Dendrimers and magnetic resonance imaging†

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The multivalent character of dendrimers has positioned these well-defined, highly branched macromolecules at the forefront in the development of new contrast agents for biomedical magnetic resonance imaging (MRI). By modifying the periphery of the dendrimer with gadolinium(III) chelates, the relaxivity of the resulting MRI contrast agent is increased considerably, compared to low molecular weight Gd(III) chelates. The monodisperse character of dendrimers creates a unique opportunity to introduce dendritic MRI contrast agents into clinics. In addition, a prolonged vascular retention time is obtained due to the larger size of the dendritic molecules. By using dendrimers as multivalent scaffolds carrying multiple ligands, the interaction between ligand and marker can be enhanced through multivalent interactions. Current research focuses on the combination of multivalent targeting and enhanced relaxivity. This paper describes the application of dendrimers in biomedical MRI.

Introduction

At the start of the twentieth century, the field of biomedical imaging emerged as a result of Röntgen’s discovery of X-rays in 1895. With the sophisticated imaging tools of today, such as magnetic resonance imaging (MRI), computed tomography (CT), positron emission tomography (PET) and ultrasonography (US), the diagnosis and recognition of disease has evolved tremendously.1,2 Traditionally, diagnostic imaging has focused on the detection and visualization of the ultimate effects of a disease. The rapidly growing discipline of molecular imaging aims to probe fundamental molecular processes at the early stage of diseases, leading to efficient therapy.2,6 Through early diagnosis, the need for exploratory surgery would also decrease, if not be completely eliminated, thereby improving patient care and reducing medical costs. Molecular imaging uses molecular probes in vivo. The attachment of various labels to target-specific ligands permits in vivo diagnosis based on a combination of existing imaging tools, resulting in an increased understanding of the pathophysiology on a molecular level.1, 2,7

MRI has become one of the prominent non-invasive imaging techniques for disease diagnosis. Its advantages include a high spatial resolution, a non-ionizing radiation source, and the ability to extract, simultaneously, physiological and anatomical information of soft tissue. However, a major limitation of MRI remains its inherent low sensitivity. To increase the sensitivity of MRI, scientists have developed non-toxic contrast agents over the last few decades. So far, the Federal Drug Agency (FDA) and European Medicines Agency (EMEA) have approved only low molecular weight (MW)
Gd(III) complexes of precise structure as MRI contrast agents, such as Gd(III)DTPA (DTPA = diethylenetriaminepentaacetic acid) and Gd(III)DOTA (DOTA = 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid) (Fig. 1).

These Gd(III) complexes are currently the most widely applied contrast agents for general MRI and are appreciated for their predominant positive signal enhancement. Nowadays, approximately one-third of MRI studies are performed using low MW Gd(III) complexes. Unfortunately, the non-specificity, low contrast efficiency and fast renal excretion of these MRI contrast agents require a high dosage. These aspects severely limit the utility of these materials for molecular MRI. One method to increase the contrast and reduce the required dosage is to attach multiple MRI labels to a single scaffold.

This concept led to research being undertaken in the area of functional polymers bearing multiple contrast agent moieties. The large MW distribution in synthetic linear polymers pre-

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Fig. 1

Chemical structures of (a) Gd(III)DTPA and (b) Gd(III)DOTA.

Fig. 2

Multivalent target-specific MRI contrast agents for the specific accumulation of MRI contrast agent at a region of interest.
Dendrimers are multivalent macromolecules with a regular, highly branched structure, and nanoscopic dimensions (2–10 nm) resembling those of proteins. These structures are synthesized via a “cascade” synthesis using an iterative sequence of reaction steps. In the early 1980s Denkewalter et al. patented the synthesis of L-lysine-based dendrimers. The first dendritic structures that were thoroughly investigated and received widespread attention were Tomalia’s poly(amidoamine) (PAMAM) dendrimers and Newkome’s “arborols.” All of these dendrimers are synthesized according to a divergent synthetic approach, in which the synthesis is started from a multifunctional core and is elaborated to the periphery. Later on, Hawker and Fréchet introduced the convergent approach for the synthesis of aromatic poly(ether) dendrimers. In the convergent procedure, the dendritic wedges are first synthesized and subsequently attached to a multifunctional core. Although the yields obtained using the convergent procedure are, in general, lower than for the divergent procedure, the purity of the dendrimers is higher.

