Water Gated Contrast Switching with Polymer-Silica Hybrid Nanoparticles

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Bioresponsive contrast agents can provide smart signaling in MRI. In wrapping pore functionalised Gd-DOTA mesoporous silica nanoparticles with pH-responsive poly(acrylic acid), exchange between pore and bulk water can be reversibly switched by local pH. The generated high contrast then exhibits a large amplitude switch in a manner relevant to environmentally responsive imaging.

Magnetic Resonance Imaging (MRI) is a non-invasive imaging technique with excellent spatial resolution and deep tissue penetration.¹ In recent years focus has progressively moved towards the development of responsive MRI contrast agents (CAs) where developed signal intensity reports on their microenvironment.²⁻⁵ Among these, those based on Gd(III) are of particular interest because of their high associated positive contrast.^{6, 7} A responsive character can be integrated by engineering the CA such that a structural or dynamic parameter that sensitively governs its relaxivity, is pH-responsive. These are most typically hydration state, water exchange rate and rotational correlation time with relaxivity modulated as predicted by the Solomon-Bloembergen-Morgan (SBM) equations.⁸ A macrocyclic tetraamide phosphonate functionalised Gd³⁺ complex (DOTA-4AmP) has, for example, been reported to have an increased longitudinal relaxivity value (r_1) under acidic conditions in a manner related to the formation and disruption of a hydrogen bond network between phosphonate side arms and water associated with Gd³⁺ affecting prototropic exchange and thus, relaxivity.⁹ Other reported examples have used macrocyclic Gd³⁺ complexes with side arms functionalised with arylsulfonamide or aminoethyl moieties that ligate to the Gd³⁺ center in a pH dependent manner (changing metal hydration state).^{10, 11} These molecular strategies, though effective, are associated with only moderate relaxivities (2 – 10 mM⁻¹ s⁻¹) with the subsequent pH-dependent

change being < 50 %. It is also the case that, in the absence of detailed *in vivo* biodistribution data, it becomes difficult to ascertain the extent to which contrast effects are reporting of microenvironment rather than different concentration levels in the specific tissue. There is, then, substantial room to improve both the CA relaxivity and the degree to which it is responsive in species that additional provide a more facile route to ratiometric imaging. Although nanoparticulate species can be associated with challenges of reticuloendothelial system uptake, tissue penetration and biocompatibility, many of these are now addressable at the point of synthesis.^{12, 13} They also present an opportunity to access markedly higher levels of environmental response than is possible with traditional chelates. In relatively early work it was shown that amphiphilic Gd³⁺ complexes internalised into liposomes generated negligible contrast but switch under acidic conditions where an increased bilayer permeability and metal hydration turns “on” the T_1 contrast.¹⁴ There have also been several reports of a pH triggered irreversible particulate disassembly with a concurrent modulation of water access;^{15, 16} this has been with both Gd chelate modified polymeric nanoparticles¹⁷ and hydrazone cross linked iron oxide nanoparticle assemblies (disassembly in the latter case promoting a change in contrast from T_2 to T_1).¹⁸

The confinement of paramagnetic centres to nanoparticles of tuneable size and payload brings with it both notable increases in contrast and new mechanisms of generating a responsive modality.^{19, 20} The high surface area, tuneable pore size and accessible surface chemistry of mesoporous silica nanoparticles (MSNs) has made them a focal point in much model environmentally responsive drug delivery work,^{21, 22} including that based on the use of pH responsive polymers such as poly(L-histidine), chitosan or poly(acrylic acid).²³⁻²⁶ The integration of contrast generating lanthanide complexes, manganese or iron oxide nanoparticles into these for MRI is well established.^{27, 28} There are few examples of MSNs loaded with manganese or iron oxide nanoparticles that have been reported as pH-responsive CAs for MRI.^{29, 30} These are based on the irreversible release of the manganese or iron oxide nanoparticles loaded into MSNs under an acidic microenvironment. In these cases,

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moderate longitudinal relaxivities up to $12 \text{ mM}^{-1} \text{ s}^{-1}$ are achieved with an < 2-fold increase at switching pH.

In this work, we have functionalised Gd-DOTA integrated MSNs with a pH responsive polymer to generate a *fully reversible* (non-destructive) pH responsive T_1 contrast switch of an unprecedented magnitude. Poly(acrylic acid) (PAA) is known to present, in aqueous solution, a globular conformation at $\text{pH} < 5.5$, swelling at higher pH.³¹ Our hypothesis was that the integration of PAA across the surface of a paramagnetically doped MSN would reversibly gate water exchange with the bulk water and consequently affect the T_1 MRI relaxivity (Figure 1).

