

Gadonanotubes as new high-performance MRI contrast agents

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Abstract: Gadolinium-based carbon nanostructures are poised to make a significant impact as advanced contrast agents (CAs) for magnetic resonance imaging (MRI) in medicine. This paper reviews and forecasts gadonanotubes as synthons for the design of high-performance MRI CA probes with efficacies up to 100 times greater than current clinical CAs. This level of performance is vital for achieving the goal of cellular and molecular imaging with MRI. These new materials will be useful for in vivo MRI applications as circulating drug nanocapsules because of their low toxicities, extremely high relaxivities, and potential for cellular targeting and induced cell death by magnetic hyperthermia.

Keywords: nanotechnology, carbon nanotubes, gadonanotubes, MRI contrast agents

Introduction

Magnetic resonance imaging (MRI) is one of the most powerful and central techniques in diagnostic medicine and biomedical research (Mansfield 2004). Primarily, MRI non-invasively produces anatomical details for improved diagnosis of many diseases. Secondly, MRI also provides valuable information about the physiochemical state of tissues and blood flow. Thus, MRI is the method of choice for the diagnosis of many types of injuries and conditions, and it is not surprising that the use of MRI scanners is growing at an explosive rate. Today more than 22 000 MRI systems operate worldwide.

In clinical MRI, the nuclear magnetic resonance signal from water protons in living tissue are used to image organs and disease sites, such as tumors, in 3D. The intensity of this MR signal depends on three important intrinsic tissue factors: the proton density, the longitudinal relaxation time, T_1 , and the transverse relaxation time, T_2 . Thus, various mathematical techniques have been developed to highlight the differences in T_1 or T_2 to obtain good contrast, ie, the difference in appearance of different tissues in an MR image. Otherwise, MR images would be fairly featureless since the amount of water does not vary significantly in the various tissues of in the body.

The development of MRI has also concurrently led to the development of chemical contrast-enhancement products called contrast agents (CAs). MRI CAs are used primarily to improve disease detection by increasing sensitivity and diagnostic confidence. There are several types of MRI CAs including extracellular fluid space (ECF) agents, extended-residence-intravascular agents (blood pool), and tissue (organ)-specific agents. Nearly all commercial CAs available today are ECF agents that distribute extracellularly and excrete exclusively via the kidney. Annually, approximately 60 million MRI procedures are performed worldwide; 30–35% of these procedures use MRI CAs.

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CAs used in clinical MRI procedures operate by changing the proton nuclear spin relaxation times of water molecules in their vicinity, enhancing the detected MR signal in tissue (Lauffer 1987; Caravan et al 1999; Merbach et al 2001; Krause and Editors 2002). Thus, the most commonly used clinical CAs decrease T_1 relaxation times (also referred to as spin-lattice relaxation) of water protons in living tissue in the vicinity of the paramagnetic CA. All CAs are paramagnetic (with unpaired electrons) because paramagnetic CAs generate very large lattice fields (magnetic and nuclear environments with which the protons interact during T_1 relaxation) in the immediate neighborhood of the CA, which greatly shorten the T_1 of any water molecule that approaches the paramagnetic center. The term “relaxivity” (r_1 for T_1 relaxation) is the key determinant for evaluating the efficacy of any MRI CA. It is defined as the change in the relaxation rate of the water protons per molar concentration of the paramagnetic CA, and is expressed in units of $\text{mM}^{-1} \text{sec}^{-1}$.

The high-spin paramagnetic gadolinium (Gd^{3+}) metal ion is the most effective T_1 relaxation agent. It has seven unpaired f-electrons, the greatest number of unpaired electrons exhibited by any atom or ion, a large magnetic moment ($\mu^2 = 63\mu_B^2$ where μ_B is the Bohr Magneton), and a highly symmetrical, slowly relaxing ground state (^8S -state) which produces strong oscillations near the ^1H Larmor frequency, and thus a strong T_1 effect.

The aquated Gd^{3+} ion is toxic, and therefore for medical use, its toxicity is usually sequestered by chelation with multidentate (linear and macrocyclic) ligands (Lauffer 1987; Caravan et al 1999; Merbach et al 2001). Despite the impressive progress in the design and synthesis of Gd^{3+} chelates for advanced CA applications, the resulting Gd^{3+} complexes still possess limitations. For example, magnetic resonance angiography (MRA or blood-pool imaging) requires long retention times in the circulatory system and almost all Gd^{3+} chelates are distributed extracellularly, with a low retention time in the blood pool (about 60 seconds), which makes them unsuitable for MRA. In addition, MRI of individual cells and their tissues (MR molecular imaging) requires that each Gd^{3+} center possess an extremely large relaxivity in order to induce sufficient signal intensity. This requirement is due to the fact that the concentration of Gd^{3+} centers that can be delivered or attached to the surface of a specific cell is largely limited to the nanomolar (nM) range by biological constraints (Nunn et al 1997). Gadolinium CAs in current clinical use with $r_1 \sim 4 \text{mM}^{-1} \text{s}^{-1}$ do not possess sufficiently large relaxivities for molecular imaging since r_1 values greater than $100 \text{mM}^{-1} \text{s}^{-1}$ are generally required (Nunn et al 1997).