In 1993, in a continuation of the original work of Voegtle et al., Müller et al. and Meijer et al. independently reported a divergent approach for the synthesis of poly(propylene imine) (PPI) dendrimers (Fig. 3).

Even though many other types of dendritic systems have been synthesized, the dendrimers mentioned above are the most frequently used and well-studied. The developed synthetic strategies to dendrimers allow the introduction of a precise number of functional groups to the core, within the branches and/or along the periphery. The introduction of functional groups into the dendritic framework can have a great impact on its physicochemical properties, such as its rigidity and solubility. Many of the fascinating properties, as well as the synthesis and possible applications of dendrimers, have been described in books and reviews by various experts in the field.

Dendrimers for MRI

The well-defined nature of dendritic architectures and their multivalent properties has intrigued researchers into using dendrimers in the biomedical arena. Dendritic structures have been actively applied for diagnostic and therapeutic purposes, as well as for drug delivery vehicles.

and other applications such as tissue engineering and light harvesting.

In recent years, a number of research groups have explored the use of dendrimers as a new class of macromolecular MRI contrast agents. The efficiency of MRI contrast agents is often expressed in terms of their longitudinal relaxivity (r1/mM⁻¹ s⁻¹), i.e. their ability to shorten the longitudinal relaxation time of protons of water molecules (T₁/s). In eqn. (1), (1/T₁)observed is the observed longitudinal relaxation rate in the presence of contrast agent, [Gd(III)] is the concentration of Gd(III) and (1/T₁)dia = (1/T₁)diamagnetic is the diamagnetic longitudinal relaxation rate (in the absence of paramagnetic species).

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\frac{1}{T_1}_{\text{observed}} = \frac{1}{T_1}_{\text{diamagnetic}} + r_1 [\text{Gd(III)}] \quad (1)
\]

In seminal work, Wiener et al. reported the synthesis of different generations of Gd(III)DTPA-based PAMAM dendrimers (Fig. 4(a), Gd(III) complex of 1). Their sixth generation dendritic MRI contrast agent (MW = 139 kg mol⁻¹) displayed an r₁ of 34 mM⁻¹ s⁻¹ (0.6 T, 20 °C), which was six times higher than the r₁ of Gd(III)DTPA (MW = 0.55 kg mol⁻¹, r₁ = 5.4 mM⁻¹ s⁻¹). This strong increase in r₁ was ascribed to the lower molecular tumbling rate of the Gd(III)DTPA complex at the periphery of the dendrimer, as evidenced from the increase in the rotational correlation times. Interestingly, no increase in r₁ value was observed for flexible macromolecular polymers of comparable molecular weight, implying that segmental motion dominates the rotational correlation time. Bryant et al. investigated the relationship between r₁ and the molecular weight of the dendritic MRI contrast agent using different generations of Gd(III)DOTA-based PAMAM dendrimers. In that case, a plateau value for r₁ of 36 mM⁻¹ s⁻¹ (0.47 T, 20 °C) was reached for the seventh generation of Gd(III)DOTA-based dendrimer (MW = 375 kg mol⁻¹). Moreover, it was demonstrated that r₁ of the seventh generation dendrimer increases with increasing temperature, indicating that slow water exchange limits the relaxivity. Rudovský et al. studied the effect on r₁ of the ionic interactions between negatively-charged Gd(III)-based PAMAM dendrimers (Fig. 4(b), Gd(III) complex of 2) and positively-charged poly(aminoacids). Titrination experiments on the second generation dendritic contrast agent with poly(arginine) showed an increase in r₁ from 20 to 28 mM⁻¹ s⁻¹ (0.47 T, 20 °C). This effect was attributed to a decrease in the mobility of the Gd(III) complex, induced by interactions between the anionic dendrimer and the cationic poly(arginine).

