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Dy-DOTA integrated mesoporous silica nanoparticles as promising ultrahigh field magnetic resonance imaging contrast agents

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Integrating Dy-DOTA motifs into mesoporous silica nanoparticle scaffolds generates remarkable ultrahigh field T_2 relaxivities for a well-defined and tailorable contrast agent, attributed to enhanced Curie outer-sphere contributions and supported by simulation.

Magnetic resonance imaging (MRI) is a powerful noninvasive imaging technique in clinical diagnosis and biomedical research.^{1, 2} Currently a moderate magnetic field strength (0.5–3 T) dominates in clinical examination.³ However, since the signal-to-noise and, therefore, spatial/temporal resolution natively increase with field strength, ultrahigh field (>3 T) MRI is increasingly utilized,^{4, 5} especially in preclinical applications where the use of 21 T has been reported⁶ and 7.0–11.7 T is common. It has been suggested that the future of MRI resides in high field imaging where anatomical detail replicates that associated with classic histological slices.³ Exogenous contrast agents (CAs) can shorten the T_1 and T_2 relaxation times of local protons and, thus, are often introduced to focus and increase image contrast.^{7–9} Currently, Gd-based T_1 and iron oxide nanoparticle (IONPs)-based T_2 CAs are predominately employed, but their efficacies generally decline markedly at higher field.^{10, 11} Gd³⁺ is well known for its T_1 relaxation enhancing capability due to its isotropic 4f electron configuration and long electronic relaxation time (τ_S), but the r_1 relaxivity of Gd-chelates T_1 agents rapidly drops at higher fields (as much as one-third).^{4, 10, 12} Similarly, the r_1 value of GdOF nanoparticles decreases from 58.6 mM^{−1} s^{−1} at 3 T to 25.2 mM^{−1} s^{−1} at 7 T.¹³ IONPs accelerate the dephasing of adjacent protons through induced strong magnetic field gradients but their magnetization usually saturates below 1 T, resulting in no significantly improved r_2 relaxivity at higher fields.^{11, 14} For

instance, with field strength increasing from 0.5 to 4.7 T, the r_2 of Feridex[®] does not increase but decreases from 152 to 105 mM^{−1} s^{−1}. There is, then, substantial room for, and value in, generating CAs which enhance contrast performance at high field.

Among the paramagnetic lanthanide ions (Ln³⁺), Dy³⁺ has the highest effective magnetic moment ($\mu_{\text{eff}} = 10.6 \mu_B$) and shortest electronic relaxation time ($\sim 10^{-13}$ s),⁵ making it a promising candidate for T_2 contrast enhancement at ultrahigh fields.^{15–17} Molecular Dy-complexes (e.g. Dy-DOTA, Dy-DTPA and Dy-DTPA-BMA),^{5, 18–20} as the analogues of classical Gd-complexes, have been investigated, but are associated with very small r_2 relaxivities (less than 20 mM^{−1} s^{−1} at 7 T) and a short retention time in the body.²¹ Dy-based inorganic nanocrystals (e.g. Dy₂O₃,²² NaDyF₄,^{16, 23} and DyF₃²⁴) have been widely studied with an emphasis on their tunable size and morphology, and exhibit large r_2 relaxivities (>100 mM^{−1} s^{−1}) at high field. In contrast to the high thermodynamic and kinetic stability afforded by Dy-molecular complexes, the pharmacokinetics and biosafety profiles of Dy-based nanocrystals have not, however, been addressed.^{25, 26}

Recently, Dy-DOTA moieties immobilized on tobacco mosaic virus nanotubes were reported to present an r_2 relaxivity >300 mM^{−1} s^{−1} at 7 T,²⁷ suggesting the Dy³⁺ tethering to nanoscale bulk as a route to augment T_2 contrast. The immobilization of molecular Gd-species onto larger scaffolds has become a somewhat standard means of increasing r_1 relaxivity, largely by virtue of greatly increased hydrodynamic radius and rotational correlation time (τ_R).^{28–30} However, the principle of relaxation enhancement by Dy-based systems differs from that of Gd-based systems, and the underlying mechanism of such tethering-induced relaxivity enhancements has not been demonstrated. To develop a generalized relaxivity-enhancing strategy, relevant to high field performance, herein we employ Dy-DOTA decorated mesoporous silica nanoparticles (MSNs), tuning and optimising the location of the metal and systematically investigate performance across a range of external field strengths. The

