Biodegradable dendritic polymersomes as modular, high-relaxivity MRI contrast agents†‡

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A modular approach combining nanoscale components that each contribute to an enhancement in Gd(III) relaxivity is described here. Small molecule and dendritic alkyne derivatives of a Gd(III) complex were conjugated to the surfaces of biodegradable polymersomes resulting in ionic relaxivities of 10.6 mM$^{-1}$ s$^{-1}$ and 26.1 mM$^{-1}$ s$^{-1}$ respectively.

The early detection and diagnosis of disease is currently one of the major challenges in medicine. Clinical imaging plays a significant role in this process. Among the various imaging modalities, magnetic resonance imaging (MRI) has become a well-established and powerful tool, due to its excellent spatial resolution and soft tissue contrast. To aid in the differentiation between healthy and diseased tissues, small molecule Gd(III) chelates such as Magnevist™ are used in approximately 50% of MRI scans. While the availability of these agents has enabled significant developments in MRI, they do suffer from some significant limitations. For example, they typically possess longitudinal relaxivities ($r_1$) in the range of 3–5 mM$^{-1}$ s$^{-1}$, only a small fraction of the theoretically possible values.1 This results in the requirement for very large doses of these agents and also limits their applicability in molecular imaging.2 In addition, most of these agents are non-targeted and have very short circulation half-lives in the blood.

To address these limitations, Gd(III) complexes have been conjugated to a wide variety of macromolecular scaffolds including dendrimers,3,4 linear polymers,5 proteins,6 viral particles,7 micelles,8,9 liposomes,10,11 and polymersomes.12–14 This can result in improvements in $r_1$ values due to the slower tumbling rates of macromolecules and the resulting increases in the rotational correlation times of the Gd(III).15,16 In addition, macromolecular systems can exhibit prolonged blood circulation times, enabling the targeting of tissues either passively or actively through the conjugation of targeting ligands. Among the available macromolecular assemblies, block copolymer vesicles, commonly referred to as polymersomes, have attracted significant attention due to their potential multifunctionality. They possess an aqueous core capable of encapsulating water-soluble species, a hydrophobic membrane that can encapsulate hydrophobes, and a surface to which specific targeting ligands or other species can be conjugated. Thus far, there are very few reports of polymersome-based MRI contrast agents.12–14 Cheng et al. have investigated porous polymersomes containing Gd(III)-labeled dendrimers within their aqueous cores, and have obtained an $r_1$ of 7.5 mM$^{-1}$ s$^{-1}$ (60 MHz, 40 °C) on a per Gd basis.12,13 More recently, Grull et al. incorporated Gd(III)-labeled lipids into a polymersome membrane, resulting in an $r_1$ of 22 mM$^{-1}$ s$^{-1}$ (20 MHz, 25 °C).14

Our group has developed approaches for the functionalization of polymersome surfaces with dendritic groups.17,18 It was shown that this is an effective method for tuning the surface chemistries of polymersomes in a single step, resulting in properties such as enhanced target binding and cell uptake.19,20 For the current work, it was proposed that polymersome-immobilized dendrons functionalized with Gd(III) complexes may serve as highly efficient MRI contrast agents. While conjugation of Gd(III) complexes to high generation dendrimers is known to enhance their relaxivities,3,4 immobilization on the polymersome surface should provide further enhancements, at the same time opening the possibility to exploit the multifunctional properties of polymersomes. We describe here the preparation of the dendron and polymersome components, and studies of their relaxivities. It is demonstrated how nanoscale components can be readily combined to provide additive enhancements in relaxivity.

Fig. 1 depicts the general approach for the preparation of the polymersome MRI contrast agents. Polycaprolactone-poly(ethylene oxide) (PCL-PEO) block copolymers were selected due to the biodegradability of the PCL block and the well demonstrated biocompatibility of PEO in various applications.21 Methoxy- (I) and azide-terminated (2) PCL-PEO were prepared as previously reported and were assembled into azide-functionalized vesicles (3).18 The z-average diameter of the polymersomes was 140 nm, as measured by dynamic light scattering (DLS). Gd(III)-functionalized dendron 4, having a focal point alkyne, was prepared starting from the third generation polyester dendron 8.17 As shown in Scheme 1, the peripheral amine groups of 8 were reacted with the commercially available diethylenetriaminepentacetic acid (DTPA) isothiocyanate...
derivative 9. The resulting dendron 10 was then treated with GdCl₃ to provide the target dendron 4.

As shown in Fig. 1, in order to determine the contribution of the dendron versus polymersome to the relaxivity, it was also desirable to prepare a non-dendritic alkyne derivative of the Gd(III) chelate (5). As shown in Scheme 2, this was accomplished by the reaction of propargyl amine with 9 to provide 11, followed by chelation of Gd(III).