A series of Gd(III)DTPA-functionalized PPI dendrimers was reported by Kobayashi et al. (Fig. 4(c), Gd(III) complex of 1). The authors demonstrated that r₁ almost linearly increased with the molecular weight of the dendrimer without reaching a plateau value, eventually resulting in a r₁ value of 29 mM⁻¹ s⁻¹ (1.5 T, 20 °C) for the fifth generation of dendritic contrast agent. Later on, we reported a novel series of Gd(III)DTPA-based PPI dendrimers utilizing a different linker between the Gd(III) complex and the dendrimer (Fig. 4(c), Gd(III) complex of 3). Also, for these dendrimers, a significant increase in r₁, though not as pronounced as for the dendritic MRI contrast agents reported by Kobayashi et al., was observed, while the...
molecular weights of both systems were comparable (fifth generation: \( r_1 = 20 \text{ mM}^{-1} \text{s}^{-1}, 1.5 \text{ T and } 20 \text{ °C} \)). Furthermore, in our case, “saturation” of \( r_1 \) upon increasing the generation of the dendrimer was observed, in contrast to the study of Kobayashi et al.\(^{55} \) This suggests that the linker between the Gd(III) complex and the dendrimer has a large effect on the overall relaxivity.

Researchers at Schering AG (Berlin, Germany) have developed a lysine-based class of dendritic contrast agents: Gadomer-17\(^{68–73,75–81} \) (\( r_1 = 15.2 \text{ mM}^{-1} \text{s}^{-1}, 1.5 \text{ T and } 37 \text{ °C} \)) and Gd(III)DTPA-24-cascade-polymer. These macro-molecular MRI contrast agents were synthesized from a trimesoyltriamide central core, to which 18 lysine amino acid residues were introduced. Gadomer-17\(^{68–73,75–81} \) consists of 24 N-nanosubstituted Gd(III)DO3A moieties (DO3A = 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid) (Fig. 4(d), Gd(III) complex of 4), whereas Gd(III)DTPA-24-cascade-polymer contains 24 Gd(III)DTPA complexes.

In the previous examples, dendrimers have shown to be suitable synthetic scaffolds for the incorporation of multiple Gd(III) moieties, leading to an improved sensitivity for MRI in terms of \( r_1 \). These conclusions are based on measurements at current magnetic fields of 0.5–1.5 T. The comprehensive studies of Merbach et al. have shown that dendritic MRI contrast agents exhibit NMRD profiles with maximum \( r_1 \) values at these magnetic fields.\(^{87,88} \) However, at high magnetic fields of 10 T, the \( r_1 \) values of dendritic contrast agents are substantially lower, not exceeding the \( r_1 \) values of low molecular weight Gd(III)-based complexes.

### Biocompatibility of dendrimers

The biocompatibility of dendrimers is an important issue when in vivo applications are considered.\(^{38} \) Recently, in vitro studies have shown that amine-terminated PPI and PAMAM dendrimers are cytotoxic, in particular the higher generations of