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dramatically enhanced T_2 relaxivity of Dy-loaded MSNs is presented, utilising Dy-DOTA as a reference. Through accompanying theoretical simulation, the underlying mechanism is analysed. Although it has been noted that Curie effects are important in inner sphere relaxivity at high solution concentrations of molecular Dy(III) complexes³¹, the impact herein on outer sphere contrast is, we believe, without precedent and a reflection of both reduced inner sphere contributions in spatially restricted nanoparticle pores and the high levels of paramagnetic loading.

Dy-DOTA was attached onto MSNs by a covalent coupling using surface amino group anchor points (Fig. 1a, see ESI† for details). Pristine MSNs were synthesized by a classical aqueous Stöber method²⁸ using cetyl trimethylammonium bromide (CTAB) as a surfactant template. Aminated MSNs were acquired by 3-aminopropyltriethoxysilane (APTES) addition into the reaction solution to form internally localized amino groups.

Fig. 1 Schematic representation (a) of Dy-loaded MSNs and (b) anchor point localisation. Dy-MSNs-S are prepared using a “Short delay” co-condensation method localising anchor points deep in the particle, while “Long delay” Dy-MSNs-L have amine anchor points nearer to the pore openings. Dy-MSNs-P, prepared by post-grafting, have externally localised anchor points.

In utilising co-condensation with a short delay (10 min), amino groups are localised deeply in particle channels, while an analogous long delay (60 min) results in a location nearer to the pore openings (Fig. 1b).³² Alternatively, APTES can also be post-grafted onto the surface of pristine MSNs to form externally localised anchor points (Fig. 1b). Transmission electron microscopy (TEM, Fig. S1) images show that the obtained MSNs are uniform with an average particle diameter of 55.7 ± 5.6 nm (Fig. S2). Nitrogen adsorption-desorption isotherm measurements confirm an average pore size of ca. 3.2 nm (Fig. S3). Importantly, particle size, morphology and pore size are retained across the tuning of amino localisation. No characteristic peaks of CTAB can be observed by Fourier-

transform infrared spectroscopy (FT-IR), confirming that templating surfactant removal is complete after several cycles of washing (Fig. S4). Dynamic light scattering (DLS) analysis confirms that all prepared Dy-loaded MSNs can be readily dispersed in aqueous solution and have similar hydrodynamic diameters (160.8 ± 1.9 to 175.3 ± 1.2 nm) as well as zeta potentials (-32.0 ± 0.6 to -32.8 ± 0.5 mV; note that these zeta

potentials are unaffected by the 0.15 mol% levels of amination; Table S1).

Fig. 2 a) Transversal relaxivities of Dy-DOTA and Dy-loaded MSNs measured at 11.7 T, b) Image phantoms obtained by MRI at 7 T for H₂O, Dy-DOTA and Dy-loaded MSNs samples (all samples in 10 mM phosphate buffer pH = 7.2 at [Dy] = 0.01 mM in all cases).