Three different samples were prepared according to the synthetic scheme and protocols described in the ESI. First, MSNs were synthesised using a brief modification of the Stöber method.³² As described in our previous work, long delay co-condensation reaction conditions can provide a high number of amino group anchor points inside the MSN but near to the opening of the pore channels.²⁷ These are subsequently modified with an N-hydroxysuccinimide ester derivative of 1,4,7,10-Tetraazacyclododecane tetraacetic acid (DOTA) prior to metallation with GdCl_3 (to obtain high relaxivity Gd-DOTA loaded MSNs, Gd-MSNs, **1**). The remaining pore-peripheral amino groups were converted to carboxylates with succinic anhydride and stepwise functionalised with di-amino pegylated chains to increase stability (Gd-MSNs-Pg, **2**) and PAA (Gd-MSNs-PgPAA, **3**).

Figure 1. Schematic representation of the pH-gated water exchange of Gd-MSNs-PgPAA (τ_m : water exchange rate, r_1 : longitudinal relaxivity).

The specificity and quantification of metallation was resolved by ICP-OES (ESI). Dynamic light scattering (DLS) and TEM analyses confirmed high colloidal stability in all samples with average hydrodynamic sizes of $126.5 \pm 5.6 \text{ nm}$ for Gd-MSNs, $140.7 \pm 3.9 \text{ nm}$ for Gd-MSNs-Pg and $154.4 \pm 4.5 \text{ nm}$ for Gd-MSNs-PgPAA (Fig. S1). Interestingly, no significant differences between Gd-MSNs and Gd-MSNs-Pg were

observed (two-tailed t -test, $n.s.$, $P = 0.0718$) whilst the PAA coating brought a significant change (two-tailed t -test, **, $P = 0.0048$) (Figure S1). TEM images also showed a slight density increase in Gd-MSNs-PgPAA due to the presence of PAA (Fig S2). (Fig. S2). The polymer generated slight darkening of particle contrast is more clearly resolved in the nanoparticles pre incubated at low pH, an observation consistent with the the formation of a more compacted conformation. N_2 adsorption-desorption measurements resolved an average pore size of $3.2 \pm 0.2 \text{ nm}$ by the Barrett-Joyner-Halenda (BJH) method for Gd-MSNs whilst Gd-MSNs-PgPAA showed a pore size of $3.0 \pm 0.1 \text{ nm}$ with a slight decrease in accessible total pore volume due to the presence of the polymer (Fig. S3). ζ -Potential measurements yielded an average positive value of $42.96 \pm 1.07 \text{ mV}$ for Gd-MSNs due to amino group anchor points and a mean of -44.54 ± 4.55 for Gd-MSNs-PgPAA in line with expectations for PAA functionalisation (Fig. S4). Fourier Transform Infrared (FT-IR) spectroscopy analysis confirmed the absence of the CTAB template in all derivatised samples (Fig. S5) with the major resolved resonance that of the Si-O-Si stretching mode at 1076 cm^{-1} reflecting the silica nanoparticle formation. The band resolved at 1630 cm^{-1} , is assigned to the amides generated by carbonyl coupling on PAA tethered and the characteristic C-O mode of PAA.³³

Since (irreversible) changes in particle aggregation have been presented as a prior means of pH regulated MR contrast (*vide supra*), we initially confirmed that Gd-MSNs-PgPAA were of consistently high colloidal stability across the $3.0 - 11.0 \text{ pH}$ range (Fig S6). Significant DLS resolved size decreases between neutral or basic ($152.4 \pm 5.8 \text{ nm}$ at $\text{pH} = 7.0$) and acidic conditions ($131.1 \pm 3.3 \text{ nm}$ at $\text{pH} = 5.0$) were noted, fully consistent with an expected distinct polymer conformational switch. To confirm the ability of PAA to reversibly gate diffusive exchange between the pore channels of the particle and bulk solution the nanoparticles were loaded with fluorescein (protocol in ESI).³⁴⁻³⁶ Although fluorescein presents some pH-dependent emission this is known to be moderate and far below the changes resolved herein;³⁷ the relative fluorescence recovery in the supernatant solution after incubation at $\text{pH} = 4.0$ (SP-4, globular conformation) and $\text{pH} = 9.0$ (SP-9, open conformation) is sufficiently large as to confirm conformational gating (Fig. 2).

Figure 2. Fluorescence resolution of pH gated fluorescein release into supernatant (reported as recovered fluorescein after incubation of Gd-MSNs-PgPAA at $\text{pH} = 4.0$ (SP-4) and $\text{pH} = 9.0$ (SP-9) ($n = 3$, two-tailed t -test, ****, $P < 0.0001$).