Gadonanotubes

Gd^{3+} -based carbon nanotube structures with their unique nanoscalar properties and superior performance as MRI CAs show promise for molecular imaging and other advanced applications.

Single-walled carbon nanotubes (SWNTs) possess unique characteristics that make them desirable for biomedical applications (Martin and Kohli 2003). The ideal length for medical applications is uncertain, but ultra-short nanotubes (20–100 nm) or US-tubes (Gu et al 2002; Mackeyev et al 2005) are probably best suited for cellular uptake, biocompatibility, and eventual elimination from the body. Additionally, the US-tube exterior surface provides a versatile scaffold for attachment of chemical groups for solubilizing or targeting purposes, while its interior space allows for encapsulation of atoms, ions, and even small molecules (Suenaga et al 2000; Monthieux 2002; Mackeyev et al 2005) whose cytotoxicity may be sequestered within the short carbon nanotube. Finally, medical-imaging agents derived from US-tubes hold promise for intracellular imaging, since carbon nanotubes have been shown to translocate into the interior of cells with minimal cytotoxicity (Dugan et al 1997; Wang et al 1999; Kam et al 2004; Lu et al 2004; Pantarotto et al 2004).

Recently, the first carbon nanotube-based CA called a “gadonanotube” (Figure 1 inset) was reported (Sitharaman et al 2005). This CA harnessed the ability of ultra-short single-walled carbon nanotubes (US-tubes) to encase smaller ions or molecules within their framework by successfully loading and confining aquated Gd^{3+} -ion clusters within the nanoscalar confines of the US-tubes. Relaxometry and magnetometry

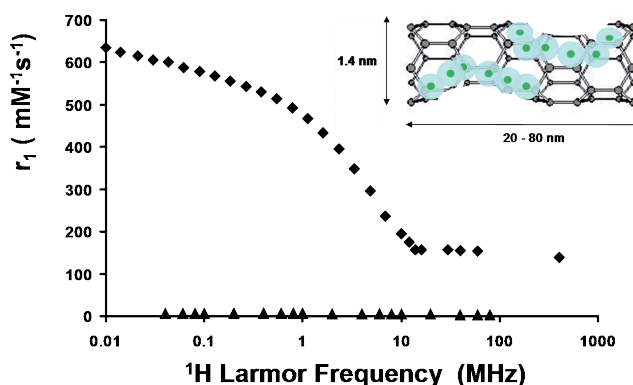


Figure 1 Nuclear magnetic relaxation dispersion profile measured for $\text{Gd}^{3+}@$ US-tubes in a 1% sodium dodecylbenzene sulfonate solution ($c_{\text{Gd}} = 0.044 \text{mM}$; $T = 37^\circ\text{C}$) (\blacklozenge). For comparative purposes, data for the commercially available CA, MagnevistTM or $(\text{Gd}[\text{DTPA}])^{2-}$ (\blacktriangle), are also shown. Inset: Depiction of a single US-tube loaded with hydrated Gd^{3+} ions through sidewall defects created by cutting full-length nanotubes to produce US-tubes (not to scale and Cl^- anions and atoms attached to dangling C bonds not shown).

studies revealed that these $\text{Gd}^{3+}_n\text{@US-tube}$ species are linear superparamagnetic molecular magnets with MRI efficacies 40–90 times larger than any current Gd^{3+} -based CA in clinical use. As such, gadonanotubes, with their embedded 2–5 nm superparamagnetic Gd^{3+}_n -ion clusters, demonstrate potential as a radically new synthon for the development of high-performance MRI CAs.