To evaluate the proton relaxation enhancing performance of Dy-loaded MSNs, Dy-DOTA was used as a reference. First, r_2 and r_1 relaxivities were measured at 7 T (calculated per mM of Dy³⁺, Fig S5). Dy-loaded MSNs show a 20-fold increase over Dy-DOTA for r_2 whilst r_1 is negligible in all cases as it expected. As shown in Fig. 2a, the r_2 relaxivity of Dy-DOTA at 11.7 T is only $10.02 \pm 2.9 \text{ mM}^{-1} \text{ s}^{-1}$, in agreement with previous reports.³² In contrast, all of Dy-loaded MSNs show a dramatically enhanced r_2 values regardless of the Dy³⁺ localisation. For instance, the r_2 value of Dy-MSNs-L is almost 20-fold of that of Dy-DOTA at the same field strength. Among the Dy-loaded MSNs, Dy-MSNs-L and Dy-MSNs-P exhibit notable r_2 values of 143.5 ± 8.2 and $140.03 \pm 5.3 \text{ mM}^{-1} \text{ s}^{-1}$ respectively, while Dy-MSNs-S shows a slightly lower relaxivity of $124.02 \pm 6.4 \text{ mM}^{-1} \text{ s}^{-1}$. This phenomenon is qualitatively the same as that previously reported in analogous Gd structures,³³ where the diminished r_2 relaxivity of Dy-MSNs-S can be attributed to the reduced water access when Dy is deeply immobilized in the channels. These r_2 values are more notable still when compared to the limited prior reports of conventional IONPs at high fields; no data at 11.7 T has been reported for these.³⁴ To facilitate a broader comparison to other works, r_2 values were also calculated per mM of nanoparticle (Table S2).

To confirm the ability of Dy-loaded MSNs to produce effective negative contrast, phantom images were acquired by MRI at 7 T. A high contrast is produced by Dy-loaded MSNs whilst no

contrast is observed for Dy-DOTA (Fig 2b). Dy-MSNs-L and Dy-MSNs-P show higher contrast in agreement with the observed r_2 values. A semi-quantitative analysis of the images confirms these results; a comparison between H₂O and Dy-DOTA intensities shows insignificant contrast whilst Dy-loaded MSN samples exhibit very significant intensity differences in comparison with water or DOTA-Dy (Fig S6, two-tailed t -test, ****, $P < 0.0001$).

The r_2 relaxivity of paramagnetic lanthanides (except Gd³⁺) should increase linearly with the square of external magnetic field strength (B_0^2).^{15–17} To investigate this here, the magnetic character of Dy-loaded MSNs was first examined. No magnetic transition down to 5 K can be observed from the M – T curve (Fig. S7a) and both coercivity and remanence are close to zero in the hysteresis loop (Fig. S7b), confirming a paramagnetic character. In subsequently assessing relaxivities, a wide range of field strengths from 1.4 to 11.7 T were applied (Fig. 3). For both Dy-DOTA and Dy-loaded MSNs, r_2 increases with field strength proportional to B_0^2 ($R^2 > 0.95$ for all). The r_2 value of Dy-DOTA increases to 10.0 ± 2.9 mM^{−1} s^{−1} at 11.7 T with a slope of 0.07, whilst that of Dy-MSNs-L increases to 143 ± 8.2 mM^{−1} s^{−1} at the same field with a 15-fold greater slope. The latter suggests that the field influence on relaxivity is more pronounced for Dy-loaded MSNs. Moreover, as the magnetization of Dy³⁺ is far from saturation at 11.7 T at room temperature,^{17, 22} one would expect yet higher r_2 relaxivity to be accessible. The progressively increasing r_2 with field strength endows these Dy-loaded MSNs with notable advantages over conventional T₂ CAs.^{11, 14}

Fig. 3 Transverse relaxivity of Dy-DOTA and Dy-loaded MSNs vs. the square of external magnetic field strength (1.4, 7, 9.4 and 11.7 T). The corresponding linear fitting is also shown. Dy-DOTA: slope = 0.07, $R^2 = 0.954$; Dy-MSNs-S: slope = 0.93, $R^2 = 0.952$; Dy-MSNs-L: slope = 1.07, $R^2 = 0.965$; Dy-MSNs-P: slope = 1.05, $R^2 = 0.954$.