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**Fig. 4** Dendritic MRI contrast agents with multiple Gd(III) complexes along the periphery.
protonated (cationic) dendrimers (IC\textsubscript{50} for DAB-dendr-(NH\textsubscript{2})\textsubscript{64} < 5 \mu g mL\textsuperscript{-1}).\textsuperscript{93} These results are in agreement with the haematotoxicity studies of Malik \textit{et al.}\textsuperscript{94} In their work, it was concluded that the haemolytic effect of PAMAM and PPI dendrimers on rat blood cells increased considerably as a function of generation.\textsuperscript{94} These effects may be attributed to favorable interactions between positively-charged dendrimers and the negatively-charged cell membranes.\textsuperscript{95} On the other hand, PPI and PAMAM dendrimers functionalized with carboxylate end groups at the periphery are neither cytotoxic nor haemolytic up to a concentration of 2 mg mL\textsuperscript{-1}. This suggests that the overall toxicity of dendritic structures is strongly determined by the functionalities along the periphery. To date, only a few systematic studies on the \textit{in vivo} toxicity of dendrimers have been reported. Remarkably, the general trend is that PAMAM dendrimers (up to the fifth generation), either unmodified or modified with chemically inert surface moieties, do not appear to be toxic in mice.\textsuperscript{96} Furthermore, peptide-functionalized poly(lysine) dendrimers were also found to be biocompatible.\textsuperscript{97}

\textit{In vivo} MRI

The aforementioned dendritic MRI contrast agents have been evaluated in animal models for high resolution MRI.\textsuperscript{51,55-71,75,98} Several \textit{in vivo} MRI studies have shown that the higher generations of dendritic MRI contrast agents, in contrast to low MW Gd(\textit{iii}) chelates, remain in high concentrations in the bloodstream for longer periods of time. This results in an improved visualization of vascular structures. Due to the fact that high MW contrast agents show little extravasation and intravascular retention, they are commonly referred to as blood pool agents, while low MW contrast agents are referred to as extravascular agents (Fig. 5).\textsuperscript{52,54}

Kobayashi \textit{et al.} demonstrated that Gd(\textit{iii})DTPA-terminated PPI dendrimers are suitable for \textit{in vivo} MR angiography, lymphography, the evaluation of MRI contrast agent distribution and clearance, and as biometric nanoprobes to detect vascular permeability.\textsuperscript{55,64-67,83} Gadomer-17\textsuperscript{68} is currently in clinical development for blood pool imaging.\textsuperscript{68-71,75}

Clearance by the kidneys to prevent the undesired accumulation of Gd(\textit{iii}) in the body is important for the \textit{in vivo} application of MRI contrast agents. From several studies, it has become clear that the pharmacokinetic properties of dendritic MRI contrast agents strongly depend on the generation, the nature of the dendritic scaffold and its overall charge.\textsuperscript{55,60,66,98} For instance, dynamic contrast-enhanced MR images with different generations of Gd(\textit{iii})DTPA-terminated PPI dendrimers (Fig. 4(c), Gd(\textit{iii}) complex of 3) have shown that the first generation dendritic contrast agent is rapidly cleared from the renal system and accumulates in the bladder, whereas higher generations are cleared from the renal system at a slower rate (Fig. 6).\textsuperscript{98,99}

\textbf{Dendritic target-specific MRI contrast agents}

The dendritic MRI contrast agents discussed in the previous paragraphs are excellent blood pool agents. However, these structures lack the specificity required for molecular MRI.\textsuperscript{100} The development of target-specific MRI contrast agents, directed to defined molecular markers, could dramatically improve the imaging of a specific disease, due to the accumulation of MRI contrast agent at the region of interest.\textsuperscript{101} For molecular MRI, the local concentration of receptors is often too low to reach detectable concentrations of monovalent target-specific contrast agent. A strategy to compensate for insufficient accumulation is to attach multiple MRI labels to the targeting unit. Important classes of targeting units that have already been introduced at the periphery of dendrimers are polysaccharides,\textsuperscript{102-105} oligopeptides,\textsuperscript{97,106-108} proteins,\textsuperscript{106,109} antibodies,\textsuperscript{110} oligo-nucleotides\textsuperscript{111} and folic acid.\textsuperscript{112-115} So far, only a few examples of target-specific dendritic MRI contrast agents have been described.
Konda \textit{et al.} reported a Gd(III)DTPA-based PAMAM dendrimer with, on average, one or two folate moieties (Fig. 7).\textsuperscript{114} \textit{In vivo} MRI in mice with ovarian tumors expressing the folate receptor resulted in a significant signal enhancement using this dendritic contrast agent, while no enhancement was observed for mice with folate receptor negative tumors.\textsuperscript{112–115}