Prior to Gd(III) insertion, compounds 10 and 11 were characterized by ¹H and ¹³C NMR spectroscopy. In addition, infrared (IR) spectroscopy was informative for this class of compounds. For example, upon conversion of dendron 8 to 10, the absence of the characteristic C=S stretch of the isothiocyanate functional group that was present in compound 9 confirmed the successful removal of excess 9 (ESI†). After insertion of Gd(III), NMR spectroscopic analysis was no longer possible due to the paramagnetic nature of the Gd(III) ion. However, inductively coupled plasma mass spectrometry (ICP-MS) was performed, confirming the successful insertion of Gd(III) into dendron 4 and compound 5. In addition, IR spectroscopy demonstrated that the peaks corresponding to the C=O stretches of the ligand shifted to significantly lower frequencies in compounds 4 and 5 relative to 10 and 11, respectively. This is also an indication of successful coordination of the carboxylate groups to Gd(III).²²

The next step was to conjugate 4 and 5 to the polymersome surfaces. This was accomplished by a Cu(I) mediated 3 + 2 "click" cycloaddition to provide the dendritic Gd(III)-functionalized polymersomes 6, and non-dendritic Gd(III)-functionalized polymersomes, 7, respectively. Unreacted 4 and 5 were removed by dialysis. ICP-MS measurements were performed on the products and the results indicated that 38% of the azide groups were functionalized in polymersomes 6 and 26% in polymersomes 7. The sizes and morphologies of the resulting polymersomes were evaluated by DLS and transmission electron microscopy (TEM). Small increases in the z-average diameters to 158 and 156 nm were found for vesicles 6 and 7, respectively (ESI†). TEM (ESI†) showed that the vesicular morphology was preserved and the contrast was enhanced upon incorporation of the Gd(III) in both dendritic and non-dendritic polymersomes.

The properties of the three newly developed contrast agents (4, 6, and 7) were then assessed in phosphate buffer (0.1 M, pH 7.4) at 298 K (Fig. 2) and 310 K (ESI†) between 0.01 and 42 MHz using a field cycling relaxometer. On a per Gd(III) basis, dendron 4 and polymersomes 6 and 7 exhibited r₁ values of 12.1 ± 0.3, 26.1 ± 1.2, and 10.6 ± 0.4 mM⁻¹ s⁻¹, respectively (20 MHz, 298 K). In comparison with the clinical agent Magnevist® (Gd(III)-DTPA) which has a reported relaxivity of 4.6 mM⁻¹ s⁻¹ under the same conditions,²³ this corresponds to 2.6-, 5.7-, and 2.3-fold increases in r₁ for dendron 4, and polymersome 6 and 7, respectively. All of the systems exhibit an r₁ versus frequency curve shape that is characteristic of restricted tumbling motion of the Gd(III) complex.¹

While polyester dendrons such as 4 have not previously been investigated as MRI contrast agents, the r₁ value of 12.1 mM⁻¹ s⁻¹ is within the range expected for a third generation dendrimer.³,⁴ As
with other dendrimers, this enhancement can likely be attributed to the crowded nature of the dendron periphery, which inhibits the free rotation of the Gd(III) complexes. The enhanced $r_1$ value of 10.6 mM$^{-1}$ s$^{-1}$ obtained for the non-dendritic polymersomes 7 is also likely a result of the hindered motion of the Gd(III) complexes at the vesicle surface, as well as the slow tumbling rate of the entire vesicle system. This $r_1$ value is lower than the value of 22 mM$^{-1}$ s$^{-1}$ at (20 MHz, 25 °C) obtained by Grüll et al. with lipid functionalized Gd(III) chelates incorporated into polymersomes. This is probably because their chelates were attached directly to the lipids, rather than through a long linker. The PEO chains in the current work introduce flexibility, which can decrease the rotational correlation time. PEO surrounding the chelate may also slow water exchange. However, $r_1$ is higher than for the system reported by Cheng et al. which contained Gd(III) complexes within the polymersomes. This can be attributed to our selective attachment of the chelates to the periphery, where they are easily accessible to bulk water. When both the dendritic component and the polymersome component are combined in polymersome 6, the resulting $r_1$ of 26.1 mM$^{-1}$ s$^{-1}$ is the highest reported relaxivity for a polymersome system. This additive effect can result from the availability of chelates at the vesicle surface for water exchange, hindered motion of the Gd(III) complexes imparted by the dendron at a local level, and the large size and slow tumbling rate of the polymersome at the nanoscale level. Thus, this work elegantly demonstrates that different components can be combined through rational design to obtain additive effects on the relaxivity. An additional feature of the current system relative to those previously reported is the biodegradability imparted by the PCL block of the copolymer and the polyester dendron which is known to break down over a period of several days in physiological conditions. This should enable the release of low molecular weight Gd(III) complexes and polymer products from the body, an important consideration for MRI contrast agents.

In conclusion, through the synthesis of dendritic and non-dendritic Gd(III) chelates and their conjugation to polymersome surfaces, three new MRI contrast agents were developed. Using these systems, the effects of the dendritic and polymersome components on the relaxivities of the agents were elucidated. They were found to have an additive effect, resulting in the highest currently reported $r_1$ for a polymersome system. In addition, this system possesses the advantage of being composed of PEO and biodegradable polyester components. Future work will be aimed at exploring the biodegradability and \textit{in vivo} properties of the system as well as exploiting the multifunctional capabilities of polymersomes.

References