To shed further light on the underlying mechanism associated with high r_2 here, theoretical simulations were employed. Generally speaking, relaxation rate enhancements induced by paramagnetic species are composite sums of inner-sphere (IS) and outer-sphere (OS) contributions.³⁵ The influencing parameters for inner-sphere contributions include the number of water coordinated to Ln³⁺ (q), the residence time of coordinated water before exchanging with bulk water (τ_M), the rotational correlation time (τ_R), the electronic

relaxation time (τ_S), the distance between Ln³⁺ and the Ln-bound water protons (r), all of which were integrated in simulating the inner-sphere contribution to r_2 (Fig. S8, see ESI† for simulation details). It is clear that, for both Dy-DOTA and Dy-loaded MSNs, the inner-sphere component only partially contributes to the overall r_2 relaxivity (Fig. 4). For instance, the inner-sphere contribution at 11.7 T represents 16% of r_2 relaxivity for Dy-DOTA and 20% for Dy-loaded MSNs. In both cases, the outer-sphere contribution is the predominant source of relaxation enhancement.

Fig. 4 Comparison between simulated inner-sphere contribution and experimentally observed total r_2 relaxivity for (a) Dy-DOTA and (b) Dy-loaded MSNs. The key simulation parameters are: $q = 1$, $\tau_M = 0.1$ μ s, $r = 0.3$ nm, $\tau_S = 0.33$ ps, $\tau_R = 56$ ps (for Dy-DOTA) and $\tau_R = 910$ μ s (for Dy-loaded MSNs).

Outer-sphere contributions comprise dipolar and Curie elements,¹⁷ the former being a spatial effect resulting from the dipolar coupling between the protic nuclear spins and the unpaired electrons of Ln³⁺, while the latter originates from the dipolar interaction between the nuclei and the thermal average of electron spin polarization, *i.e.* Curie spin. The simulation data reveal that the dipolar component is largely independent of magnetic field and has negligible contribution under high field (Fig. S9). Curie outer-sphere contributions thus dominate the r_2 relaxivity for both Dy-DOTA and Dy-loaded MSNs under high field.

Dy-loaded MSNs are, of course, distinct from Dy-DOTA through both dramatically increased hydrodynamic radius and the increased local Dy³⁺ (~16,000 Dy³⁺ ions per particle). To

separately investigate these effects we consider the Curie outer-sphere contribution (R_{2C}^{OS}) to be described by equation (1)

$$R_{2C}^{OS} = K_C \mu_{\text{eff}}^4 B_0^2 \theta(B_0) / a, \quad (1)$$

where K_C is a constant, μ_{eff} is the effective magnetic moment of the agent, and a is the hydrodynamic radius. The influence of μ_{eff} on relaxivity is marked and positive whilst Curie contributions actually fall as particle size grows (Fig. S10). In spite of their larger hydrodynamic radius, then, Dy-loaded MSNs display remarkably larger r_2 than Dy-DOTA as a result of the positive effect of augmented magnetic dipoles (the net local magnetisation is much higher in Dy loaded MSNs).

In summary, we have demonstrated that Dy-DOTA macrocycle motifs integrated into MSN scaffolds support a high-field T_2 relaxivity that is >20 fold higher than the molecular counterpart. In combining experimental and simulated results, we directly ascribe this to the effects of nanoscale confinement (high local paramagnetic density) on accentuated magnetisation. Similar effects have been noted in loading a high level of ultra-small SPIONs within polymeric nanoparticles.³⁶ However, this work represents the first example, to the best of our knowledge, of a paramagnetic contrast agent where Curie spin relaxation and outer-sphere effects dominate. This study not only illustrates the distinct mechanistic aspects of Dy-based agents, but also provides a general strategy to efficiently improve the r_2 relaxivity under high field. The particles used herein encapsulate thermodynamically stable Dy, enjoy good levels of chemical definition and marked chemical tuneability. We believe them to be of some considerable value in promoting effective high field MRI.

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Conflicts of interest

There are no conflicts to declare.

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TOC

Integrating Dy-DOTA motifs into mesoporous silica nanoparticle scaffolds remarkably amplifies the ultrahigh field T_2 relaxivity via a Curie outer-sphere mechanism.