A conceptually different approach to Gd(III)DTPA-based dendrimers, using immobilized Gd(III) at the interior of the dendritic framework, has been described by Takahashi \textit{et al.}\textsuperscript{116} They reported the synthesis of dendrimers with twelve D-glucose derivates along the periphery and one Gd(III) complex at the interior (Fig. 8). The authors speculated that the high local concentration of carbohydrates might further improve the binding affinity, due to multivalent interactions between the polysaccharide and its receptor, \textit{i.e.} the glycoside cluster effect. In general, the concept of multivalency is of particular interest when the interaction between a targeted unit and its marker is rather weak.\textsuperscript{117–119}

\section*{Perspectives}

Future challenges in the field of \textit{MRI} contrast agents involve the rational design and synthesis of multivalent target-specific structures (Fig. 9).\textsuperscript{120} The combination of target-specific ligands and the attachment of multiple \textit{MRI} labels to a single scaffold is anticipated to be beneficial for the accumulation of \textit{MRI} labels at regions of interest, as well as for the generation of a detectable \textit{MR} signal. For this purpose, novel synthetic strategies have to be developed. The desired combination of size and orthogonal peripheral functionality can be obtained via a general modular synthetic approach, enabling the optimization of \textit{MRI} contrast and binding affinity. Moreover, the combination of \textit{MRI} labels and other imaging labels (\textit{e.g.} for optical imaging or PET) on a single scaffold would allow bimodality in imaging, thereby integrating the advantages of different imaging techniques.\textsuperscript{121} For these applications, not only is the synthesis a real challenge, but also novel characterization techniques to analyze these complicated architectures must be developed. Dendrimer formulations used in humans must conform to current Good Manufacturing Practice (cGMP) to ensure their correct identity, strength, quality and purity. These regulatory hurdles are often difficult to overcome.

Ultimately, by tuning the ratio between \textit{MRI} labels and targeting units, the efficacy of target-specific dendritic contrast agents may be optimized. Dendrimers are uniquely qualified to establish this in a site-specific and controlled fashion. Recently, Hawker \textit{et al.} demonstrated this concept by merging a carbohydrate-functionalized dendritic wedge with a bivalent fluorescent label using click chemistry,\textsuperscript{122} while our group demonstrated the use of native chemical ligation to construct dendrimers with multiple ligands for gadolinium and multiple oligopeptides for targeting.\textsuperscript{123} It is our strong belief that the concept of combining multivalency for targeting, nanoscale size and multiple ligands for \textit{MRI} will bring molecular

\begin{figure}[h]
\centering
\includegraphics[width=\columnwidth]{figure8}
\caption{A dendritic structure with Gd(III) at the interior and multiple carbohydrates along the periphery, as reported by Takahashi \textit{et al.}\textsuperscript{116}}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\columnwidth]{figure7}
\caption{Target-specific dendritic \textit{MRI} contrast agents based on the fourth generation PAMAM dendrimer with, on average, two folate moieties along the periphery.}
\end{figure}
For this research, target-specific, well-designed dendrimers, either covalently modified or modified by supramolecular interactions, will lead to much success in the near future.

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Fig. 9 Multivalent target-specific MRI contrast agents. (a) A supramolecular approach to multivalent target-specific contrast agents based on a biotinylated oligopeptide equipped with Gd(III)DTPA and avidin; (b) dendritic MRI contrast agents composed of Gd(III)DTPA and oligopeptides; (c) protein-based dendritic contrast agents; (d) target-specific dendritic contrast agents based on the ligation of two different dendritic wedges.