Nuclear magnetic relaxation dispersion (NMRD) profiles can be of great help in determining the parameters influencing relaxivity, and they have played an important role in the development of our understanding of proton relaxivity. NMRD profiles are proton-spin relaxivities measured as a function of magnetic field. The NMRD profile ($B = 5 \times 10^{-4}$ –9.4 T) for an aqueous solution of a $\text{Gd}^{3+}_n\text{@US-tube}$ sample in 1% SDBS (sodium dodecylbenzene sulfonate, a surfactant employed to suspend the highly lipophilic SWNTs in water) solution at 37°C, is presented in Figure 1. Also presented, for comparative purposes, are data for one of the commercially available MRI CAs, $[\text{Gd}(\text{DTPA})(\text{H}_2\text{O})]^{2-}$ (Magnevist™). For any magnetic field in Figure 1, the relaxivity for the $\text{Gd}^{3+}_n\text{@US-tubes}$ is remarkably larger than for the clinical CA. This is true at the standard MRI field strength (nearly 40 times larger) for clinical imaging of 20–60 MHz ($170 \text{ mM}^{-1} \text{ s}^{-1}$ vs $4.0 \text{ mM}^{-1} \text{ s}^{-1}$), but is even more pronounced (nearly 90 times larger) at very low fields such as 0.01 MHz ($635 \text{ mM}^{-1} \text{ s}^{-1}$ vs $7.0 \text{ mM}^{-1} \text{ s}^{-1}$).

In addition to the exceptionally large relaxivity values obtained for the gadonanotubes, the shape of the NMRD curve, as shown in Figure 1, is also considerably different from that reported so far for any other Gd^{3+} -based system. In particular, the relaxivities are continuously decreasing with increasing magnetic field at proton Larmor frequencies below 1 MHz, in contrast to the usual Gd^{3+} CAs which present constant values at these low fields. Even more remarkable is the finding that at high magnetic fields (>60 MHz), the relaxivities remain practically constant, whereas a strong decrease is observed for the usual Gd^{3+} CAs.

The Solomon-Bloembergen-Morgan theory is unable to predict the observed shape of the NMRD profile and thus does not appear appropriate for gadonanotubes. Clearly, further investigations are needed in order to explain both the extremely large relaxivities and the magnetic-field dependency of the proton relaxivities for the gadonanotubes and possibly also other nanoscale MRI CA materials. However, the data can be used to evaluate their potential efficacy as advanced MRI CAs.

To this end, we also recently conducted T_1 -weighted MRI phantom studies of the gadonanotubes at a 0.04 mM

(Gd^{3+}). Additional vials containing the commercial contrast agent Magnevist™ and deionized water solutions were also included in the imaging section as a reference for comparative purposes.

Representative T_1 -weighted MRI images of the vials are shown in Figure 2. At 0.04 mM (Gd^{3+}) the gadonanotubes showed extremely large signal enhancement compared to Magnevist™ (ca. 100 times!) at the same concentration (Figures 2 and 3).



Figure 2 T_1 -weighted magnetic resonance imaging (MRI) phantoms of the $\text{Gd}^{3+}_n\text{@US-tubes}$ solution at a 0.04 mM (Gd^{3+}). For comparative purposes, MRI phantoms of Magnevist™ at a 0.04 mM (Gd^{3+}) and pure water are also shown. All data were collected using a 1.5T Philips MRI imager.

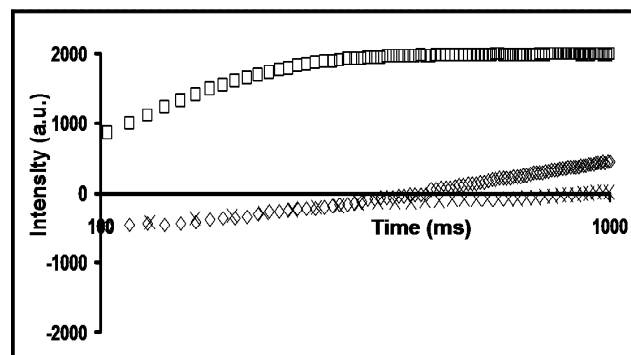


Figure 3 Magnitude signal-intensity curves for vials of $\text{Gd}^{3+}_n\text{@US-tubes}$ (\square) and Magnevist™ (\diamond) at the same Gd^{3+} ion concentration of 0.04 mM, along with that of pure water (\times). Note the substantially faster recovery time for the gadonanotubes probes when compared with the conventional CA.

Future applications

Universal MR probes

The high relaxivity values for the gadonanotubes at all field strengths (low MR fields, current clinical MR fields and high MR fields) make these new gadonanotube materials suitable for use as universal MRI CAs. At the standard MRI field strength for clinical imaging in the 20–60 MHz range, their values are nearly 40 times larger than clinical CAs ($170 \text{ mM}^{-1} \text{ s}^{-1}$ vs $4.0 \text{ mM}^{-1} \text{ s}^{-1}$). The remarkably-high values at very low fields such as 0.01 MHz ($635 \text{ mM}^{-1} \text{ s}^{-1}$ vs $7.0 \text{ mM}^{-1} \text{ s}^{-1}$) will especially benefit microtesla MRI imaging technologies (McDermott et al 2004). At high magnetic fields (>60 MHz), the ability of the gadonanotubes to maintain a nearly constant

