{IS} dominates longitudinal relaxivity in a slow tumbling construct, the r_1 of a nanostructured/particulate T_1 contrast agent can be boosted by simply increasing either the concentration of the exposed paramagnetic center or the accessibility of water molecules to the paramagnetic center. In this short review, we present recent progress on the design of new, high-efficiency T_1 contrast agents having the advantages of both T_1 contrast effect and nanostructural characteristics. Since the development of Gd^{3+}-based T_1 contrast agents has been well-documented [20–35] and the potential for causing fatal nephrogenic systemic fibrosis (NSF) limits their clinical application [36,37]. NSF can be seriously debilitating, leading to fibrotic skin contractures and in extreme cases (5%) resulting in fractured bones or death. The incurable nature of the NSF disease progression has strongly impacted the use of Gd^{3+}-based T_1 contrast agents. Therefore, we focus herein on the new generation of T_1 contrast agents that contain paramagnetic manganese and iron ions with higher biocompatibility.