To analyse the effect of polymer conformational switching on the relaxometric properties, longitudinal relaxation times were measured at 1.5 T for four different Gd³⁺ concentrations (mM). Longitudinal relaxivity values (r_1), obtained from the slope of the linear fit of the plot of the relaxation rate ($1/T_1$ in s⁻¹) versus Gd³⁺ concentration (mM) are $22.70 \pm 1.9 \text{ mM}^{-1} \text{ s}^{-1}$ for Gd-MSNs (Fig. 3a), a 6-fold increase over commercial Gd-based contrast agents and higher than many of the reported Gd-based nanoparticulate systems.^{38, 39} Relaxivity values of Gd-MSNs-PgPAA were acquired across the pH = 3.0 to pH = 10.0 window. As shown in Fig. 3a, a > 130 % increase in relaxivity ($\Delta r_1 = 11.4 \text{ mM}^{-1} \text{ s}^{-1}$) is achieved on changing the solution pH across the polymer acid pKa. Control analysis using non PAA coated Gd-MSNs confirmed a relaxivity which is reflective of the maximum water access to the internalised Gd centres and is pH independent (Fig. 3a). These relaxivity profiles are fully consistent with the zeta and dye release analyses and a water access which is gated by switching between the globular and swollen conformations of the capping PAA polymer. In considering the relatively modest resolved increase in particle hydrodynamic radius with pH (13 %, Fig S6) and knowing that the beneficial effects of rotational correlation are very moderate at low fields, the observations herein are unlikely to include significant reversible rotational effects.⁴⁰ It is notable that the Gd-MSNs-PgPAA relaxivity switch is also fully reversible (Fig. 3b). There are comparatively few examples of fully reversible switching in environmental responsive particulate contrast agents.⁵ Most of them are supported by modification of the hydration number, q , in Gd chelates.^{41, 42} The work herein represents the first fully reversible water gated high contrast nanoparticulate contrast agent.

Figure 3. a) Longitudinal relaxivity values at 1.5 T, r_1 , of Gd-MSNs (blue) and Gd-MSNs-PgPAA (green) from pH = 3 to pH = 10 ($n = 3$), b) Reversibly gated longitudinal relaxivities, at 1.5 T, of Gd-MSNs-PgPAA (from triplicate analyses of each sample).

The magnetic resonance performance of Gd-MSNs-PgPAA at different pH was additionally resolved by phantom imaging at 7 T. In accordance with the relaxivity measurements, a marked clear contrast enhancement is observed from pH = 4.0 to pH = 7.0 (Fig. 4a). Signal intensity measurements in 5 consecutive regions of interest (ROIs) confirmed contrast differences between water and Gd-MSNs-PgPAA at pH = 6.0 and 7.0 whilst no significant difference ($n.s$) is found for Gd-MSNs-PgPAA at pH = 4.0. Associated relaxivity values

are shown in Table S1 and S2 (ESI, S1 and S2).

Figure 4. a) MR image phantoms acquired at 7 T for H₂O and 1 mg/mL aqueous solutions of Gd-MSNs-PgPAA at pH = 4.0, pH = 6.0 and pH = 7.0, b) Intensities (a.u.) measured in 5 regions of interest (ROIs) of H₂O and Gd-MSNs-PgPAA at pH = 4.0, pH = 6.0 and pH = 7.0. Statistical analysis was carried out by comparing between the samples using a two-tailed t -test ($n.s$, $P > 0.05$; ****, $P < 0.0001$).

In summary, the colloidal integration of paramagnetic centres supports relaxivity values that can be an order of magnitude greater than the molecular analogues. Much can be gained by generating CA's that are reportive of their immediate environment. T_1 agents operate through a ligation-induced paramagnetic relaxation that is (necessarily) massively amplified through rapid exchange with bulk water. Although a number of responsive T_1 agents have been reported to date, none have utilised reversible steric gating of water. This novel nanoparticulate system provides higher longitudinal relaxivity values than those previously reported for pH-responsive Gd complexes and for most of the reported environmentally responsive nanoparticles.^{2, 43} The magnitude of the relaxivity switch should also be noted; the > 130% modulation magnitude herein is significantly larger than that reported for most T_1 CA's across physiologically relevant pHs. The organic polymer-inorganic scaffold platform utilised herein is almost infinitely chemically tuneable and inherently supports both the gated delivery of therapy with a simultaneous imaging modality.

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Conflicts of interest

There are no conflicts to declare.

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TOC

The polymeric capping of a paramagnetically doped MSN enables the generation of high T_1 MRI contrast which is highly pH responsive through a fully reversible change in polymer conformation.