relaxivity is particularly important (Mansfield 2004) given the current tendency to develop MRI scanners of higher and higher fields; at these same fields, the contrast-enhancing effect of traditional CAs drops off. Currently, the most efficient T_1 agents show a typical high-field relaxivity peak centered around 30–40 MHz which is characteristic of slow molecular rotation which can give maximum relaxivities of 40–50 $\text{Mm}^{-1}\text{s}^{-1}$. Above this frequency, the relaxivity quickly vanishes to very small values for current MRI CAs. In this respect, gadonanotubes may represent a significant breakthrough in CA design for high-field imaging.

Cellular- and molecular-imaging probes

Molecular imaging is a new frontier for diagnostic medicine. It aims to strengthen diagnostic accuracy of existing image modalities and their interpretation by probing unique biological signatures or sub-cellular processes that are at the cause of disease. The gadonanotube materials considered here would seem to hold exceptional promise for molecular imaging for the following reasons:

a) Their external surfaces can be used as a scaffold to attach a wide variety of agents. These agents can be water-solubilizing groups, biocompatible coverings, and even antibodies or peptides for active targeting of a specific cell type of interest, such as malignant cells.

b) Biological constraints limit targeted receptor sites on cell surfaces to very low concentrations (nM–pM/g of tissue) (Nunn et al 1997). The exceptionally large T_1 relaxivities of the gadonanotubes (Figure 1) could provide sufficient signal-to-noise to image cell-surface receptor sites in the nanomolar range. For example, for a clinical CA with $r_1 = 4.0 \text{ mM}^{-1}\text{s}^{-1}$ at clinical field strengths, the minimum detectable concentration of Gd^{3+} is 10^{-4} M (Nunn et al 1997). The increase in relaxivity provided by the gadonanotube changes this minimum concentration to approximately $2 \times 10^{-9} \text{ M}$.

c) Recently, SWNTs have been shown to translocate into the interior of cells with minimal cytotoxicity (Dugan et al 1997; Wang et al 1999; Kam et al 2004; Lu et al 2004; Pantarotto et al 2004). Thus, CA probes derived from these materials could also accumulate within targeted cells to further boost MRI signal strength. For example, we estimated that a (10 nm x 100 nm) gadonanotube probe, with $r_1 = 170 \text{ mM}^{-1}\text{s}^{-1}$ per Gd^{3+} at clinical fields, contains about 100 Gd^{3+} ions. This should give an effective $r_1 = 17000 \text{ mM}^{-1}\text{s}^{-1}$ per probe. (Here we have estimated the T_1 relaxation rate per nanoprobe, since the relaxation effect will be due to the entire densely-packed nanoprobe rather than individual Gd centers.) If just 1000 such probes were to accumulate within a single

cell, the relaxivity of the cell would be $17000000 \text{ mM}^{-1}\text{s}^{-1}$ (!) which should easily permit cellular imaging. The use of progenitor and stem cells in humans for cellular therapies will require a technique that can accurately monitor their fate and delivery noninvasively and repeatedly. MRI is ideally suited to study the migration and tissue integration of magnetically labeled adult stem cells. Early detection of cancer cells would be another very desirable application of such an intracellular molecular-imaging capability.

Guided-therapy probes

The superparamagnetic gadonanotubes also show promise for the development of the first Gd^{3+} -based MRI image-guided therapeutic agent that can be used for targeted magnetic-field-induced hyperthermia. While heating a cancer cell or tumor to the point of therapeutic destruction can be accomplished by several methods (Moroz et al 2002), the least intrusive is the use of a magnetically mediated agent. Although any magnetic material can be magnetically induced to generate heat, single domain particles (superparamagnetic materials like the gadonanotubes) are preferred because they can produce far more heat at safer (lower) magnetic fields compared with ferromagnetic materials (Moroz et al 2002). The limitation of hyperthermia for human treatment is the failure to generate appreciable heat at safe magnetic fields. However, recent results with ultra-small iron oxide particles (USPIOs) have shown promise for the first human trials (Moroz et al 2002). The gadonanotubes may make ideal MRI-delivery capsules for cancer imaging–therapy by magnetic hyperthermia due to their nanoscale size, superparamagnetism, external derivatization potential, and intrinsically intracellular nature. Furthermore, non-spherical particles such as the gadonanotubes are yet to be tested as hyperthermia agents, and it is possible that their unique rod-shaped geometry (aspect ratio of 100:1) may augment performance through non-axial Brownian relaxation (Rosensweig 2002).

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